Keynote: Michael Levin, *Tufts University*

The Bioelectric basis of morphogenetic intelligence: a roadmap for cancer

The Bioelectric basis of morphogenetic intelligence:
a roadmap for cancer medicine

Michel Levin

Tufts University

Biography

Michael Levin is the Vannevar Bush Distinguished Professor of Biology at Tufts University and associate faculty at Harvard's Wyss Institute. He serves as the director of the Allen Discovery Center at Tufts and the co-director of the Institute for Computationally Designed Organisms at Tufts/UVM. He has published over 400 peer-reviewed publications across developmental biology, computer science, and philosophy of mind. His graduate work on the molecular basis of left-right asymmetry (Cell 1995) was chosen by the journal Nature as a "Milestone in Developmental Biology in the last century". His group at Tufts works to understand information processing and problem-solving across scales, in a range of naturally evolved, synthetically engineered, and hybrid living systems. The Levin lab has pioneered approaches to organ regeneration, cancer reprogramming, non-genetic modification of the bodyplan, and the engineering of novel living proto-organisms. Using tools from behavioral and computer science, Dr. Levin seeks to understand the collective intelligence of cells and harness their problem-solving capacities for applications in birth defects, regeneration, cancer, and synthetic bioengineering.

Abstract

Why do cells ever abandon the tiny metabolic and reproductive goals of amoebas and work together on large construction projects such as maintaining healthy human organs? The failure mode of this process of coordination and collective intelligence in multicellular animals is cancer. In this talk, I will describe a view of morphogenesis and cancer from the perspective of basal cognition and the scaling of cellular competencies. I will describe our work on endogenous bioelectrical networks as the informational glue that binds cells to common purpose maintaining tissues, and thus suppresses tumorigenesis. I will describe including new ways to detect, induce, and normalize cancer in vivo in animal models by reading and writing the bioelectric pattern memories that guide large-scale growth and form. I will end with a perspective on regenerative medicine and electroceuticals: ion channel drugs that, together with AI computational models, offer a new roadmap for reprogramming the software of life toward health.

9:30 Session 1: Bioelectricity in Normal Physiology

Chair: Michael Pycraft Hughes, *Khalifa University*
The cellular zeta potential: cell electrophysiology beyond the membrane

NCI Co-Chair: Eric Johnson Chavarria, NCI, Division of Cancer Biology

Emily Anne Bates, *University of Colorado*
Mechanisms underlying influence of bioelectricity in development

Robert Gatenby, *Moffitt Cancer Center*
Modeling non-genetic information dynamics in cells using reservoir computing

Dielectrophoresis: new tools for cell biology, electromics and diagnostics.

Michael Pycraft Hughes
Khalifa University, Abu Dhabi

Biography

Michael Pycraft Hughes is a Professor of Biomedical Engineering at Khalifa University of Science and Technology in Abdu Dhabi, UAE. His research encompasses new models for cell electrophysiology, instruments for cell measurement, and electrophysiological methods of diagnosis. He received an MEng in Electronic Engineering (1992), PhD in Bioelectronics (1995), and DSc for his work on Dielectrophoresis (2023) from the University of Wales at Bangor, and held research positions at the MD Anderson Cancer Center in Texas and the University of Glasgow in Scotland, before joining the faculty at the University of Surrey in England from 1999-2021. He served as Senior Editor/Editor-in-Chief of *IEEE Transactions on Nanobioscience* for 9 years and is a Fellow of the Learned Society of Wales.

Abstract

Fundamentally, bioelectrical phenomena must arise at a cellular level; it is the interactions of ions, charge, and membranes within cells that gives rise to effects at the tissue, organ, and organism level. However, whilst advancements have been made in tools to explore the electrical properties of cells, the principal methods to study the membrane potential V_m and related phenomena remain patch clamp and fluorescence cytometry.

Recent advances in two methods measuring electrophysiology-adjacent parameters offer new insights to electrophysiology, and deeper questions on the role of these phenomena in the normal work of the cell. Dielectrophoresis (DEP) is the motion of polarizable suspensions (such as suspended cells) in non-uniform electric fields; it is analogous to the more common electrophoresis but relies on the (frequency-dependent) polarisation of the cell. The frequency-dependent nature of DEP means that analysis of the frequency response (typically 1kHz – 50 MHz) allows the determination of many cellular electrical parameters: membrane conductance and capacitance, and cytoplasm conductivity and permittivity[1]. Recent further analysis at multiple medium conductivities allows extraction of further properties, including the membrane potential V_m.

A second parameter is the ζ-potential[2]. This is the potential at the slip plane, 1-2nm outside the cell surface, which governs how the cell interacts electrically with its environment. Generally assumed to be affected only by cellular surface charge, we have shown that this is altered by up to 35% of V_m, allowing the cell to mechanistically alter its electrical microenvironment, alter ion conductance and potentially alter cell-cell interaction. Both ζ-potential and DEP have been demonstrated to link both to conventional cell electrophysiology and each other. These links have allowed us to build a model of the cellular electrome of interconnected parameters, with implications for our understanding of the role that bioelectrical phenomena play in cell function and dysfunction.

As an example, we have employed DEP for the detection of oral cancer. Using cells collected by brush biopsy, DEP analysis of 119 samples showed sensitivity of 92% and specificity of 95% detecting cancer against either healthy controls or benign lesions as confirmed by immunohistopathology [4]. Similar pilot studies showed similar statistics for bladder cancer, based on cells collected from urine samples[5], as well as other diseases. This demonstrates the potential for electromic approaches to cell-scale biomedicine in the near future.

