PHILLY MOTILITY

MAY 4, 2019

FOREWORD

Thank you all for joining Philly Motility 2019, to celebrate the newest cell motility research in the Philadelphia region. Our aim is to foster new opportunities for future collaborations amongst the area's bustling community of cell migration researchers.

The Drexel Biology and Temple College of Engineering both strongly supported our efforts to organize a local cell migration meeting. We hope to have this symposium organized biannually, and that next time, other institutions may join our efforts to catalyze future interactions.

Regards,

Bojana Gligorijevic and Ryan Petrie

SCHEDULE

Time	Activity and location	Session Chair	Speakers	Title of the talk
9:00 AM	Welcome and introduction, Auditorium		Ryan Petrie and Bojana Gligorijevic (Drexel/Temple)	
9:10 AM	Session I, Auditorium	Ryan Petrie	Ryan Petrie (Drexel)	High intracellular pressure promotes epithelial tissue integrity
9:30 AM			Ken Myers (USciences)	Septin-mediated guidance of non-centrosomal microtubules to focal adhesions
9:50 AM			Tanya Svitkina (UPenn)	Actin-Microtubule Crosstalk in Neuronal Growth Cones
10:10 AM			Peter Baas (Drexel)	KIFC1, a mitotic motor, is a new player in the mobility of post-mitotic neurons
10:30 AM			Kalpana Mandal (UPenn)	Role of the KIF20A kinesin in the mechanics of cancer cells
10:45 AM	Coffee Break I, 1st floor lobby			
11:05 AM	Session II, Auditorium	Bojana Gligorijevic	Alisa Clyne (Drexel)	Migration and metabolism in 3D in vitro vasculature-cancer models
11:25 AM			Dimitrios Vavylonis (Lehigh)	Modeling mechanisms of dendritic actin network structure and turnover
11:45 AM			Dan Hammer (UPenn)	The upstream motility of amoeboid cells.
12:05 PM			Erkan Tuzel (WPI)	Sperm selection on-a-chip: from computer simulations to the clinic
12:25 PM			Katrina Wisdom (UPenn)	Covalent cross-linking and low plasticity in basement membrane-like matrices physically restrict breast cancer cell invasion
12:40 PM	Lunch and Posters, 3rd floor auditorium			
2:00 PM	Session III, Auditorium	Ryan Petrie	Dennis Discher (UPenn)	Genomic variation in cancer - is there some basis in motility?
2:20 PM			Eti Cukierman (FCCC)	Desmoplastic extracellular matrices; wakening the nerve of pancreatic cancer
2:40 PM			Bojana Gligorijevic (Temple)	Real-time imaging of intrinsic and extrinsic control mechanisms in invasive breast carcinoma cells
3:00 PM	Cofee Break II, 1st floor lobby			
3:20 PM			Chris Janetopolous (USciences)	Tuning of Plasma Membrane Charge Regulates Cell Polarity and Tumor Progression
3:40 PM			Panagiotis Mistriotis (JHU)	Confinement Induces Nuclear Volume Expansion and Blebbing by Triggering RhoA Mediated Nuclear Influx
4:00 PM			Keynote address - Roberto Weigert (NIH)	Novel Mechanisms of Membrane Remodeling in live animals revealed by Intravital Subcellular Microscopy
5:00 PM	Wrap up and conclusions, Auditorium		Ryan Petrie and Bojana Gligorijevic (Drexel/Temple)	

KEYNOTE LECTURE

Roberto Weigert
NIH

Novel Mechanisms of Membrane Remodeling in live animals revealed by Intravital Subcellular Microscopy

We have developed Intravital Subcellular Microscopy (ISMic) to investigate the mechanisms regulating membrane remodeling in live animals. Our work has been focusing on the role of mechanical forces in deforming cellular membranes through the activation of the actomyosin cytoskeleton. Here, we show two novel machineries that control membrane remodeling in vivo during membrane trafficking and cell migration, respectively.

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INVITED TALKS

Ryan Petrie
Drexel University
High intracellular pressure promotes epithelial tissue integrity

Actomyosin contractility generated tension across cell-cell junctions is critical for forming and maintaining strong cell-cell interactions. We recently discovered that actomyosin contractility is also responsible for governing intracellular pressure in human fibroblasts. Here, we tested the hypothesis that intracellular pressure is critical for maintaining epithelial cell integrity by investigating its role in tight junction formation and preventing epithelial cell motility. Strikingly, we found that confluent epithelial cells on glass have an average intracellular pressure of 4000 Pa, compared to 500 Pa in primary human fibroblasts. This high intracellular pressure is associated with a higher transepithelial electrical resistance (TEER). Reducing actomyosin contractility, reduced intracellular pressure and TEER, while increasing cell motility. Together these data suggest that generation of intracellular pressure is important for maintaining epithelial phenotype and barrier function.

Ken Myers
University of the Sciences
Septin-mediated guidance of non-centrosomal microtubules to focal adhesions

Polarization and migration of endothelial cells requires the assembly of Golgi-derived, non-centrosomal microtubules. How cells identify and organize the non-centrosomal microtubule array is not well understood. We have identified that experimental activation of Rac1 promotes the localization of peripheral septin-7 adjacent to FAs, and that FA-associated septin-7 is critical for guiding MT assembly into FAs.

Tanya Svitkina
University of Pennsylvania
Actin-microtubule crosstalk in neuronal growth cones

The mechanisms by which microtubules regulate the actin cytoskeleton in order to enable directional cell migration or growth cone navigation remain poorly defined. We have found that branched actin networks in neuronal growth cones can be physically associated with microtubules and that formation of these microtubule-associated actin networks depend on the adenomatous polyposis coli (APC). These data suggest that microtubules enable growth cone navigation by stimulating local assembly of a branched actin network in an APC-dependent manner.

Peter Baas
Drexel University
KIFC1, a mitotic motor, is a new player in the mobility of post-mitotic neurons

KIFC1 (also called HSET or kinesin-14a) is best known as a multi-functional motor protein essential for mitosis. Here we discuss its role in regulating the sliding of microtubules in neurons, which is important for axonal growth and growth cone morphology, with special emphasis on neuronal migration during brain development.

Alisa Clyne
Drexel University/University of Maryland
Migration and metabolism in 3D in vitro vasculature-cancer models

In this talk, I will describe how our research has evolved from examining 2D endothelial cell migration on soft vs. stiff substrates to examining adhesive and migratory relationships between vascular tubes and breast epithelial spheroids. I will describe cell adhesion and migration studies from our 3D in vitro co-culture models, as well as provide an overview of computational metabolic models of endothelial cell proliferation and migration. Finally, I'll discuss future work in integrating the 3D experimental models with metabolomics, and using computational tools to better understand metabolic relationships among cells in co-culture.

Dimitrios Vavylonis
Lehigh University
Modeling mechanisms of dendritic actin network structure and turnover

We present results of three-dimensional models at the filament level aimed to understand how structural and mechanical properties are regulated within lamellipodia. We investigate the possible role of filament severing and annealing in network structure, as well as the dependence of retrograde flow on motor pulling, polymerization forces and actin network coupling to focal adhesions.

