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Abstracts Book

Morphological Complexity of Biomembranes and Synthetic Cells

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Abstract

Each eukaryotic cell is bounded by its outer plasma membrane and contains many intracellular membranes that enclose different organelles. These biomembranes exhibit a fascinating variety of different morphologies, a polymorphism that is also observed for cell-sized lipid vesicles and membrane compartments, basic modules of synthetic biology. On the molecular scale, biomembranes are molecular bilayers of lipids and proteins. The bilayers consist of two leaflets that differ in their molecular composition, which implies a certain transbilayer asymmetry. In text books on cell biology, these two properties of biomembranes - polymorphism and transbilayer asymmetry - are usually viewed as two independent and disconnected features.

In contrast, this talk will emphasize the strong interrelation between these two general features. The following aspects of this interrelation will be briefly reviewed [1]: transbilayer asymmetry and spontaneous curvature; spontaneous formation of nanotubes [2, 3, 4]; membrane necks and multispherical shapes; [5] curvature-induced fission of membrane necks and controlled division of cell-sized vesicles [6]; active shape oscillations of giant vesicles coupled to Min proteins and ATP hydrolysis [7]. At the end, a brief outlook will be given on the morphological responses of nanovesicles [8], on the composite nature of membrane tension [2, 9, 8], as well as on the interactions of membranes with liquid droplets [10, 11] and biomolecular condensates [12].

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Using Cytoskeletal Markers for Identifying and Classifying Epithelial Cells

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Abstract

Epithelial sodium channel (ENaC) activity is a significant determinant of blood pressure (BP). Enhanced expression

of ENaC leads to severe hypertension in the hereditary disorder of Liddle syndrome. Thus, determining the sites of ENaC localization could pinpoint the BP regulatory sites within the kidney. The single-cell sequencing approach used in generating the Human Cell Atlas (HCA) would not be sufficient for this purpose. ENaC activity is dependent on its concentration on the cell membrane and not on its level within the cell. Another approach would be microscopic localization by immunofluorescence techniques. This is also insufficient to identify cell types because of the kidney's structural complexity, composed of about a million nephron units.

We developed a new technique to circumvent these limitations. We can classify the epithelial cells in the kidney (proximal, distal, thin loops, collecting duct cells, etc.) by actin cytoskeleton patterns. We map whole tissue sections by confocal microscopic imaging of fluorescent phalloidin, which binds to actin filaments. In tile-scans (composed of hundreds of images) of these sections, the cortex and the medullary regions (outer and inner stripes of the outer medulla and inner medulla) could be easily identified by their cytoskeletal patterns. We used additional markers to verify our findings, including aquaporin isoforms, cytokeratin 8-18, and WGA lectin. The simple approach we employed using phalloidin fluorescence of actin filaments can be used to identify and classify epithelia in other mammalian tissues and, at higher resolution, identify even the cell types within the epithelia.

Biography

Israel Hanukoglu is a Professor of Biochemistry and Molecular Biology. He received his Ph.D. from the University of Wisconsin-Madison for research on P450 system enzymology. In postdoctoral studies, he determined the first sequences and structures of keratins (Fuchs lab, University of Chicago) and developed structural models for intermediate filament proteins. His recent work has concentrated on the genetics of hereditary diseases that result from mutations in genes that encode epithelial sodium channel (ENaC) subunits and the structure-function of ENaC. His list of awards includes Lindner Prize from the Israel Endocrine Society and Lubell Award from the Weizmann Institute of Science.

Nutrient Regulation of Signaling and Gene Expression by O-GlcNAc

Gerald W. Hart

GRA Eminent Scholar, Complex Carbohydrate Research Center & Biochemistry Department, University of Georgia, Athens, GA

Abstract

O-GlcNAcylation cycles on thousands of nucleocytoplasmic proteins and has extensive crosstalk with phosphorylation. O-GlcNAc is abundant on nearly all proteins involved in transcription, where it regulates gene expression in response to nutrients. O-GlcNAc regulates the cycling of the TATA-binding (TBP) protein on DNA during the transcription cycle. Targeted deletion of the O-GlcNAc Transferase (OGT) in excitatory neurons of adult mice results in a morbidly obese mouse with a satiety defect. Thus, O-GlcNAcylation not only serves as a nutrient sensor in all cells, but also directly regulates appetite. O-GlcNAcylation also regulates the trafficking of AMPA receptors in neurons and the development of functional synaptic spines. More than two-thirds of human protein kinases are O-GlcNAcylated and all kinases that have been tested are regulated by the sugar. Abnormal O-GlcNAcylation of CAMKII contributes directly to diabetic cardiomyopathy and to arrhythmias associated with diabetes. Prolonged elevation of O-GlcNAc, as occurs in diabetes, contributes directly to diabetic complications and is a major mechanism of glucose toxicity. Targeted over-expression of OGT to the heart causes severe heart failure in mice, which is reversed when they are crossed with mice having O-GlcNAcase over-expressed in their hearts. Drugs that elevate O-GlcNAcylation in the brain, which prevents hyperphosphorylation, appear to be of benefit for the treatment of Alzheimer's disease in animal models. To date, all cancers have elevated O-GlcNAc cycling. Supported by NIH P01HL107153, R01GM116891, R01DK61671. Dr. Hart receives a share of royalty received on sales of the CTD 110.6 antibody, managed by JHU.

Biography

Gerald Warren Hart, Ph.D. GRA Eminent Scholar & Professor of Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, University of Georgia. Honors: Director, Biol. Chem. Johns Hopkins (1997-2018), President, ASBMB (2018-2020), 2019 President's Innovator Award, Society for Glycobiology (SFG), 2018 Herb Tabor Award, ASBMB, 2018 Yamakawa award, Japan Consortium for Glycobiology and Glycotechnology, Karl Meyer Award, SFG in 2006, IGO Award (1997). Associate Editor, J. Biological Chemistry and Molecular and Cellular Proteomics. Founded journal Glycobiology in 1989. Discovered O-GlcNAcylation in 1983, he co-led elucidation of GPI anchor biosynthesis with Paul Englund's group. ~311 publications; Google H-factor = 122; i10-index = 323.

Epigenetic Modifications in the Regulation of Developmental Timing and Rate by Thyroid Hormone Receptor

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Abstract

Thyroid hormone (T3) plays important roles in regulating vertebrate development and pathogenesis. We have been studying T3-dependent Xenopus metamorphosis as a model to investigate the function of T3 and its underlying molecular mechanisms during postembryonic vertebrate development, a period around birth in human when plasma T3 level also peaks. T3 exerts its metamorphic effects through T3 receptors (TRs), which are sequence-specific DNA binding transcription factors. We have previously proposed a dual function model for TRs during Xenopus development. That is, unliganded TRs represses T3-inducible genes during premetamorphosis to prevent premature metamorphosis while liganded TR is required for metamorphic transformation of a tadpole to a frog when T3 is present. I will present some of our studies to show that knockout of TRα and TRα genes, the only TR genes in all vertebrates, leads to premature metamorphosis but reduces the rate of metamorphic progression once metamorphosis begins, indicating that TR controls metamorphic timing and the rate of metamorphosis in the unliganded and liganded state, respectively. Mechanistically, we show that unliganded TRs recruit histone deacetylase-containing corepressor complexes during premetamorphosis while liganded TRs recruit coactivator complexes during metamorphosis to induce chromatin remodeling and regulate histone modifications. We further present data showing that one of the T3 target genes encodes a histone methyltransferase that in turn feeds back positively to enhance histone methylation and T3-target gene activation during metamorphosis. Our findings provide important mechanistic insights on how chromatin remodeling affects gene regulation *in vivo* and animal development.

Biography

Dr. Yun-Bo Shi is a senior investigator at NIH, USA. He received his PhD degree from University of California, Berkeley, and established his laboratory at NIH in 1992. Dr. Shi studies thyroid hormone regulation of vertebrate development and has published over 230 articles. Dr. Shi has received many awards and recognitions, including the Van Meter Award by American Thyroid Association and an elected Fellow of American Association for the Advancement of Science. Dr. Shi has served as an official of several organizations, including North American Society for Comparative Endocrinology, where Dr. Shi is the President-Elect.