1. MP Hughes, *Nanoelectromechanics in Engineering and Biology*, CRC Press, **2002**.
2. MP Hughes, SP Clarke, R Hoque et al. Sci. Rep. **2024** 14 18477
3. KF Hoettges, EA Henslee, RM Torcal Serrano, *et al*. Sci. Rep. **2019** 9 19153
4. MP Hughes, FH Labeed, KF Hoettges,*et al*. Journal of Oral Pathology & Medicine **2023** 52 305-314
5. R Hoque, H Mostafid, MP Hughes. IEEE J. Trans. Eng. Health Med. **2020** 8 4300405

Mechanisms Underlying Influence of Bioelectricity in Development

Emily Bates, PhD

University of Colorado Anschutz Medical Campus

Biography

Dr. Emily Bates is an Associate Professor in the Department of Pediatrics at the University of Colorado Anschutz Medical Campus. Dr. Bates has led research that defines mechanisms by which ion channel activity contributes to morphological development for the past 14 years. She pioneered the use of mice and flies to study how ion channels regulate the development of tissues that most people do not consider excitable (e.g. palate mesenchyme tissue). Dr. Emily Bates started her research training as an undergraduate in the lab of Dr. Anthea Letsou at the University of Utah using genetics to define mechanisms in developmental biology. She earned her PhD in genetics from Harvard Medical School with a focus on ion channels and neurodegeneration in the lab of Dr. Ann Hart. Her postdoc was performed at UCSF where she studied episodic neurological disorders. Dr. Bates training in developmental biology and neuroscience combined to uniquely qualify her to address the question of how do ion channels contribute to cell-to-cell signaling for the development of complex structures. These studies have implications for cancer development and spread as ion channels control signals that regulate angiogenesis, cell migration (metastasis), and proliferation.

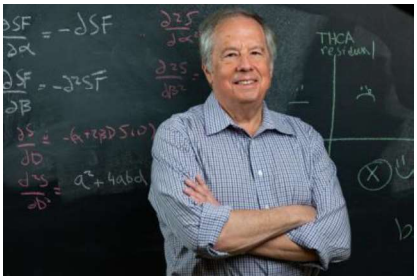
Abstract:

Bone Morphogenetic Protein (BMP) signaling is a key cell-to-cell signal for the embryonic development of complex structures ranging from limbs to the mammalian face. In addition, BMP can promote and suppress tumor growth and metastasis depending on the tissue context. However, little is known about the mechanisms that govern BMP secretion. We show that depolarization induces calcium-dependent BMP4 release from mouse embryonic palate mesenchyme, a tissue that was previously considered non-excitable. We show endogenous transient changes in intracellular calcium occur in cranial neural crest cells, the cells from which embryonic palate mesenchyme derives. Waves of transient changes in intracellular calcium suggest that these cells are electrically coupled and may temporally coordinate BMP release. These transient changes in intracellular calcium persist in palate mesenchyme cells from embryonic day 9.5 to 13.5 mice. Disruption of a potassium channel called *Kcnj2* significantly decreases the amplitude of calcium transients and the ability of cells to secrete BMP. *Kcnj2* knockout mice have cleft palate and reduced BMP signaling. Our data suggest that temporal control of developmental cues is regulated by ion channels, depolarization, and intracellular calcium for mammalian craniofacial morphogenesis. These data have broad implications for BMP signaling in other contexts because BMP signaling regulates angiogenesis, tumor growth, and cancer metastasis.

Non-genomic cellular information dynamics based on transmembrane ion gradients

Robert A. Gatenby, MD
H. Lee Moffitt Cancer Center and Research Institute

Biography



Bob received a B.S.E. in Bioengineering and Mechanical Sciences from Princeton University and an M.D. from the University of Pennsylvania. He completed his residency in radiology at the University of Pennsylvania where he also served as chief resident. Bob remains an active clinical radiologist specializing in body imaging. While working at the Fox Chase Cancer Center after residency, Bob perceived that cancer biology and oncology were awash in data but lacked coherent frameworks of understanding to organize this information and integrate new results. Reaching back to his training in engineering and physical sciences, Bob recognized that cancer was a complex dynamic system (similar, for example, to weather) and that understanding the often non-linear interactions that govern such systems requires mathematical models and computer simulations. As a result, most of Bob's subsequent research has focused on exploring mathematical methods to understand the first principles and key parameters that govern cancer biology and treatment. In 2008, Bob joined Moffitt as chair of radiology and convinced the leadership to add a group of mathematicians to the faculty and form the Integrated Mathematical Oncology (IMO) department. Now numbering 8 faculty mathematicians and over 20 post docs and grad students, the IMO has catalyzed formation of several disease-oriented teams of oncologists, surgeons, pathologists, radiologists, mathematicians, physicists, cancer biologists, imaging scientists and evolutionary biologists. These multidisciplinary groups are investigating virtually every aspect of cancer biology and therapy. In fact, IMO members are co-PIs of two ongoing clinical trials that use evolutionary dynamics and computational models to guide therapy. There is no other cancer center in the world that has so completely integrated mathematical modeling and computer simulations into basic science and clinical research.

Bob's interest in information theory dates back to high school when he read a Scientific American article on Maxwell's demon and the intellectual arc from Maxwell's gedanken to development of information theory 50 years later. He discovered, using a simple "back of the envelope" calculation, that the information content of the *E coli* genome was nearly 5 orders of magnitude smaller than the information content of the single cell organism. A similar calculation found the missing information was in the membrane and the high level on non-randomness in the differences of intracellular and extracellular concentrations of Na^+, K^+, Cl^-, Mg^{++}, and Ca^{++}. This led to a decades-long investigation of non-genetic cellular information built upon ion fluxes across the cell membrane and along elements of the cytoskeleton.