Dan Hammer
University of Pennsylvania
The upstream motility of amoeboid cells

It was recently discovered that some of the amoeboid cells of the immune system can crawl upstream under flow, like a salmon swims upstream in a river. This phenomenon, first discovered in T-lymphocytes, is dependent on the binding a particular integrin receptor - Late Functioning Antigen-1 (LFA-1) - to intracellular adhesion molecule-1 (ICAM-1). We have since show that hematopoietic stem cells and neutrophils - both which bear LFA-1 - can crawl upstream as well. In neutrophils, upstream migration is possible only when all other ICAM-1 receptors are blocked. We also show evidence that transendothelial migration is facilitated by upstream migration.

Erkan Tüzel
Worcester Polytechnic Institute
Sperm selection on-a-chip: from computer simulations to the clinic

Human infertility is a reproductive disease that affect millions of people worldwide. According to the NIH, one-third of infertility cases are caused by male reproductive issues, and existing clinical solutions for this condition fall short when sorting for sperm free of reactive oxygen species, DNA damage, and epigenetic aberrations. Although several microfluidic platforms exist, they suffer from structural complexities, i.e., pumps or chemoattractants, setting insurmountable barriers to clinical adoption. Utilizing hydrodynamic coupling between sperm cells and boundaries, we developed a pillar array design that efficiently and noninvasively isolates highly-motile and morphologically normal sperm, with lower epigenetic global methylation, from raw semen. The Simple Periodic ARray for Trapping And isolatioN (SPARTAN) modulates the directional persistence of sperm, increasing the spatial separation between progressive and nonprogressive motile sperm populations, with over 99% motility of sorted sperm, a 5-fold improvement in morphology, 3-fold increase in nuclear maturity, and 2–4-fold enhancement in DNA integrity.

Dennis Discher
University of Pennsylvania
Genomic variation in cancer - is there some basis in motility?

Migration through small constrictions can rupture nuclei, mis-localize key nuclear proteins, and increase DNA damage marked by γH2AX. Complete rescue is achieved by combining myosin inhibition with antioxidant or overexpressed DNA repair factors, with a threshold dependence of cell cycle on DNA damage also evident with custom-etched pores of ~4-um - implicating nuclear curvature. Increased genomic variation after constricted migration is also quantified in expanding clones, consistent with (mis)repair of excess DNA damage.

Eti Cukierman
Fox Chase Cancer Center
Desmoplastic extracellular matrices; wakening the nerve of pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is predicted to soon become the second killer cancer in the USA. One of the hallmarks of PDAC is desmoplasia, which mostly consists of an expansion in activated stellate cells, known as cancer associated fibroblasts (CAFs), and remodeling of their extracellular matrix. Desmoplasia is a major culprit for the unique PDAC microenvironment, which both collapses blood vessels restricting nutrients and fosters immunosuppression. Crosstalk between CAFs, PDAC, and immune cells, within this microenvironment, is not fully understood. This seminar will shed light on how CAF-derived matrices trigger a new signaling axis, akin to excitatory synaptic stress, responsible for CAF support of tumor cells under nutritional restriction as well as fostering pro-tumoral immunosuppression. The study identifies a novel potential target for PDAC, a cancer that lacks effective therapies.

Bojana Gligorijevic
Temple University
Real-time imaging of intrinsic and extrinsic control mechanisms in invasive breast carcinoma cells

Tumor cell structures that have long been hypothesized as necessary for metastasis are invadopodia, invasive protrusions rich in structural proteins (Tks5, actin), adhesion proteins (eg. integrin β1), and metalloproteases. Using our unique intravital imaging approaches, we previously demonstrated that invadopodia in vivo are necessary for intravasation and consequent lung metastasis. In primary tumors, we found that cells which assemble invadopodia migrate at slow speeds, in perivascular niches where the ECM is cross-linked. Outside of these niches, no invadopodia were observed and cells migrated at high speeds, via contact guidance along collagen fibers. The invadopodia-driven motility can be switched to contact guidance by reducing the ECM cross-linking or by knocking down Tks5, which in turn reduces intravasation and metastasis. We next deduced that invadopodia-driven motility consists of two oscillating states: i. Invadopodia state, in which a cell is relatively sessile while it assembles invadopodia and degrades ECM; ii. Locomotion state. State balance is regulated by integrin β1 activation levels. Importantly, the Invadopodia state only occurs in early G1, whereas the Locomotion state can be seen throughout the entire cell cycle, suggesting that the cell cycle controls invadopodia assembly. Using FUCCI markers, we next show that Invadopodia state occurs during the G1 phase of the cell cycle. A close look at the regulators of G1 revealed that the cell cycle regulator $p27^{kip1}$ localizes to the sites of invadopodia assembly and overexpression of $p27^{kip1}$, but not $p21^{cip1}$, causes faster turnover of invadopodia and increased ECM degradation. Taken together, these findings suggest that invadopodia function is controlled by specific cell cycle regulators.

Chris Janetopolous
University of the Sciences
Tuning of Plasma Membrane Charge Regulates Cell Polarity and Tumor Progression

Cell polarity is regulated by the local changes in lipid molecules that contribute to membrane charge, and this plays a critical role in activating effector molecules that organize the actin cytoskeleton and directed cell motility. Our laboratory uses the model system Dictyostelium to glean insight into the control of these molecules and have applied this knowledge to our understanding of cancer progession. This works suggests that the tight regulation of these molecules are critical for polarity in general, and directly contribute to the metastatic state of cancer cells.

SELECTED SHORT TALKS

Role of the KIF20A kinesin in the mechanics of cancer cells

Kalpana Mandal[1*], Katarzyna Pogoda[1], Satabdi Nandi[2], Samuel Mathieu[3], Amal Kasri[3], François Radvanyi[3], Bruno Goud[3], Jean-Baptiste Manneville[3*],Paul A Janmey[1*]

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Molecular motors play important roles in force generation, cell migration and intracellular trafficking. Changes in specific motor activities are altered in numerous diseases. KIF20A, a motor protein of the kinesin-6 family, is overexpressed in bladder cancer, and KIF20A levels correlate negatively with the clinical outcome. We report here the role of the KIF20A kinesin motor protein in intracellular and cortical mechanics probed using optical tweezers and surface AFM respectively. We show that bladder cancer cells are softer than normal urothelial cells and that cell mechanics changes with cancer grade. Inhibition of KIF20A slows down the migration of bladder cancer cells and softens the intracellular environment in both high and low grades of cancer. Surprisingly, while cell surface stiffness measured by AFM also decreases upon KIF20A inhibition in low grade bladder cancer cells, the cortex of higher-grade malignant cells stiffens. We show that changes in cortical stiffness correlate with the spatial localization of acto-myosin and the interaction of KIF20A with myosin II. Our results provide the first evidence for a central role of a kinesin motor in cancer cell mechanics, with opposite effects on intracellular and cortical mechanics.