Excitable Networks in Directed Cell Migration

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Abstract

Directed cell migration is critical for an extensive range of physiological events. During development, concerted cellular movements bring form to the embryo and, in the adult, migration is critical for immune response, wound healing, stem cell homing, and neuronal wiring. When these orchestrated movements occur improperly or are subverted, disease results. The molecular components involved in cell migration are remarkably conserved between the social amoeba, Dictyostelium and mammalian cells. It is generally believed that cytoskeletal activities drive random cell migration whereas signal transduction events initiated by receptors regulate the cytoskeleton to guide cells. However, using amoebae, neutrophils, and mammary epithelial cells, we found that the cytoskeletal network, involving SCAR/WAVE, Arp 2/3 and actin-binding proteins, is capable of generating only rapid oscillations and undulations of the cell boundary. The signal transduction network, comprised of multiple pathways including Ras GTPases, multiple phosphoinositides, and Rac GTPases, is required to generate the sustained protrusions of migrating cells. The signal transduction network is excitable, exhibiting wave propagation, refractoriness and maximal response to suprathreshold stimuli, even in the absence of the cytoskeleton. We propose that cellular protrusions that underlie cell migration, cell division, macropinocytosis, and other cell morphological events result from coupling of signal transduction and cytoskeletal networks. Whereas the cytoskeletal events provide force perpendicular to the membrane, signal transduction waves traveling laterally along the membrane provide global control of the location, dimensions, and dynamics of protrusions. We have been able to exploit the excitable nature of the signalling network to force cells to assume different morphologies and modes of migration from amoeboid to keratinocyte-like to oscillatory. The application of these concepts to the diverse migratory profiles exhibited by different cells and the ability of cells to detect and integrate extracellular cues is discussed.

Gene interactions in Drosophila without contacts and chemical intermediaries

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Abstract

Conditional mutations are the mutations in the genes of a special category, the ontogenes. These mutations manifest themselves under certain genetical conditions and do not under other genetical conditions. Three phenomena have been discovered in the Drosophila melanogaster flies carrying conditional mutations: (1) a high rate of meiotic nondisjunction; (2) zygotic selection; and (3) a disturbance of bilateral symmetry. The chromosome nondisjunction suggests the absence of homologous pairing. The cases of elimination of some zygotes indicates the absence of "recognition" of chromosome sets in the formation of the zygote. The disturbance of symmetry points at the loss of commutation between the ontogenes residing in different cells. All three phenomena demonstrate the existence of a special type of gene interaction, namely, a "remote" interaction without contacts and chemical intermediaries (RNA and protein molecules).

The phenomenon named the paradox of homologous pairing is described. The available data on ontogenes allows this paradox to be resolved. It is assumed that the sequence of each ontogene possesses a factor that (1) is a product of this nucleotide sequence; (2) is co-located with this sequence; and (3) generates approaching independently of nucleotide sequence positions in space. The sole candidate to the role of this factor is the DNA conformation of ontogene. The conformation in the form of a solenoid of DNA is able to generate electromagnetic field independent of the orientation of the DNA sequence itself. The proposed resolution of the paradox is considered in terms of the problem of "remote" interaction without contacts and chemical intermediaries.

Biography

Nina B. Fedorova graduated from the faculty of biology of Tomsk State University in 1996 on specialty biologist, chemist, teacher of biology and chemistry. In 2002 she finished post-graduate studies at Institute. During post-graduate she also got a scholarship of George Soros. Nina received her Ph.D. on specialty "genetics" in 2007. The subject for her dissertation was "Genetic Instability in Drosophila Lines Containing Facultative Dominant Lethals". It is dedicated to studies the transpositions of mobile genetic elements in Drosophila lines containing conditional mutations. Her research interests are related to the fundamental science: relationship between facts and concepts of classical genetics and modern investigations in epigenetics, molecular and cellular biology.

Spatiotemporal Organization of the E. coli Transcriptome: Insights into RNA-mediated Regulation

Orna Amster-Choder

Professor, Incumbent of Dr. Jacob Grunbaum Chair in Medical Sciences, Department of Microbiology and Molecular Genetics, The Hebrew University Faculty of Medicine, Israel

Abstract

Until recently bacterial RNAs were not assumed to have distinct localization patterns. We have previously demonstrated that E. coli mRNAs may localize to where their products localize in a translation-independent manner in a translation-independent manner [1]. These findings challenged the transcription-translation coupling dogma, although the scope of RNA localization in bacteria remained unknown. Recently we developed a protocol that assigns subcellular localization

data to each transcript to either the membrane, the cytoplasm or the poles [2]. Our results revealed asymmetric distribution of RNAs on a transcriptome-wide scale, which significantly correlates with proteome localization. The results further demonstrate that translation-independent RNA localization is prevalent in E. coli.

The polar transcriptome turned out to be the most unique, enriched with stress-related mRNAs, including small regulatory RNAs (sRNAs). Upon stress, two thirds of the cellular sRNAs became dramatically enriched in the poles via a mechanism that depends on the Hfq chaperon. The remarkable polar enrichment of most sRNAs upon stress supports a polygenic plan for sRNA activity, i.e., a significant fraction of the sRNAs assemble at the poles to orchestrate cell adaptation to stresses. This plan explains the enigmatic subtle effect of deleting single sRNA genes, despite their alleged importance for most aspects of bacterial physiology, including virulence and antibiotic resistance. I will present results that support the existence of such a plan and suggest that sRNAs exert their effects cooperatively.

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Vascular Colonization as a Trigger for Meningococcal Purpura fulminans

Valeria Manriquez[1], Pierre Nivoit[1], Tomas Urbina[1], Hebert Echenique-Rivera[1], Keira Melican[1], Patricia Flamant[2], Taliah Schmitt[3], Patrick Bruneval[4], Dorian Obino[1] and Guillaume Duménil[1*]

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[3]Paris Saint-Joseph Hospital, France
[4]Service d'Anatomie Pathologie, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France

Abstract

Neisseria meningitidis, a human-specific bacterium, is responsible for meningitis and fatal fulminant systemic disease. Bacteria colonize blood vessels, rapidly causing devastating vascular damage despite a neutrophil-rich inflammatory infiltrate. How this pathogen escapes the neutrophil response is unknown. Using a humanized mouse model, we show that vascular colonization leads to the recruitment of neutrophils, partially reducing bacterial burden and vascular damage. This partial effect is due to the ability of bacteria to indiscriminately colonize capillaries, venules and arterioles, as observed in human samples. In venules, potent neutrophil recruitment allows efficient bacterial phagocytosis. In contrast, in infected capillaries and arterioles adhesion molecules such as E-Selectin are not expressed on the endothelium and intravascular neutrophil recruitment is minimal. These results show that colonization of capillaries and arterioles by N. meningitidis create an intravascular niche that preclude the action of neutrophils, resulting in immune escape and subsequent fulminant progression of the infection.

Biography

Guillaume Duménil is the head of the pathogenesis of vascular infections laboratory at the Institut Pasteur in Paris France. He became interested in the mechanisms underlying infectious diseases during his PhD with Philippe Sansonetti. He pursued his career as a postdoctoral fellow in Ralph Isberg's lab in Boston and then came back to France where he obtained a position at INSERM, initially in X. Nassif's lab. Creating his how lab he moved to the Paris Cardiovascular Research Center (PARCC) and then to the Institut Pasteur. He was laureate of an ERC starting grant and recipient of several scientific prizes.