ABSTRACT

Uniquely in nature, living systems use information to maintain a stable, highly ordered state while far from thermodynamic equilibrium. Investigations of cellular information dynamics are typically limited to genetics. However, information in the genome is fixed and requires time to transcribe while optimal evolutionary fitness demands continuous, often rapid, adaptations to diverse opportunities and hazards in the environment. Thus, spatially focused, and rapid responses to changes in the environment requires information flow to and from the plasma membrane, which is the cell's primary interface with the external environment. We propose cells possess a non-genetic information network built upon transmembrane ion gradients. Cells typically use about 1/3 of their energy budget to maintain large transmembrane gradients of Na^+, K^+, Cl^-, Mg^{++}, and Ca^{++}. This non-random distribution of ions represents Shannon information that is quantitatively (in bits) far larger than that of the genome, but the corresponding evolutionary benefit remains unclear. We propose transmembrane ion gradients enable a dynamic and versatile biological communication system that acquires, analyzes, and responds to environmental information. We hypothesize environmental signals are transmitted into the cell by ion fluxes along pre-existing gradients through gated ion-specific membrane channels. The consequent local changes of cytoplasmic ion concentration can generate a local response since the function of many membrane and peripheral membrane proteins are dependent on local cation concentration. Ion and electron fluxes along elements of the cytoskeleton (which is observed experimentally) allow information exchange between the nucleus, endoplasmic reticulum, and the cytoplasm adjacent to the cell membrane enabling an integrated global/regional cellular response. We have framed these non-genetic information dynamics through a quasi-physical (Cell-Reservoir) model that demonstrates the proposed ion dynamics permit rapid dissemination of information and response to extrinsic perturbations consistent with experimental observations. We conclude previously unrecognized information dynamics based on ion fluxes permit learned complex nonlinear cellular behaviors that separates living systems from all other constituent of nature.

11:00 Session 2: Mechanisms of Bioelectricity

Chair: Marco Rolandi, *University of California Santa Cruz*
Bioelectric Signaling: Role of Bioelectricity in Directional Cell Migration in Wound Healing

NCI Co-Chair: Sean E Hanlon, NCI Center for Strategic Scientific Initiatives

Joao Carvalho, *University of Coimbra*
A computational model of organism development and carcinogenesis resulting from cells' bioelectric properties

Xi Huang, *University of Toronto*
EGF receptor signaling is essential for electric-field-directed migration of breast cancer cells

Directing homeostasis in cells to control cell cycle and fate

Marco Rolandi

Department of Electrical and Computer Engineering University of

California, UC Santa Cruz, mrolandi@ucsc.edu

Biography

Marco Rolandi, Ph.D., is Professor of the Department of Electrical and Computer Engineering Department at the University of California, Santa Cruz. He is also co-founder and Chief Scientific Advisor of CruzFoam that makes sustainable packaging solutions. Marco received his PhD in Applied Physics from Stanford University and a LEAD Certificate from the Stanford Graduate School of business. His research focuses on bioelectronic systems and devices, biological control in regenerative medicine and synthetic biology, and their translational applications. His work on bioelectronic transistors and shark electrosensors has been highlighted in *The New York Times, the Washington Post, New Scientist, MRS 360, IEEE Spectrum, Materials Views, Engadget, Popular Science*, and several others. He currently leads one of the teams performing on the DARPA BTO BETR program to accelerate wound regeneration using bioelectronics.

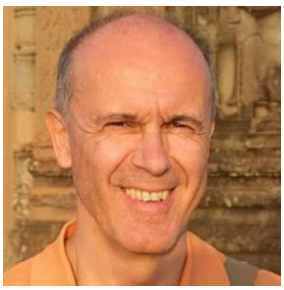
Abstract

Homeostasis is essential in biological processes and to life itself. To maintain homeostasis receptors and effectors connected in a closed control loop achieve and maintain a dynamic equilibrium. Examples include cardiac rhythms, blood glucose regulation, thermoregulation, and cell-lifecycle. Bioelectronic devices are now able to sense and actuate physiological processes by translating biological signals in the form of ions and small molecules into electronic signals that can be analyzed and processed by information technologies. I will present our work that merges AI based closed loop control with bioelectronic sensors and actuators to direct physiological processes. I will focus on our proof-of-concept aimed at directing stem-cell fate by controlling cell membrane potential. We used fluorescent reporters and ion pumps that can change extracellular ionic concentration to dial membrane potential in pluripotent stem cells. Membrane potential is a key variable that drives cell function and morphogenesis. With this bioelectronic toolbox we can open opportunities to direct cell fate, organ shape, and potentially limit abnormal cell-proliferation such as in cancer.

A bioelectric computational model of cell membrane depolarization and carcinogenesis Joao Carvalho

University of Coimbra

Biography



Dr. Carvalho is a Professor of Physics at the University of Coimbra, Portugal. He is a member of the Soft and Biological Matter group in the Center of Physics of the University of Coimbra (CFisUC). His research work focuses on developing computer models for simulation of carcinogenesis and organism development, in particular the influence of bioelectricity in cell communication and tissue organization. Dr. Carvalho received a BSc in Physics from Coimbra University and a PhD in Particle Physics from the University of Liverpool, UK. There he studied symmetry breaking in the neutral kaon system. Later he moved to modeling of biological systems at CFisUC.

Abstract

A robust theory of biological organization is needed for better interpretation of observational results and to advance our understanding of life's complexity. Such a theory would significantly aid in explaining both normal and pathological organism development. As the main theory of carcinogenesis, the Somatic Mutation Theory faces increasing challenges in explaining some experimental observations, and different theories are being proposed. One major alternative is the Tissue Organization Field Theory, which views cancer as a disease of tissue regulation rather than a primarily cellular issue. This work supports this hypothesis, suggesting that the bioelectric field - particularly cell membrane polarization and ionic exchange through ion channels and gap junctions - is crucial for cell communication and tissue regulation.

Carcinogenesis is introduced through localized depolarized cells or random depolarization within the tissue, returning cells to a uncontrolled state. A computational model of cancer initiation was developed, based on some principles of organism development theory, suggesting that cells' default state is proliferation and motility, regulated by bioelectric contexts, and incorporating the propagation of a cell depolarization wave within the tissue. This depolarization changes the cell state by activating and deactivating various regulatory pathways, decreasing the cell proliferation control, with no need of genomic mutations. Intercellular communication via gap junctions can propagate this bioelectric state to neighboring cells, creating a chain reaction until an electric discontinuity is reached. However, this process is reversible, as normalizing the bioelectric condition can restore homeostasis, and experimental evidence shows that targeted therapy on ion exchange channels can repolarize cells and reverse the uncontrolled depolarized state. This mechanism could be significant in cancer prevention, diagnosis, and therapy, warranting further experimental investigation. Even in its simplified form, the model offers insights for new approaches in complex systems and suggests experimental tests focused on its predictions.