Covalent cross-linking and low plasticity in basement membrane-like matrices physically restrict breast cancer cell invasion

Katrina M. Wisdom[1], Kolade Adebowale[2], Dhiraj Indana[1], Julie Chang[3], Joanna Y. Lee[1], Sungmin Nam[1], Pei-En Chou[4], Rajiv Desai[5], Ninna Struck Rossen[6], Marjan Rafat[6], Robert B. West[7], Louis Hodgson[8], Taeyoon Kim[9], and Ovijit Chaudhuri[1]

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Breast cancer cells must breach nanoporous basement membranes (BM) to initiate the metastatic cascade during ductal carcinoma progression. Prior research on cancer cell migration has shown that the two predominant modes involve one that is protease-dependent, involving cells degrading matrix with invadopodia, and one that is protease-independent, requiring pre-existing, micron-sized channels or pores for cells to squeeze through. However, many extracellular matrices exhibit mechanical plasticity, irreversibly deforming in response to force, so that pore size may be malleable. To investigate the impact of matrix plasticity on cancer cell invasion in confining microenvironments, we recently developed nanoporous, reconstituted basement membrane (rBM)-alginate interpenetrating network (IPN) hydrogels in which plasticity could be modulated independent of stiffness. Strikingly, cells in high plasticity IPNs used invadopodia protrusions to physically open up channels for migration, independent of proteases. By contrast, cells in low plasticity IPNs were rounded and did not migrate. While these IPN hydrogels consisted of ionic cross-links, native BM contains both ionic and covalent cross-links, yet the role of BM covalent cross-linking on invadopodia extension into the BM remains unclear. We now address this open question using two separate materials systems as well as a computational model. We find that increased covalent cross-linking of rBM matrix diminishes its mechanical plasticity. Covalently cross-linked rBM matrices, as well as low plasticity IPN hydrogels incorporating bio-orthogonal covalent cross-links, both restrict cell spreading and protrusivity, independent of proteases. Mechanistically, our computational model shows that the reduction in plasticity due to covalent cross-linking is sufficient to physically inhibit the extension of invasive protrusions, independent of the impact of covalent cross-linking or matrix plasticity on cell signaling pathways. These new findings highlight the biophysical role of covalent cross-linking in regulating BM plasticity and invasion of these confining tissue layers, and suggest a therapeutic approach toward the inhibition of this plasticity-mediated migration mode.

Confinement induces nuclear volume expansion and blebbing by triggering RhoA-mediated nuclear influx

Panagiotis Mistriotis[1*], Emily Wisniewski[1*], Kaustav Bera[1], Jeremy Keys[2],Soontorn Tuntithavornwat[1], Robert A. Law[1], Yuqi Zhang[1], Runchen Zhao[1], Petr Kalab[1], Jan Lammerding[2], Konstantinos Konstantopoulos[1]

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Cell migration through tissues is a critical step during the metastatic spread of cancerous cells from primary tumors to distal organs in the body. Cancer cells in vivo must migrate through complex confining microenvironments that initiate intracellular signaling cascades distinct from those experienced during 2D migration. As the largest and stiffest cellular component, the nucleus poses a significant barrier to confined cell migration. As such, confined migration induces significant nuclear deformation that may lead to nuclear blebbing and nuclear envelope rupture with detrimental consequences in genomic stability. While actomyosin contractility has been implicated in regulating nuclear envelope integrity, the underlying mechanisms of nuclear. envelope blebbing and rupture remains unknown. In this study, we combine microfluidic migration assays with molecular biology techniques and high-resolution imaging to elucidate how contractile forces specifically promote nuclear bleb formation. We demonstrate that confinement-induced activation of RhoA/myosin-II contractility at the cell posterior locally increases cytoplasmic pressure, and through nucleo-cytoskeletal bridges formed by the LINC complex, promotes passive influx of cytoplasmic constituents into the nucleus without altering nuclear efflux. Photoablation of cortical actomyosin contractility or inhibition of the RhoA/ROCK pathway suppresses confined migration-induced nuclear influx. Elevated nuclear influx is accompanied by nuclear volume expansion, blebbing and rupture. Moreover, inhibition of nuclear efflux is sufficient to increase nuclear blebbing on two-dimensional surfaces, and acts synergistically with RhoA/myosin-II contractility to further augment blebbing in confinement. Nuclear volume expansion and blebbing ultimately reduce cell motility. Cumulatively, our results demonstrate that confinement regulates RhoA activation and perturbs nuclear flux homeostasis with significant consequences for nuclear size, integrity and cell motility.

POSTER ABSTRACTS

Poster #1 - Quantification of intracellular N-terminal beta actin arginylation

Li Chen and Anna Kashina
Department of Biomedical Sciences, University of Pennsylvania

Actin is a ubiquitous, essential, and highly abundant protein in all eukaryotic cells that underlies muscle contraction, as well as cell adhesion, migration, and leading edge dynamics in many types of non-muscle cells. The two non-muscle actins, beta and gamma, are ubiquitously present in every cell type and are nearly identical to each other at the amino acid level, but play distinct intracellular roles. The mechanisms regulating this distinction have been the focus of recent interest in the field. Work from our lab has previously shown that beta, but not gamma, actin undergoes N-terminal arginylation on Asp3. While functional evidence suggest that this arginylation may be important to actin's function, progress in these studies so far has been hindered by difficulties in estimating the abundance of arginylated actin in cells, and its occurrence in different tissues and cell types. The present study represents the first quantification of the percentage of arginylated actin in migratory non-muscle cells under different physiological conditions, as well as in different cells and tissues. We find that while the steady-state level of arginylated actin is relatively low, it is consistently present in vivo, and is especially prominent in migratory cells. Inhibition of N-terminal actin acetylation dramatically increases the intracellular actin arginylation level, suggesting that these two modifications may directly compete in vivo. These findings constitute an essential step in our understanding of actin regulation by arginylation, and in uncovering the dynamic interplay of actin's N- terminal modifications *in vivo*.

Poster #2 - Discrete mechanical model of dendritic actin networks undergoing retrograde flow

David M. Rutkowski , Danielle Holz , Dimitrios Vavylonis
Department of Physics, Lehigh University, Bethlehem, PA

Determining the force distribution within the lamellipodial actin network as it passes over focal adhesions is important for understanding cell migration. Both motor-based pulling from the lamellar region as well as pushing on the membrane at the leading edge by actin polymerization contribute to the actin retrograde flow and the advancement of the leading-edge of the cell forward. Several computational and mathematical models have been used to investigate the overall stress profile of lamellipodial actin networks, including around focal adhesions, using a continuum approximation. One study (Schreiber et al. PNAS 2010) calculated the force-velocity curve for a discrete model of an actin network passing over adhesive sites. However, the forces and deformations of individual actin filaments important in lamellipodial mechanics have not been considered. We developed a computational model of a discrete actin network as it passes over nascent focal adhesions as determined by 3d Brownian dynamics simulations. The actin network used in our simulations is a dendritic model that has the correct density of actin and has a network topology as revealed in prior electron micrographs. Focal adhesions in our simulations are modeled as regions of increased friction. We describe the stress distribution, deformations, and associated retrograde flow as function of polymerization and pulling forces.