Expression Profile of Sporadic Cerebral Cavernous Malformations Endothelial Cells by Whole RNA Sequencing

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[2]Department of Biomolecular strategies, genetics and avant-garde therapies, I.E.ME.S.T., Palermo, Italy

Abstract

Cerebral Cavernous Malformation (CCM, OMIM#116860) affects brain microvasculature. Capillaries appear enlarged, tangled and lacking of pericytes. Lesions may occur sporadically or be inherited following germline mutations at the KRIT1, CCM2 and PDCD10 loci. The encoded proteins regulate cell junction maintenance, oxidative stress response, apoptosis. CCM patients harbouring no CCM genes mutations have been reported. Two hypotheses can be considered: i) causative genes not yet discovered; ii) tissue-specific alterations of expression patterns of genes involved in angiogenesis. The study draws up the molecular signature of endothelial cells isolated from CCM specimens.

Endothelial cells (CD31+) were isolated from 2 CCM specimens by the MidiMacs cell separator. Whole transcriptome analysis was performed. Human brain microvascular endothelial cells (HBMECs) were used as negative control. Functional enrichment analysis was performed according to the Gene Ontology "Biological process" annotation terms.

By comparison with HBMECs, 1325 genes were differentially expressed (Bonferroni p-Value<0.05) in both CCM samples. Functional enrichment analysis clustered these genes in 80 terms related to neuroinflammation, extra-cellular matrix remodelling, cell junction impairment, oxidative damage. However, two novel pathways were dysregulated in both samples. These are related to the "non-canonical Wnt5a/Planar Cell Polarity pathway" and to the "ion homeostasis and transport". According to our results, the molecular shift from canonical to non-canonical Wnt pathway might be a key event in CCM pathogenesis, causing loss of endothelial cell polarity. Moreover, ion transport imbalance also contributes to increase blood brain barrier permeability. Therefore, our results provide new further molecular cascades to investigate in order to comprise CCM pathogenesis.

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Unraveling the Molecular Bases of Human Congenital Disorders of Glycosylation using Fission Yeast as Experimental Model

Cecilia D'Alessio

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Abstract

Congenital Disorders of Glycosylation (CDGs) are a group of human inherited multi-systemic diseases. Many are due to defects in protein N- glycosylation, in which an evolutionary conserved pre-assembled glycan Glc3Man9ClcNAc2 (G3M9) is transferred by the oligosaccharyltransferase (OST) from a donor lipid to proteins that are entering into the endoplasmic reticulum (ER). Defects in glycan remodeling afterwards produce CDG Type II. Glucosidase I (GI) is the first

glycan-remodeling enzyme that removes the outermost glucose, and mutations in GI-encoding gene (gls1+) result in CDG-IIb. Using the fission yeast Schizosaccharomyces pombe lacking GI as a model organism we demonstrated that the main cause of the morphological and growth defects observed in mutant cells was the persistence of G3M9 structures in glycoproteins, as a second mutation in alg10+ gene (responsible for the addition of the last Glc during the lipid-linked G3M9 synthesis) substantially suppressed the observed defects. The sick phenotype of Δgls1 mutant cells could not be ascribed to a product inhibition of OST transfer reaction, to the inability of glycoproteins to enter into calnexin-folding cycles, or to a potentially reduced ER-associated degradation. Further analysis showed that the endomembrane system was altered in cells lacking GI, as cell wall glycoproteins region was wider in Δgls1 cells than in WT ones and as the lack of GI produces cells with highly fragmented vacuoles. Collectively, these results suggest the occurrence of alterations in the secretory/endocytic pathway in cells lacking GI and shed light on the underlying molecular and cellular mechanisms of CDG IIb disease.

Biography

Cecilia D'Alessio is Licenciada in Biological Sciences with a specialization in Molecular Genetics (1995) and Ph.D in Chemistry (2001), University of Buenos Aires, Argentina. Head of the Cell Glycobiology and Applied Yeast Genetics laboratory at the Department of Physiology, Molecular and Cell Biology, Facultad de Ciencias Exactas y Naturales, University of Buenos Aires, the main interest areas are cell Glycobiology related with glycosylation disorders using fission yeasts as model organisms and yeast glycol-engineering for biotechnological purposes. Currently she is Adjunct Professor at the University of Buenos Aires and Independent Researcher of the National Research Council (CONICET), Argentina.

Easy and Efficient Delivery of Cells to the Bone Marrow in Mice

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Abstract

Bone marrow is a key organ to understand mechanisms underlying hematopoiesis, leukemia and cancer metastasis. However, current *in vitro* culture techniques are not sufficient to recapitulate tissue microenvironments of bone marrow. This prompts us to adopt murine models in hematopoiesis and cancer-related studies. In these studies, cell transplantation assay is indispensable to assess stem cell/cancer cell behavior in the bone marrow. Here, we developed a new systemic transplantation pathway to deliver the cell to bone marrow in mice. Our intra-caudal arterial (CA) transplantation delivered the cells >10-fold more efficiently than current standard transplantation methods [1,2]. We demonstrated that CA transplantation is a promising approach to construct murine bone metastasis models with various cancer cell lines.

References

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[2] http://sites.google.com/view/takahirokuchimaru/protocol

Biography

Takahiro Kuchimaru was raised in Kobe, Japan. He received his Ph.D. in Electric Engineering from Osaka University in 2009. Then, he experienced multidisciplinary postdoctoral works in the field of medical biology at Tokyo Institute of Technology, Kyoto University and Massachusetts General Hospital. Since 2018, Takahiro Kuchimaru has been an assistant professor in the Center for Molecular Medicine at Jichi Medical University. His current research focuses on the development of bioluminescence/fluorescence imaging tools for biomedical researches and understanding the molecular basis of bone metastasis.

Activation of the EGF Receptor by Ligand Binding and Oncogenic Mutations: the Rotation Model

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[2]Cellular Structural Biology Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Abstract

The epidermal growth factor receptor (EGFR) is a member of the receptor tyrosine kinase family, which plays vital roles in many cellular processes including cell survival, proliferation, differentiation, motility, and metabolism. Aberrant activation of EGFR by mutations has been implicated in a variety of human cancers. Elucidation of structures of the full-length receptor is essential to understand molecular mechanisms underlying its activation. Unlike previously anticipated, we report that full-length EGFR solubilized with detergent from the membrane exists in a homo-dimeric form *in vitro* before and after ligand binding. Cryo-electron tomography analysis of the purified receptor also shows that the extracellular domains of the receptor dimer, which are conformationally flexible before activation, are stabilized by ligand binding. This conformational flexibility stabilization is most likely to rearrange the intracellular kinase dimer into a flexible active form. Consistently, mutations in the interface of the symmetric kinase dimer spontaneously activates the receptor *in vivo*. Optical single molecule observation also demonstrates that binding of only one ligand activates the receptor dimer on the cell surface. Based on these results, we propose the "rotation model" for how EGFR dimers are activated by ligand binding and oncogenic mutations. Our results shed light on how mutations spontaneously activate the receptor and on the development of novel cancer therapies.

Biography

Ichiro Maruyama is a Professor at the Okinawa Institute of Science and Technology Graduate University (OIST). He received his Ph.D. from The University of Tokyo, Japan. Subsequently he was trained as a post-doctoral fellow at MRC Laboratory of Molecular Biology, Cambridge, UK, and then as an Associate Professor at The Scripps Research Institute, La Jolla, California, USA. At OIST, Ichiro Maruyama continues to work on molecular mechanisms underlying transmembrane signaling mediated by cell-surface receptors.

Tells of the Co-opertive Actions of TDP-43 and FMRP in Spine Transport/Translation of Specific mRNAs

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Abstract

Initial stages of mammalian brain development require a tight control over dendritic transport of mRNP granules. This ensures temporal modulation of protein synthesis inside sub-dendritic compartments and fine-tuning of spinogenesis of the neuronal dendrites. Using FISH/IF, live cell imaging, and molecular techniques like RNA-IP, polysome profiling etc. we have established that ALS related protein, TDP-43 and Fragile X mental retardation protein, FMRP co-operate with each other to regulate dendritic transport and translation of mRNAs like Rac1 and Map1b, as well as spinogenesis in primary hippocampal neurons in culture. Our results further showed that TDP-43 recruits translation inhibitory complex FMRP-CYFIP1 to the mRNPs to inhibit the translation during dendritic transport processes and translation in these mRNPs gets reactivated only after reaching the destination, eg inside the spines. Thus these two RBPs, TDP-43 and FMRP, orchestrate dendritic and spine transport, translation inhibition and reactivation to facilitate time dependent spatial translation of TDP-43 bound mRNAs. This novel regulatory mechanism might be utilized by some or all of 160 common target mRNAs of these two RBPs. This study establishes physical and functional partnership between FMRP and TDP-43 that probably

mechanistically links different neurodegenerative diseases and neurodevelopmental disorders.