Targeting fluidic force-sensing mechanism to treat brain tumor metastasis

Xi Huang

The Hospital for Sick Children / University of Toronto

Biography

Dr. Xi Huang is a Senior Scientist in the Developmental and Stem Cell Biology Program at The Hospital for Sick Children, and Associate Professor in the Department of Molecular Genetics at University of Toronto. Dr. Huang holds Canada Research Chair in Cancer Biophysics. Dr. Huang studies ion channel-mediated mechano-electrical-chemical signaling in brain cancer. Dr. Huang received B.Sc. in Biological Sciences from Xiamen University, Ph.D. in Developmental Biology from Vanderbilt University, and completed postdoctoral training in Dr. Lily Jan's lab at University of California, San Francisco.

Abstract

Brain tumor is the leading cause of cancer-related deaths in children. How brain tumour growth alters the conduit of biofluid, thereby impacting fluid shears stress (FSS)-regulated cancer progression is unknown. Medulloblastoma (MB) is the most common malignant pediatric brain tumour. Dissemination of MB cells into the highly nutrient-deprived cerebrospinal fluid (CSF) initiates metastasis within the central nervous system. I will present our simulation of the CSF dynamics based on patient magnetic resonance imaging, which shows that FSS is elevated at the cervicomedullary junction. I will show that MB-relevant FSS promotes metastasis along the spinal cord in mice. MB cells perceive FSS by mechanosensitive calcium-permeating ion channel PIEZO2, which drives actomyosin contractility-dependent GLUT1 recruitment at the plasma membrane to enhance glucose uptake. I will show that genetic targeting of PIEZO2 or pharmacologic inhibition of GLUT1 mitigates metastasis in mice. I will end by proposing a targetable FSS-activated mechano-metastatic cascade for the treatment of MB metastasis.

12:30 Session 3: Bioelectricity and cancer

Chair: Mustafa Djamgoz, *Imperial College London*

NCI Co-Chair: Miguel R Ossandon, NCI Cancer Treatment and Diagnosis

Electrical signaling in cancer

Madeleine J Oudin, *Tufts University*
Potassium channel-driven bioelectric signaling regulates metastasis in triple-negative breast cancer

Michael R. King, *Vanderbilt University*
Ion channels in cancer mechanotransduction

Electrical signaling in cancer: CELEX model

Mustafa B A Djamgoz
Imperial College London, UK

Biography

Djamgoz has been interested in 'bioelectricity' since his teenage years. He studied physics at Imperial College London moving onto a PhD in biophysics. He spent some 25 years working on the electrophysiology of various model neurobiological systems including the vertebrate retina, insect neurons and giant muscles and frog oocytes. In 1995, he was made Professor of Neurobiology. Curiosity then led him to study electrical signaling in cancer cells, beginning again with model cell lines derived initially from prostate cancer. In a comparative approach, he showed for the first time (in 1995) that cancer cells of strong metastatic potential express *de novo* voltage-gated sodium channels (VGSCs). This phenomenon has now been confirmed in several carcinomas. In the following 25 years, he revealed several key aspects of the channels' molecular pathophysiology and clinical potential. In 2005, the new work led to a second Professorship in Cancer Biology. In 2020, he was appointed 'Emeritus Professor' at Imperial (a lifetime position) and remains research active. He holds several patents and a company (Celex Oncology Innovations Ltd) has been formed focused on his discoveries. He is the co-EIC of the journal, *Bioelectricity*.

Abstract

Cancer cells express a plethora of ion channels, rather like the brain! We adopted a dual strategy focusing on (i) voltage-activated ion channel (due to the massive influence of the membrane potential on cellular functioning) and (ii) those channels that may drive metastasis, the main cause of death from cancer. As cells' metastatic potential increased VGSC activity appeared whilst, in parallel, outward currents were reduced, i.e. the cells acquired electrical excitability. We call this the CELEX model (CELEX = Cellular Excitability) (Djamgoz, 2024a). Subsequent work confirmed that such cells indeed generate Hodgkin-Huxley type action potentials (APs). The results have strong clinical implications. Electrodiagnosis would be possible simply by recording APs using electrodes placed on superficial tumours, e.g. melanoma. Thus, an 'electro-oncogram', analogous to electrocardiogram, can be developed. There are also several therapeutic possibilities. First, the culprit VGSCs can be targeted by exploiting their (i) neonatal nature or (ii) the persistent current which develops in hypoxia, common to growing tumours. The latter can selectively be suppressed using the anti-angina drug, ranolazine (Djamgoz, 2024b). Yet another possibility is to use DC electric fields to impose galvanotactic movement upon VGSC-expressing cancer cells and draw them out of the tumours (Mycielska & Djamgoz, 2004). The long-term promise of our research is to control metastasis non-toxically, rather than kill cancer cells, and thus avoid issues like stemness-driven drug resistance and disease recurrence. Thus, it may ultimately become possible to live with cancer as a chronic condition.

References

Djamgoz MBA (2024a). Electrical excitability of cancer cells-CELEX model updated. Cancer Metastasis Rev. doi: 10.1007/s10555-024-10195-6. Epub ahead of print.

Djamgoz MBA (2024b). Ranolazine: a potential anti-metastatic drug targeting voltage-gated sodium channels. Br J Cancer. 2024 May;130(9):1415-1419. doi: 10.1038/s41416-024-02622-w.

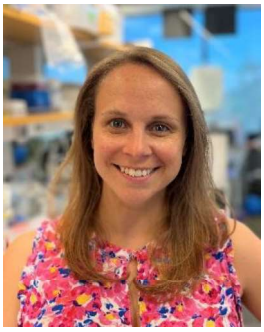
Mycielska ME, Djamgoz MBA (2004). Cellular mechanisms of direct-current electric field effects: galvanotaxis and metastatic disease. J Cell Sci. 2004 Apr 1;117(Pt 9):1631-9. doi: 10.1242/jcs.01125.