Poster #3 - β-actin and γ-actin nucleotide coding sequences regulate cell migration speed

Pavan Vedula, Satoshi Kurosaka, and Anna Kashina
University of Pennsylvania, Veterinary School of Medicine

Actin is one of the most essential and abundant eukaryotic proteins, highly conserved across the tree of life. Among the six mammalian actins, β- and γ-cytoplasmic actins are the only two that are ubiquitously expressed in every cell type and share the highest identity at the amino acid level, with only four homologous substitutions within their N-terminus. At the same time, these actins have been shown in mouse knockout models to play vastly different physiological roles. We previously showed that the β-actin nucleotide sequence, rather than amino acid sequence, is essential for embryogenesis. We now address the specific role of the coding sequence in β- and γ-cytoplasmic actins' intracellular function, using stable cell lines with exogenously expressed β- and γ- actin, as well as their "codon-switched" variants, β-coded γ- and γ-coded β- actin. We find that, when targeted to the cell periphery using the β-actin 3′UTR, β-actin and γ-actin have differential effects on cell migration in mouse embryonic fibroblasts (MEFs). Cells expressing β-actin migrated much slower than cells expressing γ-actin, and this difference proved to be directly coding sequence dependent: expression of β-coded γ-actin resulted in cell migration speeds comparable to those expressing β-actin, while cells expressing γ-coded β-actin migrated at speeds similar to γ-actin expressing cells. Since the coding sequence controls translation and protein accumulation rates, we used fluorescence recovery after photobleaching (FRAP) to demonstrate that γ-actin accumulates in cells slower than β-actin. Such slower accumulation affects the focal adhesion size, and thus the cell migration speed. These results demonstrate an essential role of coding, rather than amino acid sequence in actin isoform function and show for the first time a direct role for coding sequence-mediated actin translation and accumulation at the leading edge in cell migration.

Poster #4 - Mechanisms for dendritic actin network formation, distributed turnover, and structural remodeling

Danielle Holz, Aaron Hall, Dimitrios Vavylonis
Department of Physics, Lehigh University

The dendritic network of actin filaments provides the force for lamellipodial protrusions, driven by actin filament polymerization and branch generation by the Arp2/3 complex. Electron microscopy experiments of lamellipodia revealed that the network structure of filaments varies with distance to the leading edge. Near the leading edge there is a dense brushwork composed of short filaments. Filaments are longer and appear more linear near the center and rear of keratocyte lamellipodia. Prior modeling of FRAP and single molecule imaging experiments suggested the existence of a diffuse actin oligomer cytoplasmic pool and distributed turnover of F-actin through the lamellipodium. The precise mechanisms behind network remodeling and the role of the oligomer pool have yet to be determined. To answer this question, we created a three-dimensional stochastic model at the filament level that includes rate constants for known mechanisms of polymerization, depolymerization, branching, capping, uncapping, severing and debranching. The model reproduces the +/- 35o orientation pattern when branching occurs within 10o of the lamellipodial plane as well as the density of branches, ends and length distribution near the leading edge for both fibroblast and keratocyte lamellipodia (that differ in the magnitude of retrograde flow and polymerization rates). We show that the simplest implementations of severing, debranching, uncapping, and depolymerization from either pointed or barbed ends does not provide mechanisms for network structural remodeling at the level observed in electron micrographs. We examine and test several mechanisms that include severing and oligomer annealing that may provide an explanation.

<u>Poster #5</u> - Novel role of tRNA-derived fragments in protein arginylation

Irem Avcilar-Kucukgoze, Junling Wang, Anna Kashina

[1] *Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA.*

Posttranslational modifications are fundamental processes that expand the functional diversity of the proteome. Arginylation mediated by arginyltransferase ATE1 is a posttranslational modification of emerging importance that regulates mammalian embryogenesis, cell migration, and normal brain function, and has been recently proposed as a global biological regulator. Arginylation has been detected in the key components of cytoskeleton, including actin, tubulin, α-actinin, tropomyosin, troponin T, myosin binding protein-C, and titin. Importantly, it was found that N-terminal arginylation of beta actin regulates actin cytoskeleton and cell motility in mouse embryonic fibroblasts. However, the molecular mechanism of arginylation is elusive. Our preliminary results showed that while arginylation is very specific to Arg-tRNA Arg its reactivity is similar towards all mouse tRNA Arg isoacceptors, suggesting ATE1 interaction with Arg-tRNA Arg is determined by its targeting toward the Arg-tRNA Arg acceptor stem. In support, tRNA Arg stem structures conjugated to Arg are capable of mediating in vitro arginylation. Since these stem structures bear resemblance to predicted tRNA-derived fragments (tRF) known to be generated in cells via the action of RNaseT2, we next tested the possible involvement of tRF Arg in arginylation. Our data show that RNaseT2 is capable of cleaving the arginyl-tRNA Arg , and this cleavage product is capable of mediating arginylation with similar efficiency to the intact Arg-tRNA Arg . Moreover, our tRNA and tRF-Seq results showed that the ratio of tRNA Arg to tRNA Arg -derived tRF is significantly higher in Ate1 knockout cells compared to wild type, suggesting that lack of arginylation inhibits tRF generation. We propose that tRF Arg can potentially be dedicated solely to arginylation, which enables this modification to maintain its levels even in the high background from protein synthesis. Our study suggests that generation of physiologically important tRFs can potentially play a critical role in a switching mechanism between protein translation and arginylation in vivo, and points to the new role of tRFs in protein modifications.

Poster #6 - Reconstitution of muscle contraction on engineered substrates

Fidalgo, A.R.[1], Barnes, V.S.[1], Kelley, R.S.[1], Kogan, T.Y[1], Street, R.[2] , Chikelu, E.[2], Schauer, C.L.[2], Padrick, S.B[1]

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[2] Department of Materials Science and Engineering, Drexel University College of Engineering

A sarcomere, the basic unit of a muscle, consists of thin (actin) and thick (myosin) filaments, whose interaction is regulated by the proteins troponin and tropomyosin. The actin filaments are capped and anchored in the Z- line by the CapZ complex. Step-wise motion of myosin motor domains along actin filament tracks directly results in muscle contraction, including cardiac muscle, and mutations in either actin or myosin lead to a variety of cardiomyopathies and cardio-hypertrophies. Existing molecular studies have mainly focused on proteolytically isolated monomeric myosin motor domains. While these studies have been revealing, there is a knowledge gap because myosin thick filaments (motor head and tail), composed of hundreds of myosin dimers, are the functional motor unit in vivo. My project looks to develop a new observational platform for measurement of myosin thick filament contractile forces on actin filaments. Using purified proteins (actin, myosin, troponin and tropomyosin) and an engineered CapZ complex that binds chitin, we are reconstituting calcium-regulated, actin-filament – myosin thick-filament contraction on chitin nanofilaments. To measure contraction, we will exploit the piezoelectric property of chitin nanofilaments, which means the chitin filaments generate measurable electrical potentials upon compression. Over the course of my project, I have improved myosin thick filament production, developed a new co-expression and purification method for the human cardiac troponin complex, purified tropomyosin, and optimized CapZ concentration for chitin-CapZ-Actin-Myosin assembly. This work accomplishes several important milestones needed for contraction measurement using this novel system.