Biography

Pritha Majumder: Currently Post Doc under Dr. C.-K. James Shen in Academia Sinica, Taiwan. PhD in Molecular Biology from University of Calcutta, India (Year: 2008). Total publications (journals+book chapter+ meeting proceeding, 15 (Total impact factor: 84.681).

Specific RNA Binding of BICC1 and Its Regulation

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Abstract

Defective signal transduction by primary cilia can provoke a spectrum of ciliopathy syndromes, including perturbations of left-right asymmetry during embryogenesis or tubule dilation and the formation of fluid-filled cysts in kidneys. Situs defects are provoked by failure of cilia to bias SMAD2,3 activation to the left side, whereas kidney cyst formation is stimulated by the transcription factors YAP/TAZ, c-Myc and PPARα and by associated metabolic changes. However, the mechanisms that link the regulation of any of these transcription factors to cilia remain obscure. Among candidate effectors of cilia signaling, we have studied a molecular network of the ciliopathy proteins ANKS6 and ANKS3 with the RNA-binding protein Bicaudal-C1 (Bicc1) that is organized by multivalent interactions, presumably to stimulate or inhibit mRNA silencing. Depending on the context, Bicc1 has been reported to inhibit translation and/or promote mRNA decay after recruiting target RNAs to one or several K-homology (KH) domains, followed by self-polymerization. Using electromobility shift assays to monitor competitive *in vitro* binding, combined with CRISPR editing of Bicc1 in cell-based assays, we here delineate structural requirements for specific RNA binding and its regulation by interacting factors. We propose that ciliary proteins may employ Bicc1 to regulate gene expression post-transcriptionally, and that kidney cysts form due to the loss of this regulatory switch.

Biography

After doctoral research at ETH Zürich and postdoctoral studies at Harvard University, Daniel Constam became a group leader at the Swiss cancer research institute ISREC, and then Associate Professor at EPFL. His research concentrates on the regulation of TGFα signaling by proprotein convertases and its role in development and cancer. His lab also discovered a role for the RNA-binding protein Bicc1 in left-right development and renal tubule morphogenesis. Besides his laboratory, he currently directs the EPFL doctoral program in Molecular Life Sciences.

The Impact of Magnesium(II) Ions and Sugar Puckering on the Formation of Tertiary Contacts of Nucleic Acids – Fundamentals in (self)splicing and Potentially Reverse Transcription

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Abstract

Our research is focused on the molecular mechanism of self-splicing ncRNA, in particular, the group IIB intron of S. cerevisiae. Here, we use smFRET in combination with global hidden Markov modeling and molecular dynamics (MD) as a hybrid approach [1]. In our comparative study, we look at an obligate tertiary contact common to all classes of group II introns: the exon and intron binding site 1 (EBS1/(d)IBS1) with known NMR structure [2]. Single-molecule detection

gives us access to different subpopulations which show the same FRET efficiency but differ kinetically. A characteristic of such "degenerate" FRET states is their multi-exponentiality [3]. Here, we fully resolve for the first time a degenerated, heterogeneous nucleic acid system using a global HMM on an ensemble of single-molecule FRET trajectories [1]. Interestingly and in contrast to the RNA-RNA contact, the RNA-DNA contact displays homogenous unbinding kinetics. Our all-atom MD simulations show the structural origin of the observed kinetic heterogeneity in a uniform (RNA-RNA) and hybrid duplex (RNA-DNA). In this way, we found that fast sugar puckering in the heteroduplex relieves molecular strain at the binding interface, which in turn makes the RNA-DNA contact more labile and kinetically homogenous [1, 3].

In the context of group II introns, our study suggests a possible coevolution of intron-encoded proteins to stabilize labile RNA-DNA contacts in the event of an intron invasion. Such cooperative binding, where RNA and proteins act in concert, might be a general mechanism to overcome weak interactions under physiological ionic conditions, thus, at low concentration of divalent metal ions.

References:

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Biography

Richard Börner is a (bio)physicist by training and his research is focused on RNA folding kinetics and structure. He did his PhD at the University of Lübeck, where he got in contact with single molecule spectroscopy studying single organic fluorophores. Moving as a Postdoc to Zurich, he switched gears and started using single-molecule FRET in TIRF microscopy to follow domain motions of (RNA) biomolecules such as riboswitches and ribozymes in time. We share our recent advances in the development of our software package MASH-FRET to analyze single-molecule-videos (including molecular sorting and resolving degenerate FRET states) on github: https://github.com/RNA-FRETools/MASH-FRET.

Cell-type-specific Genomics Reveals Histone Modification Dynamics in Mouse Meiosis

Gabriel Lam*, Kevin Brick, Gang Cheng, Florencia Pratto and R. Daniel Camerini-Otero

Genetics and Biochemistry Branch, National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Bethesda, MD

Abstract

Meiosis is the specialized cell division during which parental genomes recombine to create genotypically unique gametes. Despite its importance, mammalian meiosis cannot be studied *in vitro*, greatly limiting mechanistic studies. *In vivo*, meiocytes progress asynchronously through meiosis and therefore the study of specific stages of meiosis is a challenge. Here, we describe a method for isolating pure sub-populations of nuclei that allows for detailed study of meiotic sub-stages. Interrogating the H3K4me3 landscape revealed dynamic chromatin transitions between sub-stages of meiotic prophase I, both at sites of genetic recombination and at gene promoters. We also leveraged this method to perform the first comprehensive, genome-wide survey of histone marks in meiotic prophase, revealing a heretofore unappreciated complexity of the epigenetic landscape at meiotic recombination hotspots. Ultimately, this study presents a straightforward, scalable framework for interrogating the complexities of mammalian meiosis.

Biography

Gabriel earned his PhD in the University of Leicester in the UK. He is currently a post-doc research fellow in Dr. Camerini's lab in the NIDDK in the NIH. His research focuses on studying the biochemical mechanism of genetic recombination in mammals and developing novel genomic approaches to explore epigenetic changes and DNA-protein interactions throughout meiosis.

Structural and Biochemical Studies on Trimethoprim Resistant DHFR from Pathogenic Bacteria

Dennis L. Wright

Professor of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Groton, CT

Abstract

The binary antibiotic trimethoprim/sulfamethoxazole (TMP/SMX, co-trimoxazole) was introduced in 1974. Since then it has become one of the most widely used agents for the treatment of Gram-positive Staphylococcus aureus infections. The combination of TMP and SMX inhibits two consecutive steps in the folate pathway, with TMP targeting the essential enzyme dihydrofolate reductase (DHFR), required for the biosynthesis of thymidine, purines and methionine. Our heavy reliance on TMP/SMX for the management of these infections leaves us vulnerable to the emergence of drug-resistance. We set out to develop second-generation antifolates, based on our propargyl-linked antifolate (PLA) scaffold, that would provide coverage against both S. aureus and S. pyogenes and retain potency against the major chromosomal DHFR mutations that were considered to be the primary clinical manifestation Central to the design was the installation of a carboxylate moiety on a PLA scaffold to mimic a key ionic interaction made by the natural cofactor, dihydrofolate. s of TMP-resistance. As part of this work, we conducted a clinical surveillance of current TMP^R-MRSA isolates and were surprised to discover that resistance to TMP was exclusively mediated by two plasmid-encoded DHFR genes (dfrK and dfrG), previously undetected in the US. These transposable elements not only conferred resistance to trimethoprim but also the newer antifolate iclaprim. Using a structure-based design strategy, we developed a new class of antifolates that display remarkable potency against these resistant enzymes. The lecture will discuss structural and biochemical studies on these new TMP resistance elements.