Potassium channel-driven changes in the membrane potential regulate metastasis

Madeleine Oudin

Tufts University

Biography:



Madeleine completed a BSc in Biochemistry at McGill University, a PhD in Neuroscience from King's College London, UK. She was a post-doctoral fellow working in Prof. Frank Gertler's lab at the Koch Institute for Integrative Cancer Research at MIT for 6 years. She received a Breast Cancer Research Department of Defence Post-doctoral Fellowship and a K99/R00 Pathway to Independence from the NCI. She started her own lab at Tufts University in the department of Biomedical Engineering in 2018, where research focuses on understanding the mechanisms by which the tumor microenvironment contributes to cancer metastasis and resistance to drugs. She has received numerous awards for her research such as the DP2 New Innovator Award in 2021 and the 2020 CMBE Rising Star Award and was voted Exemplary Engineer by the graduate students in her department 3 years in a row.

Abstract:

Metastasis, driven by the local invasion of tumor cells to secondary sites in the body, is the leading cause of cancer deaths. We have found that on average, 40% of triple-negative breast cancer (TNBC) patients have gain-of-function mutations or gene amplification in the 50 K^+ channels expressed in breast tumors and that K^+ ion flow regulates the RMP of TNBC cells. Overexpression of K^+ channels leading to hyperpolarization in human TNBC cells significantly increased tumor cell invasion *in vitro* and tumor growth and metastasis *in vivo* via upregulation of pro-invasive cytoskeletal genes. Further, we found that the FDA-approved K^+ channel blocker Amiodarone induced depolarization in human TNBC cells, leading to decreased proliferation and reduced metastasis *in vivo*. K+ channel blockers can synergize with existing chemotherapy treatments used for TNBC treatment to further reduce cell survival and invasion. Our work provides insight into how the bioelectric properties of cells regulate cell migration and metastasis and identifies novel treatment strategies for metastatic disease that target endogenous bioelectrical control mechanisms through drug repurposing.

Cancer Mechanotherapy: Harnessing Cellular Mechanotransduction

to Understand and Treat Metastatic Cancer

Michael King

Rice University

Biography

Michael R. King is the E.D. Butcher Chair of Bioengineering at Rice University, and Special Advisor to the Provost on Life Science Collaborations with the Texas Medical Center. He is also a Scholar of the Cancer Prevention and Research Institute of Texas (CPRIT). Previously, King was the J. Lawrence Wilson Professor and Department Chair of Biomedical Engineering at Vanderbilt University, and before that was the Daljit S. and Elaine Sarkaria Professor at Cornell University. He completed a PhD in chemical engineering at the University of Notre Dame and postdoctoral training in bioengineering at the University of Pennsylvania. He has written textbooks on the subjects of statistical methods and microchannel flows, and has received several awards including the NSF CAREER Award, Outstanding Research Awards from the American Society of Mechanical Engineers and the American Society of Clinical Chemistry, and the Christopher Jacobs Award for Excellence in Leadership. King is a Fellow of the American Institute of Medical and Biological Engineering (AIMBE), Biomedical Engineering Society (BMES), International Academy of Medical and Biological Engineering (IAMBE), American Association for the Advancement of Science (AAAS), and the National Academy of Inventors (NAI), and served as founding Vice President of the International Society of Bionic Engineering. Since 2013 he has been the Editor-in-Chief of Cellular and Molecular Bioengineering, an official journal of the BMES. He previously served as Chair of the BME Council of Chairs, and Chair of the AIMBE College of Fellows.

Abstract

Many types of cancer metastasize via the bloodstream, where circulating tumor cells (CTCs) originating from the primary tumor can travel through the circulation or lymphatic system and engraft in distant organs. Previously, our laboratory found that cancer cells exposed to physiological levels of fluid shear stress (FSS) are dramatically more susceptible to undergoing apoptosis via TRAIL protein, inspiring a new therapeutic drug delivery approach to target metastatic cells in the circulation. The FSS response of CTCs and their neutralization by nanoscale liposome conjugation to the surface of circulating immune cells has been demonstrated with in vitro cell line experiments, orthotopic mouse models of metastasis, and analysis of primary CTC aggregates isolated from metastatic cancer patients. We learned that this shear stress response is primarily mediated by Piezo1 activation, and is modulated by interactions with aggregated stromal cells such as cancer-associated fibroblasts. Interestingly, we also discovered that FSS activation of Piezo1 dramatically enhances the activation of T cells and dendritic cells, which may have important implications for various immunotherapy applications. Our ongoing research is also exploring whether cellular mechanosensors can be non-invasively stimulated using focused ultrasound, to improve clinical outcomes in cancer.

2:00 Session 4: Bioelectricity potential clinical and translational research

Chair: Norbert Perrimon, *Harvard Medical School*
Bioelectric-dependent intestinal regeneration

NCI Co-Chair: Linda Zane, NCI Small Business Innovation Research (SBIR)

Donglu Shi, *University of Cincinnati*
Bioelectricity in nano-bioprobing and cancer diagnosis

Dany Spencer Adams, *Tufts University*
Cell membrane voltage imaging to identify cancer in biopsies and surgical specimens

Rosalia Moreddu, *Instituto Italiano di Tecnologia*
Nanotechnology and cancer bioelectricity: bridging the gap between biology and translational medicine

Bio-Electrical Manifestation of the Warburg Effect: Glycolytically-Regulated Cancer Cell Surface Charge

Donglu Shi

University of Cincinnati

Dr. Donglu Shi is a professor of Materials Science and Engineering at the University of Cincinnati. His research focuses on nanoscience-based precision medicine, cancer diagnosis and therapeutics, and biomaterials, which involve designs of unique nanostructures that not only interface with biological systems but also offer new bio-chemical-physical properties for fundamental studies. Dr. Shi has conducted research across diverse fields such as nanoscience, energy materials, biomedical engineering, precision medicine, and condensed matter physics. His efforts have led to over 300 peer-reviewed journal publications, with some of his works appearing in leading journals like Nature, Physical Review Letters, Advanced Materials, and ACS Nano.