Poster #7 - Myosin II governs intracellular pressure and traction force by distinct tropomyosin-dependent mechanisms

Kimheak Sao[1], Tia Jones[1], Andrew Doyle[2], Galina Schevzov[3], Peter Gunning[3], Ryan Petrie[1]

[1] *Drexel Biology, Philadelphia, PA*
[2] *National Institutes of Health, Bethesda, MD*
[3] *University of New South Wales, Sydney, Australia*

During wound healing by dermal fibroblasts and tissue invasion by metastatic cells, the actin-binding proteins tropomyosin (Tpm) and non-muscle myosin II control the contractile forces that drive cell motility. Classically, two-dimensional (2D) substrate rigidity promotes myosin II activity to increase traction force and enlarge focal adhesions in a process coordinated by Tpm 2.1. We recently discovered that actomyosin contractility can pull the nucleus forward like a piston in 3D matrices to increase intracellular pressure and switch fibroblasts from low-pressure lamellipodia to high-pressure lobopodial protrusions. However, it remains unclear whether these myosin II-generated cellular forces are produced simultaneously, and by the same molecular machinery. We found adhesion size decreased and intracellular pressure increased on soft 2D material, suggesting traction force and pressure can be controlled independently. We identified Tpm 1.6 as a positive regulator of intracellular pressure and confirmed that Tpm 2.1 is a negative regulator of traction force. Although these Tpms can independently regulate pressure and traction, both pathways target the mechanosensitive transcription factor YAP/TAZ to the nucleus. While Tpm 1.6 and 2.1 each contributed to efficient 3D cell movement, Tpm 1.6 was specifically required for the generation of pressure and lobopodial protrusions by the nuclear piston mechanism. Both Tpms associate with actin filaments in response to matrix stiffness, with Tpm 1.6 uniquely regulating the recruitment of the regulatory myosin light chain to these structures. Taken together, our work suggests that actomyosin contractility generates intracellular pressure and traction by distinct mechanisms and these mechanisms can be integrated to control complex cell behaviors on 2D and in 3D environments.

Poster #8 - A unique role for clathrin light chain a in cell spreading and migration

Oxana M. Tsygankova and James H. Keen

Department of Biochemistry and Molecular Biology, Cell Biology and Signaling Program of the Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia

Clathrin heavy chain is the structural component of the clathrin triskelion, but unique functions for the two distinct and highly conserved clathrin light chains (CLCa and CLCb) have been elusive. Here we show that following detachment and replating, CLCa is uniquely responsible for promoting efficient cell spreading and migration. Selective depletion of CLCa, but not of CLCb, reduced the initial phase of isotropic spreading of HeLa, H1299 and HEK293 cells by 60-80% compared to siRNA controls, and wound closure and motility by ≈50%. Surface levels of b1-integrins were unaffected by CLCa depletion. However, CLCa was required for effective targeting of FAK and paxillin to the adherent surface of spreading cells, for integrin-mediated activation of Src, FAK and paxillin, and for maturation of focal adhesions but not their microtubule-based turnover. Depletion of CLCa also blocked interaction of clathrin with the nucleation promoting factor WAVE, and altered actin distribution. Further, preferential recruitment of CLCa to budding protrusions was also observed. These results comprise the first identification of CLCa-specific functions, with implications for normal and neoplastic integrin-based signaling and cell migration.

Poster #9 - Modulation of T-cell activation by dendritic cell stiffness

Blumenthal, D, Burkhardt, J.K.

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Dendritic cells (DCs) responding to inflammatory stimuli undergo a process of maturation, through which they become highly effective APCs specialized for T-cell priming. Activation of T-cells takes place at a specialized cell-cell contact site termed the immunological synapse (IS), where multiple receptor-ligand interactions work in concert to direct the T-cell response. Signaling events at the IS depend on forces exerted on the DC by the T-cell actin cytoskeleton. We hypothesize that maturation-associated changes in the DC cytoskeleton alter the biophysical properties of the DC cortex, thereby serving as a platform for enhanced mechanotransduction in interacting T-cells. Using atomic force microscopy, we show that upon maturation, DC stiffness increases two to three fold via an actin-dependent process. Pharmacological studies and analysis of DCs from KO mice reveal that this process depends on actin polymerization downstream of both Arp2/3 complex and formins. Using acrylamide hydrogels coated with stimulatory ligands, we find that T-cell activation is enhanced by increases in stiffness similar to those observed during DC maturation. Dose-response studies reveal that increased substrate stiffness reduces the agonist dose needed to initiate T-cell activation, indicating that mechanical cues function as co-stimulatory signals. Stiffness sensitivity is conserved in CD4 + and CD8 + T-cells, and affects both priming and effector functions. Finally, by engineering DCs with altered stiffness, we show that stiffness of mature DCs directly correlates with their ability to prime ex-vivo T-cells. Taken together, these data reveal a novel mechanism for regulation of T-cell priming by DCs and identify cortical stiffness as an unexplored control point for T-cell priming.

Poster #10 - Tunable and reversible substrate stiffness reveals dynamic mechanosensitivity of cardiomyocytes

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New directions in material applications have allowed for fresh insight into the coordination of biophysical cues and regulators. While the role of the mechanical micro-environment on cell responses and mechanics is often studied, most analyses only consider static environments and behavior, however, cells and tissues are themselves dynamic materials that adapt in myriad ways to alterations in their environment. Here, we introduce an approach, through the addition of magnetic inclusions into a soft PDMS elastomer, to fabricate a substrate that can be stiffened nearly instantaneously in the presence of cells through the use of a magnetic gradient to investigate short-term cellular responses to dynamic stiffening or softening. This substrate allows us to observe time-dependent changes, such as spreading, stress fiber formation, Yes-associated protein translocation, and sarcomere organization. The identification of temporal dynamic changes on a short time-scale suggests that this technology can be more broadly applied to study targeted mechanisms of diverse biologic processes, including cell division, differentiation, tissue repair, pathological adaptations, and cell-death pathways. Our method provides a unique in vitro platform for studying dynamic cell behavior by better mimicking more complex and realistic microenvironments. This platform will be amenable to future studies aimed at elucidating the mechanisms underlying mechanical sensing and signaling that influence cellular behaviors and interactions.

Poster #11 - Plectin links actin and vimentin filaments to polarize intracellular force and move fibroblasts directionally in 3D matrices

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The ability of fibroblasts to move through three-dimensional (3D) environments is critical for wound healing and tissue homeostasis. Heavily cross-linked matrices, such as those found in dermis, are a barrier to the movement of the bulky nucleus within dermal fibroblasts. Fibroblasts can overcome this physical barrier by using actomyosin contractility, vimentin intermediate filaments, and the nucleoskeleton-cytoskeleton linker protein nesprin 3 pull the nucleus forward. The forward movement of the nucleus causes it to act like a piston in these cells and generate compartmentalized pressure, with low pressure behind the nucleus and high-pressure in front of the nucleus generating lobopodial protrusions. Intriguingly, traditional mediators of polarity in crawling cells, specifically Rac1, Cdc42, and PIP3, are uniformly distributed in the highly polarized lobopodial cells. In addition, it is currently unknownhow the anterior myosin II contractility is physically connected to the nucleus to pull it forward. Our work tests the hypothesis that vimentin filaments are linked to contractile actomyosin filaments through the cytolinker protein plectin, and this machinery is required to polarize the movement of lobopodial fibroblasts. We found that inhibiting myosin II dismantled the nuclear piston by dissociating vimentin and nesprin 3 from actin and myosin II, as well as reducing directional 3D migration. These data suggest that myosin II activity mediates nuclear piston complex formation and the directional 3D migration of lobopodial fibroblasts. Knocking down plectin redistributed myosin IIB from the anterior to the posterior cytoplasmic compartment. Reducing plectin expression also disrupted the nuclear piston mechanism, resulting in reduced intracellular pressure. Critically, plectin knock-down and myosin II inhibition both abrogated the close association of actin and vimentin within the cytoplasm of intact fibroblasts, demonstrating that plectin and actomyosin contractility are required maintain the connections between filamentous actin and vimentin intermediate filaments. Taken together, these findings suggest that plectin connects myosin II-generated contractility to vimentin filaments in front of the nucleus. Further, these connections are required to pull the nucleus forward, polarize 3D lobopodial fibroblasts, and maintain directional cell migration. In the future, we will test the role of these plectin linkages in the lamellipodia-independent chemotaxis of high-pressure fibroblasts through 3D collagen gels.