Biography

Dr. Dennis Wright is a Professor of Medicinal Chemistry at the University of Connecticut. His research group focuses on highly drug resistant pathogenic organisms such as bacterial, fungi and viruses. The Wright lab takes an integrated approach to studying the mechanisms of drug resistance and new antimicrobial design through the use of structural, biochemical and chemical tools. Using this highly interdisciplinary approach, we work to develop next generation agents that can circumvent these important resistance mechanisms.

Ribonucleotides Embedded in Genomic DNA are not Random and Show Specific Preferences of Incorporation

Sathya Balachander, Alli Gombolay, Taehwan Yang, Penghao Xu and Francesca Storici1*

Professor, Associate Chair for Graduate Affairs, School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA

Abstract

Ribonucleoside monophosphates (rNMPs) embedded in DNA are the most frequent nonstandard nucleotides found in the genome of cells. Their presence in DNA increases DNA fragility and mutability, and alters the way DNA interacts with proteins. To study functions of rNMPs embedded in genomic DNA, we invented and developed the ribose-seq technique for capturing genomic sites of rNMPs (PMID: 25622106), and the Ribose-Map bioinformatics toolkit to analyze the genomic data (PMID: 30272244). We built and analyzed many high through put sequencing libraries of rNMPs derived from mitochondrial and nuclear DNA of budding and fission yeast. We worked with Saccharomyces cerevisiae, the closely related Saccharomyces paradoxus, and the more distantly related Schizosaccharomyces pombe, using common lab strains of wild-type and different mutant genotypes of ribonuclease H2, which is the major enzyme that initiates removal of rNMPs from DNA. We revealed both common and unique features of rNMP sites among yeast species and strains, and between wild type and different ribonuclease H-mutant genotypes. We found that the rNMPs are not randomly incorporated in DNA because there are preferred sequence contexts of rNMP presence in DNA. We discovered signatures and patterns of rNMPs. We uncovered that the sequence of the deoxyribonucleoside monophosphate (dNMP) immediately upstream

from the site of rNMP incorporation has the most impact on the frequency of rNMP incorporation. Furthermore, we found that around sites of autonomous replicating sequences in nuclear DNA of S. cerevisiae, the patterns of rNMP incorporation markedly change on the leading and lagging strands. Because the bulk of the leading and lagging strand replication is performed by distinct DNA polymerases in yeast, our results suggest a unique mechanism of accommodation of the rNMPs in the active site of the DNA polymerases that synthesize the leading and lagging strands.

This work is supported by NIH, NIEHS R01 ES026243, and Howard Hughes Medical Institute Faculty Scholars Award, HHMI 55108574 to F. Storici.

Chemoptogenetic-Mediated Singlet Oxygen Damage to Mitochondria Causes Telomere Dysfunction

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[8]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA

Abstract

Singlet oxygen plays important roles in aging, inflammation, and cancer. Mitochondria are rich in photosensitizers such as porphyrins, which are able to convert oxygen into singlet oxygen upon light exposure. However, the physiological consequences and the signaling pathways downstream of mitochondrial damage caused by singlet oxygen are elusive. By using an innovative mitochondrial targeted fluorogen activating peptide (FAP) complexed with a MG2I dye we were able to precisely control the generation of singlet oxygen exclusively to mitochondria. This organelle-targeted generation of singlet oxygen resulted in comprised respiration, mtDNA damage and mitochondrial fragmentation. The mitochondrial singlet oxygen generated by this FAP-MG2I system triggered a secondary wave of ROS generation including superoxide and hydrogen peroxide. Importantly, the hydrogen peroxide generated by dysfunctional mitochondria diffused into the nucleus and caused nuclear oxidation of cysteine residues. This wave of hydrogen peroxide also induced a cell cycle delay accompanied by DNA replication stress and activation of DNA damage repair signaling. However, a COMET assay revealed a lack of gross nuclear DNA damage and strand breaks. Surprisingly, we have found that the telomeres are especially sensitive to damage as a result of nuclear oxidation. This damage appears to be sufficient to cause ATM mediated signaling. The susceptibility of the telomeres in the response to mitochondrial targeted damage by singlet oxygen reveals a novel mechanism underlying the pathophysiological role of singlet oxygen in human diseases. Supported by NIH R33ES025606

Biography

Dr. Van Houten is the Richard M. Cyert Professor of Molecular Oncology in Pharmacology and Chemical biology and the Co-Leader of the Genome Stability Program in the UPMC-Hillman Cancer Center and the Associate Director for Basic Research in the Aging Institute. His work is focused on: 1) mitochondrial dysfunction and its role in aging and disease; and 2) the structure and function of DNA repair enzymes and their relationship to disease. Dr. Van Houten holds four patents and has authored over 225 scientific articles and has also published 28 book chapters and reviews. His Web of Science, h-index is 68.

Enhancing Chemotherapy with Small Molecule Translesion Synthesis Inhibitors

Pei Zhou

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Abstract

Chemotherapy remains an effective treatment option for many cancer patients. Despite a high rate of initial treatment success, the majority of the patients eventually relapse with resistant tumors that render subsequent rounds of treatment ineffective. It is increasingly recognized that mutagenic translesion synthesis (TLS), a DNA damage tolerance pathway, plays an important role in cancer cell survival and development of resistance after chemotherapy, suggesting that targeting TLS is an attractive avenue for improving chemotherapeutics. However, development of small molecules with high specificity and *in vivo* efficacy for mutagenic TLS has been challenging. Employing a high-throughput screening campaign, we have discovered a small molecule inhibitor, JH-RE-06, that disrupts mutagenic TLS by preventing the recruitment of mutagenic POL ζ. Remarkably, our structural analysis reveals that JH-RE-06 targets a nearly featureless surface of REV1 that interacts with the REV7 subunit of POL ζ. Binding of JH-RE-06 induces REV1 dimerization and blocks the REV1-REV7 interaction and POL ζ recruitment. JH-RE-06 inhibits mutagenic TLS and enhances cisplatin-induced-toxicity *in vitro*; furthermore, co-administration of JH-RE-06 with cisplatin suppresses the growth of xenograft human melanomas in mice. Taken together, these results establish the feasibility of developing TLS inhibitors as a novel class of chemotherapy adjuvants.

Biography

Dr. Zhou obtained his Ph.D. training in the area of chemical biology at Harvard University from 1993-1998 and postdoctoral training in the area of structural biology at Harvard Medical School from 1998-2001. In 2001, Dr. Zhou established an active research program at Duke University School of Medicine to probe the structure and dynamics of macromolecular assembly and inhibition. His research interests include enzymes and protein complexes involved in bacterial membrane biosynthesis, host-pathogen interactions, translesion DNA synthesis, and co-transcriptional regulations.

Aptamer-based Imaging of Polyisoprenoids Applied to the Malaria Parasite

Maria Belen Cassera*, Flavia M. Zimbres, Emilio F. Merino, Grant Butschek

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Abstract

Dolichols are isoprenoid end-products of the mevalonate and 2C-methyl-D-erythritol-4-phosphate (MEP) pathways. Their synthesis starts with the condensation of farnesyl diphosphate (FPP) and several molecules of isopentenyl diphosphate (IPP) to synthetize polyprenyl diphosphate catalyzed by a cis-prenyltransferase. Subsequent steps of dephosphorylation followed by reduction of the a-isoprene unit by polyprenol reductase leads to the formation of dolichol which can vary in size depending on the number of isoprene units incorporated. In eukaryotes, dolichols are synthesized as a mixture of 4 or more different lengths with one or two predominant species with the size varying among species. Their biosynthesis is predicted to occur in the endoplasmic reticulum where dolichols are essential for protein glycosylation and GPI anchor biosynthesis. However, these lipids have been detected in other subcellular localizations where their biological functions remain largely unknown. Visualization and quantification of small molecules (metabolites) temporally and spatially in a cellular context remain very limited and rely mainly in using non-natural, chemically modified metabolites. We developed a novel polyisoprenoid (PP) aptamer-based sensor for in situ imaging of linear native polyisoprenoids. The specificity profile of the aptamer was evaluated against a broad range of isoprenoid products and its potential for in situ imaging was assessed in the malaria parasite, Plasmodium falciparum. Aptamer-based microscopy analysis of these lipids revealed distinctive subcellular localizations that changed with parasite's development and in response to chemical or genetic disruption of isoprenoid biosynthesis.