Abstract

Bioelectricity plays a crucial role in understanding the fundamental behaviors of biological systems. As a dynamic process in cells, bioelectricity manifests metabolism, which cannot be accurately probed as a static electrical potential by electrophoresis. It is well-known that cancer cells exhibit the "Warburg Effect," producing energy predominantly through anaerobic glycolysis followed by lactic acid secretion. High levels of glucose uptake and lactate secretion are the most distinguishable metabolic behaviors of cancer cells, significantly higher than those of normal cells. Our studies, involving twenty-two cancer cell lines, show a close correlation between secreted lactic acid and the net negative electrical charges on cancer cell surfaces. We have found a direct relationship between cancer cell surface electrical charge and glycolysis capacity, dynamically regulated by glucose uptake. The lactate-secretion-generated negative charge can be explained by the cross-membrane movement of mobile ions. Charge neutrality in most human cells is maintained by ion pumps through the plasma membrane. In cancer cells, the cross-membrane movement of lactate is a pathway of glycolysis. The charges of immobile ions encounter the massive amount of Na+ present in the interstitial space and extracellular environment, becoming fully accessible for charge neutralization. Elevated glycolysis levels utilize available glucose and secrete large amounts of lactate as mobile anions, creating a net negative charge. Thus, perpetuating ion movement is solely responsible for the signature negative charge pattern on cancer cell surfaces. Our findings mechanistically link glycolysis, a fundamental metabolic pattern, with a hallmark characteristic of cancer cell surface charge. Additionally, this understanding aids in electro-magnetically capturing circulating tumor cells (CTCs) from whole blood for early cancer diagnosis and prognosis.

Bioelectric-dependent intestinal regeneration: A Drosophila perspective

Norbert Perrimon

Harvard Medical School, HHMI

Biography

Norbert Perrimon, PhD, James Stillman Professor of Developmental Biology in the Department of Genetics at Harvard Medical School, Investigator of the Howard Hughes Medical Institute, is a geneticist known for pioneering a number of techniques in *Drosophila*, as well as specific substantive contributions to signal transduction, developmental biology, and physiology. Among the tools that he has developed are: the FLP-FRT Dominant Female Sterile technique to generate germline mosaics, the GAL4-UAS method to control gene expression both spatially and temporally, highthroughput genome-wide RNAi and CRISPR screens, and proximity labeling methods to identify secreted molecules. These methods have had transformative impacts in signal transduction, development, physiology, neurobiology, and functional genomics. Early in his career, he identified and characterized factors involved in RTK, Wnt, and JAK/STAT signaling, contributing to the elucidation of these canonical pathways. Perrimon went on discover intestinal stem cells in the adult fly gut, opening up an entire field of study to identify factors and pathways involved in stem cell homeostasis and regeneration. In more recent years, he has taken a systematic approach to identify factors involved in inter-organ communication, which are leading to a systems wide understanding of how hormonal systems are regulated by the state of various organs in homeostatic and stressed conditions.

Abstract

A fundamental and unresolved question in regenerative biology is how tissues return to homeostasis after injury. Answering this question is essential for understanding the etiology of chronic disorders such as cancer. We used the *Drosophila* gut to investigate this question and discovered that during regeneration a subpopulation of cholinergic enteric neurons triggers Ca^{2+} currents among epithelial cells to heal the gut after injury. Specifically, we found that down-regulation of the conserved cholinergic enzyme Acetylcholinesterase in the gut epithelium enables acetylcholine from specific enteric neurons to activate nicotinic receptors in the gut. Activation of nicotinic receptors triggers high Ca^{2+} that spreads in the epithelium through gap junctions, promoting epithelial maturation followed by reduction of proliferation, inflammation and return to homeostasis. Disrupting this process causes chronic injury and makes the gut prone to hyperplasia. Altogether, our findings advance the current understanding of how a tissue returns to homeostasis after injury by highlighting the crucial role of the conserved cholinergic pathway in triggering a bioelectric mechanism that heals the gut. Intestinal stem cell proliferation in *Drosophila* is regulated by bioelectric regulators (ion channels, exchangers, receptors) such as the chloride channel CFTR (Cystic Fibrosis cystic fibrosis transmembrane conductance regulator) whose mutation we found to cause gut hyperplasia. Given our discovery that the cholinergic pathway induces a bioelectric-dependent healing intestinal mechanism, we are currently studying how interactions of Acetylcholinesterase and CFTR impact gut hyperplasia.

Kim, K., Lane, E. A., Saftien, A., Wang, H., Xu, Y., Wirtz-Peitz, F. and Perrimon, N. (2020) *Drosophila* as a model for studying cystic fibrosis pathophysiology of the gastrointestinal system. PNAS. **117**:10357-10367. PMID:32345720

Petsakou, A., Liu, Y., Liu, Y. Comjean, A. Hu, Y. and Perrimon, N. (2023) Cholinergic neurons trigger epithelial Ca^{2+} currents to heal the gut. in the intestinal epithelium restores homeostasis after injury. Nature. doi: 10.1038/s41586-023-06627-y. PMID: 37722602

Voltage Sensitive Dye Imaging for Intraoperative Cancer Detection in Surgical Margins

Dany Spencer Adams

Tufts University

Biography

Dr. Dany Spencer Adams began her work on Bioelectricity when she joined the lab of Dr. Michael Levin as a post-doctoral fellow, soon getting her own lab at the Forsyth Institute. She continued this work as a PI in the Tufts Center for Regenerative and Developmental Biology. Together Levin and Adams worked on left-right patterning, cancer, and regeneration. Dr. Adams discovered the first empirical evidence of a bioelectric pre-pattern in embryos with her "electric frog face" video. She also made the first discovery showing that voltage could initiate regeneration. As the imaging specialist and voltage dye expert at the Center, she had the opportunity to work with Levin and most members of his lab on many projects. Since leaving the Center she has focused on developing and patenting a method for using dyes to find cancer in surgical margins through her company Lucell Diagnostics.