Poster #12 - Cells under different physical environments utilize different force-generation mechanisms and consume different energy to migrate

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Cells in vivo live in diverse physical environments that provide mechanical cues for cells to deform, migrate, and carry out their biological function. For example, during wound healing cells mostly migrate on two-dimensional substrates where the focal adhesion is typically strong. In this case cell migration is likely driven by actin polymerization (actin-driven). During immune responses, however, neutrophils usually migrate within three-dimensional collagen matrices where the hydraulic resistance, defined as the hydrostatic pressure experienced by a cell during its migration, can be very high depending on the permeability and geometry of the matrices. In this case, actin-based cell migration may not be able to overcome the high hydraulic resistance and as a result water permeation (water-driven) may be needed in order for cells to move forward. Indeed, it has been found that S180 cell migration in confined channels, where the hydraulic resistance is high, is driven by water permeation instead of actin polymerization and myosin contraction. This suggests that the specific physical environments of cells largely determine the feasible mechanisms that cells can adopt for migration. In this regard, a single-mechanism model is not sufficient to describe cell migration under various physical conditions and thus a higher level mathematical framework is needed to reconcile and unify the physiology and physics behind cell migration.

We discuss a two-phase theoretical framework of cell migration which includes both actin polymerization (actin phase) and water permeation (cytosol phase). The model specially emphasizes the impact of physical environments on different mechanisms of cell migration through the fundamental physical laws including the conservation of mass, the conservation of momentum, and the appropriate boundary conditions. The model predicts that actin polymerization and water permeation are two complimentary methods of force-generation during migration: actin-driven migration is effective in strong focal adhesion and low hydraulic resistance environments, and water-driven migration is effective in weak focal adhesion and high hydraulic resistance environments. The transition from actin-driven to water-driven cell migration depends on the external hydraulic resistance, which varies with the mechanical properties of the external fluid and the geometry of the cell surroundings.

Cell migration also consumes energy, which varies based on the mechanism of cell migration, the physical environments, as well as the morphology of cells. The two-phase model we have developed satisfies energy identity and is thus well suited to study energy consumption and dissipation during cell migration. The model predicts that, in general, actin-driven cell migration consumes less energy than water-driven cell migration. However, in terms of the cell output efficiency, defined as the ratio of the maximum mechanical power output during cell migration to the input mechanical power, water-based cell migration is more efficient under higher hydraulic resistance environments. This work has implications on early embryonic development, morphogenesis, and cancer cell metastasis.

Poster #13 - Nuclear pressure and piston activation govern 3D motility

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Fibroblast migration through three-dimensional (3D) matrix environments is essential for normal wound healing. During 3D migration, cell morphology and the mechanism of migration are largely dictated by the interactions of the cell with its local microenvironment, including the ability of a cell to squeeze its bulky rigid nucleus through narrow openings. To efficiently translocate their nuclei through the small pores within heavily cross-linked matrices, fibroblasts use actomyosin contractility to pull their nuclei forward like a piston to generate compartmentalized pressure and switch from a lamellipodia- to a lobopodia-based migration. However, it remains unclear how the nuclear piston mechanism is activated in response to specific cell-matrix interactions. Recently, it was shown that the nuclear envelope can transiently rupture in cells moving in 3D environments, suggesting mechanical stress on the nucleus can lead to a dramatic increase in pressure inside the nucleus. Here, we hypothesize that cells moving through cross-linked 3D matrices activate the nuclear piston mechanism in response to increased resistance and mechanical stress on the nucleus. Mechanical stress on the nucleus was indirectly measured by quantifying changes to nuclear morphology and intranuclear pressure. Our results show cells moving from the 2D surface to the interior of 3D matrices can dramatically elongate their nuclei without activating the piston mechanism. Since physically deforming the nucleus was not sufficient to trigger the nuclear piston, we next measured changes in nuclear pressure in cells immediately before and after piston activation. Nuclear pressure was not only compartmentalized and distinct from cytoplasmic pressure, but also increased upon piston activation, suggesting the nucleus is under elevated mechanical stress when the piston is on. Next, we investigated how nuclear pressure was controlled in dermal fibroblasts. We determined myosin II activity and the nucleoskeleton-cytoskeleton linker protein, nesprin 2, are both required for the increase in nuclear pressure during piston activation. Overall, our current model of piston activation consists of three distinct steps: 1) the nucleus becomes stuck when migrating in 3D matrix, 2) tension on nesprin linkages increase nuclear pressure and 3) initiation of a signaling cascade within the cytoplasm triggers the actomyosin contractility required to pull the nucleus forward. Future work will determine the temporal coordination of pressure changes inside the nucleus and cytoplasm during piston activation.

Poster #14 - Emerin and nuclear structure of invasive breast cancer cells

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Dysmorphic nuclei are commonly used for diagnosis and prognosis of many different types of cancer. However, the underlying mechanism regulating nuclear shape changes is poorly understood. Due to the large size and relative rigidity of the nucleus, nuclear structure greatly impacts the cell's ability to migrate through small spaces. The deformability of the nucleus limits the cell's ability to pass through tight spaces, reducing or even stalling migration as the gap size decreases. It is becoming clear nuclear envelope proteins play an important role in regulating nuclear size and structure in cancer. Altered expression of nuclear lamins are found in many cancers. Increased lamin A expression is associated with better clinical outcomes. Recent studies showed expression of nuclear envelope proteins, including emerin and other nuclear lamina proteins, positively impact nuclear size, stiffness, and compliance. The molecular mechanisms behind the regulation of nuclear size and structure in invasive cancer cells will greatly contribute to understanding cancer cell migration, a key step during metastasis. Our results begin to elucidate the role of the inner nuclear envelope protein emerin in nuclear size and stiffness and how emerin expression effects invasiveness of breast cancer cells. We hypothesize emerin plays an important role in regulating nuclear size and stiffness and disruption of emerin expression contributes to the increased invasiveness of breast cancer cells by reducing nuclear size and stiffness. Our research showed nuclei lacking emerin were 44% smaller than wildtype nuclei. Invasive breast cancer cells had 1.4- to 1.7-fold smaller nuclei than normal breast fibroblasts. We found breast cancer cell lines had 1.4- to 1.8-fold decreased emerin expression compared to normal breast cancer fibroblasts. Importantly, increasing emerin expression in theses invasive cancer cells increased nuclear size by up to 1.4-fold. Emerin mutants that specifically inhibit binding to selected partners showed emerin binding to HDAC3, actin and lamin A were important for nuclear architecture, while emerin binding to transcription regulators or BAF played no role. How emerin and emerin mutants alter migration and invasion of invasive cancer cells in vitro and in vivo will be done to test how emerin regulation of nuclear size and structure effects cell invasion and migration through small pores or channels.