Biography

Dr. Maria (Belen) Cassera is an Associate Professor at the University of Georgia. She has a Doctor of Science degree in the area of Biology of Host-Pathogen Interaction from the Department of Parasitology at the University of Sao Paulo in Brazil. She learned enzymology and drug design from Dr. Vern Schramm at the Albert Einstein College of Medicine in the Bronx as a postdoctoral trainee before starting her own laboratory at Virginia Tech in 2011 where she stablished a metabolomics and drug discovery research program. In August of 2016, she moved to UGA to continue her work on drug discovery and development.

Deciphering the Metabolic Outliers in Genetic Diseases

Min Ni[1,2*], Ashley Solmonson[1], Chunxiao Pan[1], Hieu Vu[1], Ling Cai[1,3], Jimin Pei[4], Goncalo Dias do Vale[5], Dinesh Rakheja[6], Nick V. Grishin[4,11], Jeffrey G. McDonald[5], Garrett K. Gotway[2,7,8], Markey C. McNutt[2,7,8], Juan M. Pascual[2,8,9,10] and Ralph J. DeBerardinis[1,2,8,11]

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Abstract

Inborn errors of metabolism (IEMs) are genetic disorders characterized by abnormal metabolism and are diagnosed by assessing the phenotype and pedigree along with clinical biochemical and molecular testing. However, establishing a specific diagnosis is challenging because many IEMs have non-specific symptoms. Whole exome sequencing (WES) has accelerated IEM discovery, but the functional impact of many genetic variants remains unknown. Here we integrated genomics and metabolomics to identify a cause of lactic acidosis and epilepsy. The patient is a compound heterozygote for variants in LIPT1, which encodes the lipoyltransferase-1 required for 2-ketoacid dehydrogenase (2KDH) function. Plasma metabolomics revealed abnormalities in lipids, amino acids and 2-hydroxyglutarate consistent with loss of multiple 2KDHs in this patient. Homozygous knock-in of a LIPT1 mutation reduced 2KDH lipoylation in utero and resulted in embryonic demise. In patient fibroblasts, defective 2KDH lipoylation and function were corrected by wild-type but not mutant LIPT1 alleles. Isotope tracing revealed that LIPT1 supports lipogenesis and balances oxidative and reductive glutamine metabolism. Taken together, these findings extend the role of LIPT1 in metabolic regulation and demonstrate how integrating genomics and metabolomics can uncover broader aspects of IEM pathophysiology.

Biography

Min Ni is an Assistant Professor of UT Southwestern Medical Center. She is working with Dr. Ralph DeBerardinis on a clinical study of genetic and metabolic diseases, especially undiagnosed Mendelian disorders. Min received her Ph.D. from the University of Southern California at Los Angeles, and did her postdoctoral training with Dr. Myles Brown at Dana-Farber Cancer Institute and Harvard Medical School. In 2014, she joined the Children's Research Institute of UT Southwestern.

Regulation of Diacylglycerol Kinases by Membrane Shape

José Carlos Bozelli, Jr.* and R.M. Epand

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Abstract

The addition/removal of phosphate groups in lipids, as in proteins, is critical for cellular signal transduction. In this regard, one particular family of enzymes is very relevant, that of diacylglycerol kinases (DGK). DGK catalyze the phosphorylation of diacylglycerols (DAG) to produce phosphatidic acids (PA). Both are important lipid signaling molecules. The presence of different structural domains/motifs in the structure of mammalian DGK isoforms, as well as the different expression patterns, suggests that these isoforms have different biological roles, albeit catalyzing the same enzymatic reaction. Moreover, one idea that starts to emerge is that some biological phenomena rely on specific lipid molecular species. In that sense, it is interesting to mention that some DGK isoforms have been shown to bear substrate acyl chain specificity. Since DGK are interfacial active enzymes, membrane binding is a requirement for catalytic turnover and, therefore, it is hypothesized that DGK are regulated by the properties of the membrane they bind to. By use of purified DGK and model membranes with variable physicochemical properties it is shown that the substrate acyl chain specificity of two DGK isoforms, DGKe/DGKa, depends on both the enzyme structure and the shape of the membrane it binds to. The results suggest that different isoforms might regulate the levels of different molecular species of DAG/PA and, therefore, different signaling pathways. It is proposed that there is a hierarchic coupling of membrane physical and chemical properties that synergistically regulates membrane signaling events, highlighting the elegant nature of DGK role in lipid signaling events.

Research/Financial support: This work was supported by the Canadian Natural Sciences and Engineering Research Council grant RGPIN-2018-05585.

Adnp and 14-3-3 Regulate Neuronal Morphogenesis in the Developing Cortex

Kazuhito Toyooka

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Abstract

Mutations in activity-dependent neuroprotective protein (ADNP) cause various neurodevelopmental defects, including autism spectrum disorder (ASD), epilepsy, and brain abnormalities. However, little is known about ADNP functions in the developing cortex. To clarify the Adnp's functions in neurodevelopment, we used cellular and *in vivo* strategies, including primary cortical neuron culture, in utero electroporation (IUE), and live imaging on brain slices. Adnp knockdown in the developing cortex caused multiple neuronal morphological defects. Using ex vivo live imaging on brain P0 slices, we found severe flaws in cellular dynamics, including failure of neurite retraction, slow growth speed, increased neurite stabilization, and intracellular swellings on growing neurites. Also, the Adnp knockdown by IUE resulted in increased basal dendrite number, axon length, and interhemispheric axon innervation. To analyze whether excitatory connectivity was altered in Adnp deficient neurons, we utilized GPI anchored Reconstitution-Activated Proteins Highlight Intercellular Contacts (GRAPHIC), a state-of-the-art synaptic tracing technique, and found increased interhemispheric connectivity between Adnp deficient layer 2/3 pyramidal neurons in opposing cortices. Another interesting aspect of ADNP is that it is bifunctional with roles as both a transcriptional factor in the nucleus and a microtubule regulator in the cytoplasm. We found that Adnp is shuttled from the nucleus to the cytoplasm by 14-3-3 proteins as neurons differentiate. Thus, we conclude that Adnp is shuttled from the nucleus to the cytoplasm by 14-3-3, where it regulates neurite formation, maturation, and functional cortical connectivity.

Biography

Dr. Toyooka is an assistant professor in the Department of Neurobiology & Anatomy at Drexel University College of Medicine. He did postdoctoral fellowships at the University of California San Diego and the University of California San Francisco. He also served on the faculty at Osaka City University School of Medicine in Japan. He was appointed to the faculty in the Department of Neurobiology & Anatomy at the College of Medicine in 2013.