Abstract

Because standard pathological analysis takes many hours, cancer surgeons cannot know if the surgical margin is clear until well after surgery is over, leading to unacceptably high rates of positive surgical margins (35% in ovarian cancer, 9% on average) and their sequelae, chemotherapy, radiation, immunotherapy, and even repeat surgery. Intraoperative imaging is therefore a critical goal. A number of researchers have suggested that voltage imaging might be useful in cancer detection. I had successfully used voltage reporting dyes to show a role for voltage variation in the cancer-relevant process of differentiation in developing frog embryos. I had also demonstrated that voltage changes precede the appearance of tumor-like structures in frog tadpoles. Working in vitro, I showed that the dye signal from murine cancer cells is a different brightness, and shows a different pattern, from normal cells. The same was true in a human prostate cancer line compared with untransformed prostate cells. Most recently, I worked with dermatologists to try the technique in human tissues. First, I adapted a protocol for collecting cells from the margin of excised skin tissues using a touch prep with nitrocellulose (NC) membrane. Samples of suspected basal or squamous cell carcinoma were provided by surgeons. Within minutes of excision, cells were collected by adherence to the NC membrane then stained first with a nuclear counterstain, then with $DiBAC_3(4)$, an anionic dye that would glow brightly in depolarized cancer cells and dimly in normal cells, as it had in vitro. Consistent with the adage that what happens in vitro stays in vitro, the data from adult tissues were more complex. Histograms of pixel intensity showed that cancer cells had significantly more of the brightest pixels than normal cells; however, it is clear that computer aided image analysis of parameters other than brightness, for example entropy, is going to be critical for gathering more useful information from these images. Because this was expected, the NC membranes, which hold cells in the orientation they had in vivo, were fixed for staining using anti-cancer antibodies to create the ground truth for machine learning. These preliminary data are hopeful, but the analysis step needs further work before it can be translated. Nonetheless, this technique is simple, inexpensive, and does not require large or unusual equipment, or trained personnel, thus it has the potential to reach patients around the world, including those in underserved, rural ,and less affluent areas. It has been my hope all along that this straightforward, "low-hanging fruit" would also help to raise consciousness of the as yet untapped power of bioelectricity in medicine evidenced at this meeting.

Unraveling and Harnessing Bioelectricity Beyond Excitable Cells

Rosalia Moreddu

Institute of Biomedical Engineering, University of Oxford, Oxford, UK

Biography

Rosalia Moreddu is a researcher in bioelectricity at the Institute of Biomedical Engineering, University of Oxford, UK. She was previously a Marie Curie fellow at the Italian Institute of Technology, Italy. She holds a PhD in Biomedical Engineering jointly from Imperial College London and the University of Birmingham, UK, and a master's degree in Nanotechnology from Polytechnic of Turin, Italy. Her research interests are in the field of biointerfaces, biomaterials and nano-devices targeting cellular bioelectricity for fundamental and applied sciences.

Abstract

Bioelectricity is known to play a key role in regulating and driving crucial life processes at all scales: from subcellular structures to cells and cell networks, tissues, and organs. Cellular electrophysiology studies and technologies to retrieve electrical information from cells have evolved substantially over time, exploiting different materials and techniques to translate these electric charges into actionable information in healthcare and drug testing. However, most of the studies in the existing literature have been focused on recording action potentials and extracellular signals in excitable cells, i.e. brain cells and muscle cells. Yet, bioelectric charges and bioelectric gradients regulate crucial functions in all existing cells in nature, including homeostasis, cellular migration, communication, proliferation, mutation, genetic expression, cancer insurgence and progression. Cancer cells are known to exhibit different metabolism and communication mechanisms compared to their healthy counterparts, given by the alteration of ion channel functionality, ion channel expression, surface charges, and network signaling. This talk will provide an overview of this emerging topic, to then present preliminary results on the dynamic electrical activity of cancer cells recorded at different frequency ranges using microelectrodes. These findings may contribute to set the basis for further investigations on the emerging role of bioelectricity in cancer proliferation and metastasis, as well as the development of precision biointerfaces in cancer diagnostics and drug testing.

4:00 Panel Discussion: Moving cancer bioelectricity research forward

Linda Zane, NCI SBIR funding and commercialization resources for the development and commercialization of cancer technologies"

Vish Subramaniam,
EMBioSys Inc. Technologies for treatment of Metastatic Solid Tumors

Eric M Johnson,
NCI DCB technology funding

Kelly Crotty
NCI CSSI IMAT funding

Concluding remarks

NCI SBIR funding and commercialization resources for the development and commercialization of cancer technologies

Linda ZANE, PhD
Program Director at the NCI SBIR Development Center

Biography

Dr. Linda Zane is a Program Director in the Small Business Innovation Research (SBIR) Development Center at the National Cancer Institute (NCI). Dr. Zane manages a portfolio of small businesses with a focus on cancer diagnostics, therapeutics and novel tools for research and drug discovery. She provides guidance to applicants and awardees while also informing them of NIH resources to develop stronger applications and commercialization strategy. Additionally, she plays an active role and represents NCI's SBIR Development Center in some NCI and trans-NIH programs including the NCI technology interest group, the NCI Innovative Molecular Analysis Technologies (IMAT) and the Somatic Mosaicism Across Human Tissues (SMaHT) Common Fund Programs.

Prior to coming to SBIR, Dr. Linda Zane was a program director in the Division of Cancer Biology where she managed a portfolio of grants focusing on genomic variation, omics technologies, and 3D genome architecture. Before that, she was in the NCI Division of Cancer Treatment and Diagnosis (DCTD) where she first acquired some experience in regulatory affairs and technology transfer in the Regulatory Affairs Branch at the Cancer Therapy Evaluation Program (CTEP). She gained program management experience in the Diagnostic Biomarkers and Technology Branch of the Cancer Diagnosis Program (CDP), which stimulates and supports research to develop new biomarkers, diagnostic strategies, modeling, innovative technologies, and improved devices.

Dr. Zane received a Ph.D. in Oncovirology from the University Claude Bernard Lyon I, a M.Sc. in Biotechnology Engineering from the University Bordeaux II, and a B.Sc. in Biochemistry from Pierre and Marie Curie University Paris VI in France. She completed post-doctoral research in Dr. Kuan-Teh Jeang's lab at NIAID and then in Dr. Tom Misteli's lab at NCI.