Poster #15 - Invadopodia is decoupled from EMT and metastasis

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Disseminating cancer cells are posing a major clinical challenge due to their metastatic potential and the absence of efficient methods for their detection and elimination. Cancer cell dissemination is initiated by the acquisition of a motile phenotype, which can be roughly classified into collective or single cell. To move individually, epithelial cells need to detach from the surrounding tissue and it has been proposed that to do so, cancer cells hijack the developmental program called epithelial- mesenchymal transition (EMT). Numerous cues within the host tissue were proven to initiate EMT and led to cancer cell dissemination. This work has brought EMT to the forefront of metastasis research. However, recent in vivo and clinical data challenge the necessity for this transition during cancer spreading, leading to an ongoing debate in the field. Our previous work showed that mesenchymal breast cancer cells utilize proteolytically active membrane protrusions, called invadopodia, to degrade vascular basement membranes (BM) in vivo. While it is now clear that invadopodia are necessary for intravasation of mesenchymal cells, it is unknown whether invadopodia can assemble in epithelial cells and whether invadopodia are necessary for breaching the epithelial BM. We hypothesized that acquisition of invadopodia can occur independently from the EMT program. To test this, we analyzed literature on Tks5, a recently identified unique marker of invadopodia and a good predictor of tumor progression in patients. Using overexpression of Tks5, my preliminary data show that Tks5 alone is sufficient to drive invadopodia formation and associated extracellular matrix degradation in epithelial breast cancer cells. My preliminary work reinforces the notion that while EMT is permissive for metastasis, invadopodia function is necessary for metastasis. Our future work will include animal experiments towards understanding the role of invadopodia during the progression from ductal carcinoma in situ to invasive carcinoma.

Poster #16 - Mechanical plasticity of extracellular matrix regulates the invadopodial dynamics

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Invadopodia, the F-actin rich protrusions, generate local forces, create space and lead the invasion of cancer cells. Recent experiments have shown that the cancer cells can utilize invadopodia to plastically deform the extracellular matrix (ECM) and migrate through the ECM without protease degradation. Prior to the migration, invadopodia were found to go through the protrusion and retraction cycles, and the oscillatory behaviors are influenced by the plasticity. However, the mechanism of invadopodial mechano-sensitivity on plasticity remains unknown. To physically understand how the invadopodia sense the plasticity and undergo oscillatory protrusions, we built a mechanochemical model by combining the mechanical equilibrium and biochemical signaling pathway feedbacks. Our model predictions agree with the experimental findings that higher ECM plasticity leads to longer protrusion length with large oscillation amplitude. Our model is further verified by predicting similar dynamics of inhibiting different protein (i.e., integrin, myosin and ROCK) activities. Finally, we found that the oscillatory behaviors are achieved by the competition of protrusion timescale, signaling feedback timescale and myosin binding timescale. With the cooperation of myosin contractility, adhesion forces, actin polymerization, actin retrograde flow and Rho-ROCK pathways, the invasive protrusions can dynamically probe the ECM plasticity and modify their oscillatory behaviors.

Poster #17 - Invadopodia-mediated ECM degradation is enriched in the G1 phase of the cell cycle

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Invadopodia are cancer cell protrusions rich in structural proteins (e.g. Tks5, cortactin) and proteases (e.g. MT1-MMP) that are responsible for degradation of the extracellular matrix (ECM). For successful tumor growth and metastasis, cancer cells are required to be both proliferative and invasive, which comprises both migration through the tissue and invadopodia assembly. While several studies addressed how cell motility parameters change throughout cell cycle, the relationship between invadopodia and cell cycle progression has not been elucidated. In this study, using invadopodia- and cell cycle- fluorescent markers, we show in 2D and 3D cultures, as well as in vivo, that breast carcinoma cells assemble invadopodia and invade into the surrounding ECM preferentially during the G1 phase of the cell cycle. Cells synchronized in the G0/G1 phase of the cell cycle degrade at significantly higher levels compared to the cells synchronized in the S phase. Consistent with this, mRNA of Tks5 and protein levels of cortactin are elevated in G1. While it has no effect on cell doubling time, elimination of invadopodia may alter the timing of transitions between cell cycle phases. Taken together, our findings suggest that invadopodia function is tightly linked to cell cycle progression.

Poster #18 - Effect of cell curvature on tetrahymena motility

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Cilium, a complex organelle which can be found in various cell types in the human body, has a crucial contribution in generating flow of: cerebrospinal and embryonic fluids and mucus in the respiratory tract. It can also act as antenna to sense chemical and physical extracellular signals. Defected cilia can cause diseases such as polycystic kidney disease, hydrocephalus and retinal degeneration, which are common maladies affecting more than one in 1,000 individuals worldwide. Thus, understanding the sensory and motility roles of cilia is important. Here we use the tetrahymena model organism to shed light into mechanisms of ciliary beating and interactions. Motile cilia are capable to synchronize with each other and form the metachronal waves, i.e. sequential action (as opposed to synchronized) that give the appearance of a travelling wave. Although most ciliary organisms, tetrahymena included, have curved surfaces, modeling work available to date have all been done for flat surfaces. Here, our goal is to study the metachronal waves on curved surfaces, and realistic 3d organism geometries. We used Multi-Particle Collision Dynamics (MPCD)—a coarse-grained hydrodynamic solvent—to understand this coupling between surface curvature and hydrodynamic synchronization, and the role of basal bodies (BB). Starting from experimentally measured beating patterns, using realistic tetrahymena BB positions, and shapes, we show that the curvature is critical to maintain metachronal waves, and shed light into how cell shape affects ciliary cell motility.

Poster #19 - Contact guidance and chemotaxis synergize to increase cancer cell dispersion

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During tumor progression, cancer cells are simultaneously exposed to multiple guiding cues of migration, both chemical and physical, which direct cancer cell movement. While directed migration of cancer cells exposed to a single guiding cue has been widely studied, only a few studies have addressed how cells navigate migration when simultaneously exposed to multiple guiding cues.

Here, we established a model of 3D cell migration in the perivascular niche, where human breast carcinoma cells are simultaneously exposed to the chemotaxis and contact guidance, in the orthogonal or parallel arrangement. Briefly, we used ibidi 3D chemotaxis chambers as well as custom-made microfluidic devices. We embedded cells and magnetic beads in 3D collagen I, aligned collagen I fibers by exposure to magnetic field and established chemotactic gradients of FBS with varying steepness. We monitored cell trajectories and fibers over 48 hours and observed multiple cell changes in cell directionality, cell stalling and migration at changing velocities. To dissect complex cell trajectories, we utilized Hidden Markov Model, exposing that trajectories are consisting of *passive* and *active* states, and classifying trajectory fragments into these states.