RNA-binding Protein HuR Restrains Inflammatory Cytokine Production in Innate Cells

Jing Chen and Shiguang Yu*

Department of Neurology, Thomas Jefferson University, Philadelphia, PA

Abstract

Sepsis is the most common cause of mortality in many intensive care units and responsible for more than 250,000 deaths in the United States annually. The characteristic hallmark of sepsis is an exaggerated innate immune response leading to a cytokine storm, excessive vasodilation and severe vascular leakage, circulatory shock and multiple organ failure. We have found that mice with myeloid cells specifically deficient in RNA-binding protein HuR (HuR KO), exhibit exacerbated inflammation following challenges with the inflammatory stimulus lipopolysaccharide (LPS). These mice experienced a substantially enhanced cytokine storm and showed markedly increased mortality associated with vascular leak and circulatory collapse. Although HuR is critically important in controlling innate immune response, its regulation remains poorly understood. In our preliminary studies, we found that HuR KO bone marrow derived macrophages (BMDMs) were more sensitive to LPS challenge, with increased level of proinflammatory cytokine production than wild type (WT) BMDMs. In addition, HuR KO BMDMs were more susceptible to LPS plus ATP-induced pyropotosis than WT BMDMs. Knockout of HuR decreased the level of histone deacetylase (HDAC)1 and 4 in BMDMs by Western blot assay. We also found that an immunomodulator was an effective agent for sustaining HuR expression, thereby preventing the NLRP3 inflammasome activation, ultimately inhibiting proinflammatory cytokine production. Overall, our results suggested that upregulated expression of HuR in innate cells can be an effective means to attenuate inflammation. Understanding of regulation and function of HuR in myeloid cells could lead to a novel intervention on sepsis.

Biography

Dr. Jing Chen is a research instructor at the department of neurology, Thomas Jefferson University (TJU), Philadelphia, USA. Over the years, she has studied the roles of RNA-binding protein HuR in cancer cells and immune cells. Her recent work demonstrated that HuR is required for T cells to induce autoimmune neuroinflammation. Dr. Shiguang Yu is an assistant professor at the department of neurology, TJU. His research interests focus on studying regulation of autoreactive T cells in autoimmune diseases.

Use of a Tethered Ligand Signaling Mechanism by Polycystin-1

Robin L. Maser*, Jayalakshmi Ravichandran, Ericka Nevarez Munoz and Brenda S. Magenheimer

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Abstract

Polycystin-1, encoded by the PKD1 gene, is mutated in 85% of cases of autosomal dominant polycystic kidney disease (ADPKD). Polycystin-1 is a large and complex protein with 11 transmembrane domains (TM) implicated in multiple cellular functions, including the binding and activation of heterotrimeric G proteins. Studies in animal model systems demonstrate that polycystin-1-mediated G protein regulation is fundamental in the prevention of ADPKD. Notably, polycystin-1 shares an evolutionarily conserved structure, the GAIN domain, with the adhesion family of G protein-coupled receptors (GPCRs). Located within the N-terminal, extracellular region near the first TM in both protein families, the GAIN domain

is known to mediate an autocatalyzed proteolytic cleavage at a conserved sequence, the GPS, within its own structure. GPS cleavage generates an extracellular, N-terminal fragment (NTF) and a membrane-embedded, C-terminal fragment (CTF), which remain non-covalently associated with each other. One of the mechanisms utilized by Adhesion GPCRs to regulate G protein signaling involves removal of the NTF which exposes the short, N-terminal 'stalk' of the CTF (or Stachel sequence) that then functions as a tethered ligand/agonist to activate G proteins. We have tested the hypothesis that polycystin-1-mediated G protein signaling is regulated by a similar 'cryptic' tethered ligand mechanism. Transient, ectopic expression of constructs encoding full-length or CTF forms of polycystin-1 demonstrate that activation of an NFAT promoter-luciferase reporter is greater by the CTF, is dependent on the presence of the stalk, can be activated in trans by synthetic, stalk-derived peptides, and is affected by ADPKD-associated, missense mutations within the stalk.

Biography

R. Maser has been working in the PKD field for over 25 years. Her lab focuses on uncovering the structure-function relationships of polycystin-1 with respect to its ability to function as an atypical GPCR. Contributions to the field have included revealing the ability of polycystin-1 to bind and signal via heterotrimeric G proteins and providing the first experimental evidence for the 11-transmembrane domain structure of polycystin-1.

Developing Dual-Targeted Nanoparticles to Circumvent the Resistance to Src Inhibition in Head and Neck Cancer

Yong Teng[1,2,3*], Liwei Lang[1], Xiangdong Zhao[1], Yuanping Xiong[1] and Chloe Shay[4]

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[3]Department of Medical Laboratory, Imaging and Radiologic Sciences, College of Allied Health, Augusta University, Augusta, GA
[4]Department of Pediatrics, Emory Children's Center, Emory University, Atlanta, GA

Abstract

Head and neck squamous cell cancer (HNSCC) is considered one of the malignity with the most severe impact on patients' mortality, caused mainly by relatively low responsiveness to treatment and severe drug resistance. Despite Src has been implicated as a key promoter in tumor progression and metastasis of HNSCC, the clinical benefit of anti-Src drugs is significantly dampened by low response rate and therapeutic resistance. In this study, the Src inhibitor saracatinib loaded into the novel multifunctional nanoparticles exhibited superior effects on suppression of HNSCC compared with the free drug, which is mainly attributed to a highly specific and efficient tumor-targeted drug delivery system. Moreover, we identified that upregulation of the AKT/S6 is the critical mechanism for HNSCC cells to develop saracatinib resistance. Capivasertib is the selective and potent AKT inhibitor and inactivating the AKT signaling can reverse saracatinib resistance and improve the efficacy of saracatinib in 3D cell cultures and preclinical tumor-bearing mice. Most importantly, Cathepsin B-sensitive nanoparticles for codelivering saracatinib and capivasertib significantly improved the efficacy of tumor repression without increasing side effects. These findings demonstrate that the addition of AKT blockade improves anti-HNSCC efficacy of anti-Src therapy, and co-delivery of capivasertib and saracatinib by tumor-targeting nanoparticles has the potential to achieve better treatment outcomes than the free drug alone or in combination.

Biography

Dr. Yong Teng is an Assistant Professor at DCG in Augusta University, with joint appointments in MCG and Georgia Cancer Center. The main research activity in his lab is to understand and reverse mechanisms of cancer metastasis and metabolism. He received research awards from NIH, DOD, and other fund agencies, and authored more than 100 articles and book chapters. He serves as Associate Editor-in-Chief or editor for many reputed journals, as well as on several grant review panels. In addition, he was appointed as USA Bentham Ambassador and Organizing/Leadership Committee Member for many international conferences.

RNA Stabilization via Thio-phosphate and Gene Regulation

Elizabeth Frayne

University of Phoenix, Phoenix, AZ; Frayne Consultants, La Mirada, CA

Abstract

Use of the phosphate analogue, thio-phosphate, during cell culture appears to result in an increase in the cellular energy charge. This analogue reduces RNA turnover by creating phos-phorothioate linkages in RNA that inhibit cellular nucleases, which then allows the accumulation of significant amounts of mRNA and other non-ribosomal RNAs in the cell. Over time, the energy savings from reduced RNA turnover results in an enhanced biosynthetic profile, the result of transcriptional changes rather than differential mRNA stabilization. In E coli, RNA seq studies of the analogue show enhancements in the transcription of genes for RNA processing enzymes, ribosomal proteins, tRNA charging enzymes, translational proteins, as well as outer membrane, plasma membrane, and periplasmic proteins. There are also increases in transcripts for genes involved in the synthesis of amino acids, fatty acids, carbohydrates, cell structures, cofactors, vitamins, and secondary metabolites. None of these changes correlate mRNA stability, which suggests that transcriptional control mechanisms are at play, in response to the presumed increase in cellular energy. Additional evidence comes from changes in cells observed at the protein level, such as in yeast where thio-phosphate enhances total protein secretion and in HEK293 cells where it appears to enhance the expression of neurofilaments. Possible mechanisms are also discussed.

Biography

Elizabeth Frayne, PhD has over 20 yrs of R&D experience, most of this pertaining to the use of phosphate analogues in cell culture. Her consulting company supports the research and production of phosphate analogues. She is also a faculty member with the University of Phoenix where she has received some support for her work. She received her BA from UCSB, PhD from Baylor College of Medicine in Texas, and was a postdoctoral fellow at the University of Switzerland in Basel as well as UC Irvine.