Abstract

The National Cancer Institute (NCI) Small Business Innovation Research (SBIR) Development Center provides $200M in non-dilutive funding to small businesses each year in support of innovative technology development by ~350 companies. Our portfolio includes therapeutics, diagnostics, and digital health technologies. NCI SBIR leverages its awards with private sector fundraising help, has a matching fund program leveraging private sector investors, and offers several commercialization mentoring and training programs for its awardees.

Learn about NCI SBIR programs:

- NIH-wide SBIR and STTR
- Funding Opportunities for start-ups, academic spinout companies, and early-stage academic entrepreneurs (e.g., postdoctoral fellows)
- Commercialization resources for applicants and awardees

Technologies for Treatment of Metastatic Solid Tumors

Vish Subramaniam
The Ohio State University
and
EMBioSys Inc.

Biography

Dr. Vish Subramaniam is Academy Professor and Professor Emeritus in the Department of Mechanical & Aerospace Engineering at The Ohio State University (OSU). He was also a Professor in the Chemical Physics Program. He currently serves as Chief Scientific Officer at EMBioSys Inc., which he co-Founded in 2022 for commercializing induced electric field (iEF) therapy for metastatic solid tumors. His research interests in bioelectricity range from cancer biology, wound healing, to treatment of antibiotic-resistant bacterial biofilms. Dr. Subramaniam received his B.S. (specialization in applied physics) and M.S. degrees in Mechanical Engineering from Columbia University and his Ph.D. in Mechanical Engineering from Carnegie-Mellon University. He was a tenured faculty member from 1988-2021 at OSU, where he and his collaborators discovered and developed iEF therapy. He continues to actively collaborate with researchers and clinicians at OSU and other institutions.

Abstract

The interaction of electromagnetic fields with tissues and cells has been explored over the past several decades mostly from the vantage point of adverse health effects, largely related to power lines. However, use of electric fields with or without accompanying electric currents for the treatment of solid tumors is gaining traction and several approaches are in various stages of translation to the clinic. The most commercially developed product for treatment of solid tumors is Novocure's tumor treating fields (TTFs), approved for glioblastoma and mesothelioma. There are other technologies such as pulsed electric field (PEF) treatment and positive electrostatic charge therapy (PECT) that are in various stages of development. A common factor with all these techniques is that they require contact of electrodes with the skin. In contrast, induced electric field (iEF) therapy is contact-free, requires no electrodes, and is non-invasive. Induced electric fields (iEFs) on the order of µV/cm-mV/cm can be produced by time-varying magnetic inductions of order nT-µT. These iEFs have significant and selective biologic effects on triple negative breast cancer (TNBC) cells in vitro and tumors and pulmonary metastasis in vivo. MDA-MB-231 cells subjected to combination treatment of iEF and standard of care (SOC) chemotherapies for TNBC (capecitabine, paclitaxel), show significant reduction of IC50 values (from 1.11 mM to 0.68 mM for capecitabine+iEF and from 13.1 nM to 5.8 nM for paclitaxel+iEF), indicating increased potency of the two chemotherapeutic agents each in the presence of iEF. Combining a pharmacologic approach (cytotoxic chemotherapy) with a non-pharmacologic treatment (iEF therapy) that specifically targets metastasis, could be a powerful and effective tool in an oncologist's arsenal to treat TNBC and other solid malignancies. This talk will briefly discuss existing technologies but focus more in iEF therapy.

Eric Johnson Chavarria
Program Director at the NCI Division of Cancer Biology (DCB)
Biophysics, Bioengineering, and Computational Sciences Branch

Biography

Dr. Johnson Chavarria joined DCB in 2021, following a tenure as a Program Director in the NCI Center for Cancer Health Equity (CCHE) and as a AAAS Science and Technology Fellow.
Dr. Johnson Chavarria manages a portfolio of grants focused on physical sciences in oncology, technology development, and engineering approaches for cancer biology. He has expertise in the biology of the cytoskeleton, protein structure, microfluidics and microfabrication, and single molecule technologies.
Along with managing grants, Dr. Johnson Chavarria is leading the division's advocacy initiative and is a member of an NCI Equity Council Working Group, NIH QIS and Quantum Sensing in Biology Interest Group, and the DCB Data and Portfolio Analysis Group.
He is committed to increasing diversity and inclusion while leveraging open innovation approaches to foster multidisciplinary collaborations that address health and research challenges.

Research Programs

Dr. Johnson Chavarria also helps manage a DCB cooperative agreement program and a trans-agency initiative:

- Physical Sciences - Oncology Network (PS-ON)
- NSF-NCI SuPporting new AReas of Knowledge (SPARK): Cancer as a Living Material – New Ideas and New Connection

Kelly Crotty
Program Director SCCI/IMAT

Dr. Kelly Crotty is a Program Director in the Center for Strategic Scientific Initiatives (CSSI) at the National Cancer Institute (NCI). She directs the Innovative Molecular Analysis Technologies (IMAT) program, manages collaborative projects for the Informatics Technology for Cancer Research (ITCR) program, coordinates the COnsortium of METabolomics Studies (COMETS) program, and co-leads NCI's Emerging Technologies Seminar Series.

Dr. Crotty joined the National Cancer Institute in 2019 as an NCI Communications Fellow. She received her Ph.D. in biochemistry from the University of California – San Francisco where she used protein and RNA biochemical methods to investigate the Unfolded Protein Response across yeast species. In addition to her dissertation work, she organized an intramural seminar series at UCSF and volunteered with organizations supporting women and minorities in science.

Innovative Molecular Analysis Technologies (IMAT) Program

Technical innovation is critical to improving and transforming our ability to understand, prevent, diagnose, and treat human disease. The IMAT program catalyzes the development of novel technologies for cancer research by supporting early-stage projects to develop highly innovative tools that will allow us to grapple with the complexity of cancer biology and create new possibilities for the fight against cancer. The program places an emphasis on innovation and the potential impact of the technology on its intended field within cancer research and clinical care. Learn more about the program by reading the funding opportunities or browsing the funded projects from over the years.