Our results show that in the orthogonal conformation of contact guidance and chemotaxis cues, cells switch between performing contact guidance and chemotaxis, which results in the decrease of end-to-end persistence and consequently, in the increase in the cell dispersion. Cells migrating in parallel arrangement showed an increase in their instantaneous velocity and end-to-end persistence. Our results suggest that in the perivascular niche, contact guidance and chemotaxis cues work in synergy to maximize the coverage of the blood vessels, independent of the spatial conformation of the guiding cues.

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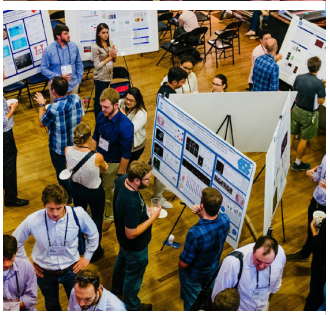

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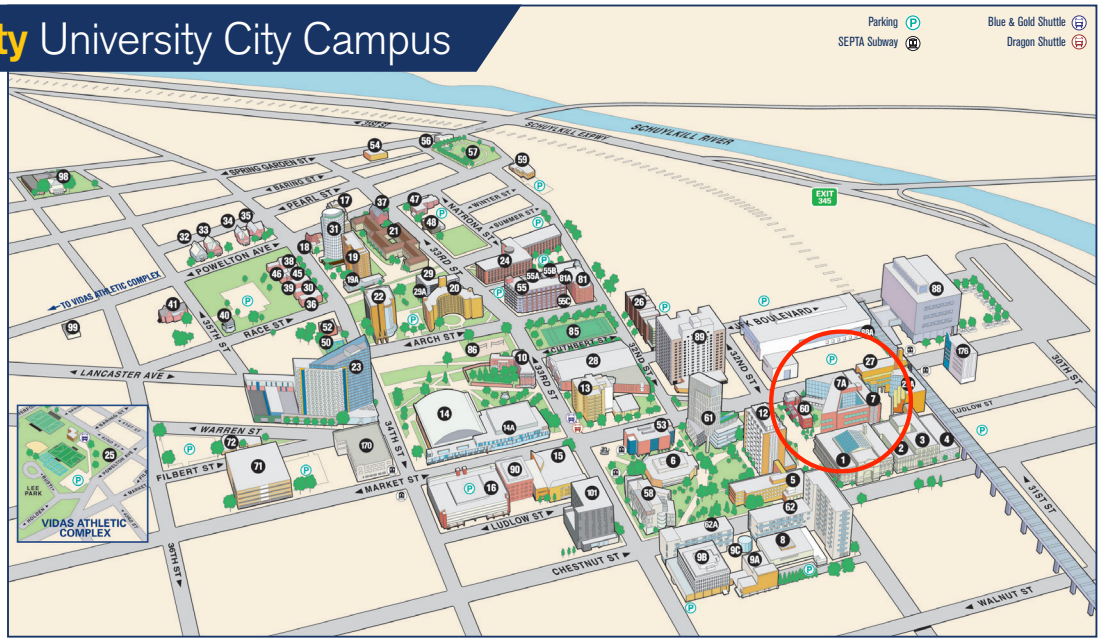

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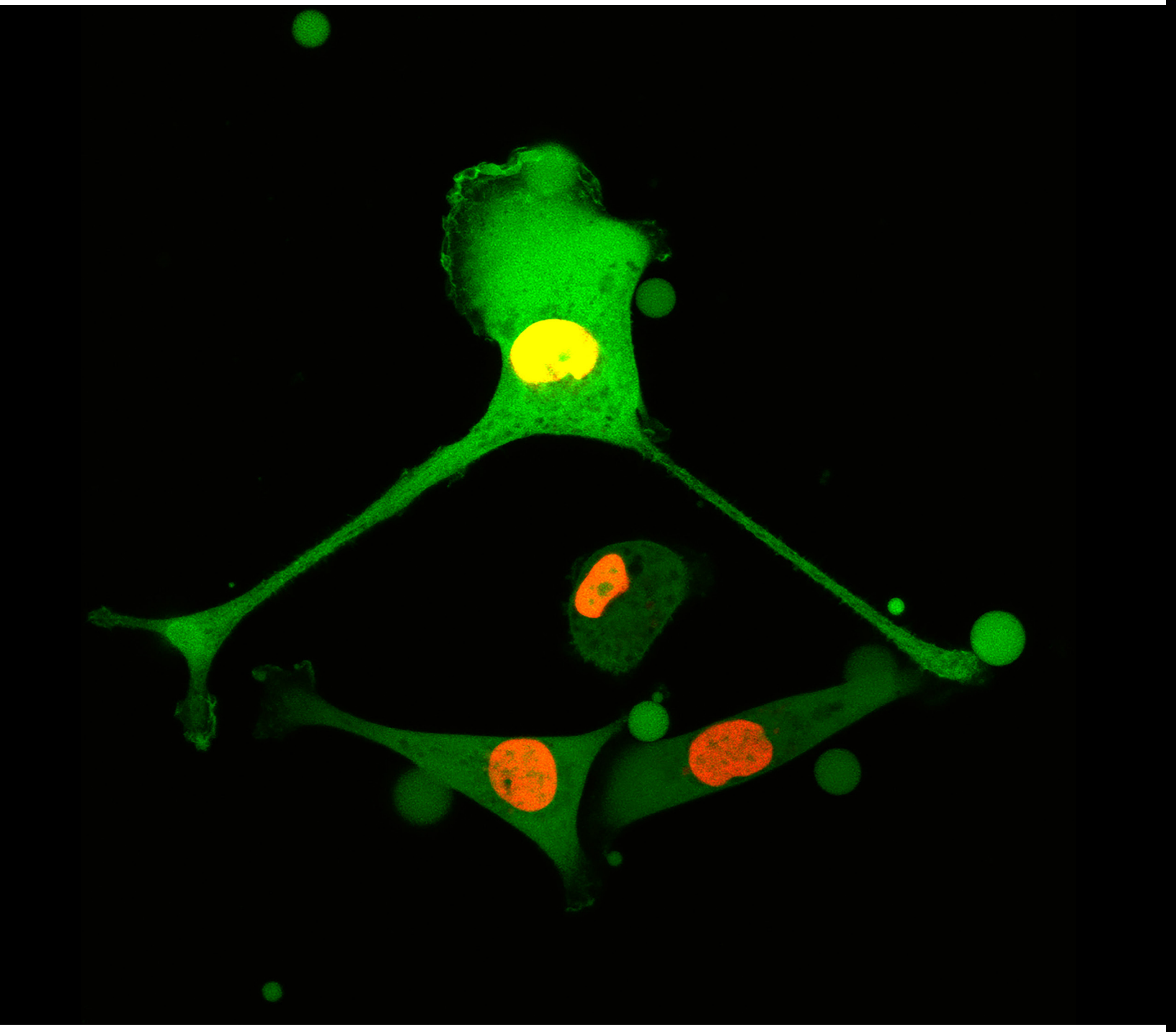

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