Elucidating XRN2-mediated DNA Repair in Glioblastoma Multiforme

Tuyen T. Dang* and Julio C. Morales

Department of Neurosurgery and Stephenson Cancer Center at OU Health Science Center, Oklahoma City, OK

Abstract

Glioblastoma multiforme (GBM) is a highly aggressive brain cancer. The standard course of treatment is a combination of radiation and chemotherapy both of which induces DNA breaks. Even with the dual treatment, the rate of patients with GBM is between 4-7%. Therefore, there is a need to develop novel therapies. A possible cause of the low survival rate is the presence of neoplastic cells with efficient DNA repair abilities, which can grow unchecked leading to lethal secondary tumors.

XRN2 is upregulated in GBMs as compared to normal brain tissue. XRN2 is a 5′-3′ exonuclease that resolve R loops that arise during transcription. R-loop biology can affect gene expression. Preliminary data have shown that loss of XRN2 sensitizes cells to a variety of DNA damaging agents. Additionally, XRN2 is required for DNA double stranded break repair.

To understand how XRN2 modulates DNA repair, we conducted RNA-Seq analyses of two GBM cell lines with and without XRN2 expression and found that XRN2 can regulate genes involved in DNA repair. We have conducted a mini-cherry picked screen of the XRN2 targets and found at least 6 genes to be required for DNA double stranded break repair. A subset of the 6 genes were found to be sensitive to DNA damage agents.

Our goal is to develop a patient signature that can better predict patient outcome and if possible a new synergetic treatment plan to increase the efficacy of radio-therapies.

Biography

Tuyen Dang received her Bachelor's degree from Oregon State University, USA in Biochemistry/Biophysics. Her PhD in Cancer Biology is from UT Southwestern Medical Center, USA. Her doctoral mentor was Dr. Gray W. Pearson and her research topic was studying breast cancer cell invasion. Her current post-doctoral mentor is Dr. Julio C. Morales and her research topic is studying XRN2's mechanism of DNA repair.

Novel Mechanism of Manganese Homeostasis Regulation

Ningning Zhao*, Ivo F. Scheiber, Yuze Wu and Shannon E. Morgan

Department of Nutritional Sciences, The University of Arizona, Tucson, AZ

Abstract

Manganese transporters play important role in regulating manganese homeostasis. As a newly identified manganese importer, ZIP14 is abundantly expressed in the liver and small intestine, the two major organs involved in the control of manganese metabolism. Patients with loss-of-function mutations in ZIP14 developed severe childhood-onset neurological disorder due to manganese hyper-accumulation in the brain; similarly, mice with whole-body Zip14 knockout displayed manganese loading in the blood and brain, indicating an indispensable role for ZIP14 in maintaining systemic Mn homeostasis. Through the deletion of ZIP14 in enterocytes, we have identified ZIP14 as the major transporter mediating basolateral manganese uptake. Lack of ZIP14 severely impaired basolateral-to-apical manganese transport, but strongly enhanced manganese transport in the apical-to-basolateral direction. Mechanistic studies demonstrated that ZIP14 limits manganese absorption via direct reuptake of freshly absorbed manganese. we propose a novel model for the control of systemic manganese homeostasis by ZIP14 that takes into account both manganese absorption by enterocytes and manganese clearance from the portal blood by hepatocytes.

Biography

Ningning Zhao received his Ph.D. in Nutritional Science from the University of Florida. His postdoctoral training at Oregon Health & Science University was focused on molecular cell biology of metal metabolism. The research in his lab has been focused on examining the basic cell biology of membrane proteins involved in metal metabolism and investigating the role of these proteins in human diseases including hereditary hemochromatosis, cancer, and metal-related neurodegeneration.

Diagnostic Exploitation of the Circulating Rare Cell Population. Systemic Cytology for Systemic Pathologies

Stefan Schreier[1] and Wannapong Triampo[2]

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[2]*Thailand Center of Excellence in Physics, Ministry of Higher Education, Science, Research and Innovation, Thailand*

Abstract

Cell-based liquid biopsy (cbLB) has never lost its innovative potential yet, remains limited to biomarker applications of prediction and prognosis in the late stage cancer setting. We are challenging this stagnation in and limitation to cancer, respectively by moving cbLB to the next level herein, referred to as systemic fluorescence cytology. Key to the advancement is the blood circulating rare cell population (RCP) comprising various bone-marrow and tissue-derived progenitor-, stem-, and somatic cells. The RCP inherits information about ones general health status presenting comprehensive cellular evidence of certain pathologies at the systemic level. We have developed and using a specialized rare cell population detection platform comprising steps of unmasking desired cells and cytological analysis based on fluorescence microscopy. Prove of concept tests confirmed distinction between healthy and various diseased individuals and showed individual rare cell population profiles being positively correlated with disease severity. The read-out of the CRP provides sufficient cellular evidence of damage, repair, maintenance or even malignancy to move liquid biopsy from prediction to confirmation. Therefore, we entertain the thought that systemic fluorescence cytology is a superior fit to systemic pathology diagnostic care.

Biography

Stefan Schreier is currently a lecturer and researcher at Mahidol University and internationalstart-up entrepreneur dedicated to advance liquid biopsy in the academic and commercial sector. He graduated from Munich University of Applied Sciences in Bionengineering with a German Diploma degree in 2008 then, continued education a year later to a doctoral degree at Mahidol University, Thailand in pathobiology and graduated in 2012. After graduation, Stefan worked in a Mahidol university spin-off company as product developer for over 3 years.

Functional Genomics of Cystic Fibrosis: Illuminating Pathways and Therapies

Margarida D. Amaral

BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Portugal

Abstract

Cystic Fibrosis (CF), the most common life-threatening genetic disease in Caucasians, is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene encoding a chloride/bicarbonate channel expressed at the apical membrane of epithelial cells. Despite intensive symptomatic treatments, individuals with CF have progressive lung disease due to major airway mucus obstruction, recurrent bacterial infections and chronic inflammation, conducing to shortened life expectancy.

F508del-CFTR, the most common CF-causing mutation is associated with a traffic defect due to misfolding recognized by the endoplasmic reticulum quality control (ERQC) and thus targeted for degradation. Despite the recent therapeutic successes in rescuing this mutant, a global mechanistic view of this process is still missing.

We have used a functional genomics approach (high-content siRNA screen) for a global characterization of mechanisms and pathways associated with this trafficking defect This consisted in the development of a high-throughput microscopy assay in human bronchial epithelial cells to identify factors that rescue traffic of F508del-CFTR to the cell surface [Botelho et al, Sci Rep 2015]. This pipeline was applied to screen a library of 27,312 siRNAs targeting the druggable genome (~9,000 genes), i.e., about half of the human genome.

Our data show the involvement of a complex network of several cellular functions in the regulation of the F508del-CFTR traffic indicating that there are multiple ways to correct the primary cause of this disease and unravel novel potential drug targets.

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Biography

Margarida D. Amaral is Full Professor of Biochemistry/ Molecular Biology at the Faculty of Sciences, University of Lisboa (Portugal) and Coordinator of BioISI - Biosystems & Integrative Sciences Institute. MDA is alumna of EMBL-European Molecular Biology Laboratory (2008-10;2016) and of IGC - Gulbenkian Institute of Science (1983-93). EMBO member (2014).

The Amaral lab focusses on the molecular and cellular mechanisms of biogenesis, traffic and degradation of normal and mutant protein CFTR, which when mutated causes the genetic disease Cystic Fibrosis (CF). To understand CF mechanisms globally we use transcriptomics, proteomics and functional genomics (functional siRNA screens).

Novel Mechanisms of Post-Translational Regulation of Autophagy

Alexander Agrotis, Niccolo Pengo and Robin Ketteler*

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Abstract

Autophagy is a cellular stress response that is tightly regulated by the controlled activity of AuTophaGy (ATG) genes. A key step in the formation of an autophagosome is the conjugation of LC3/GABARAP proteins to phosphatidyl-ethanolamine on the membrane of autophagosomes to allow cargo selection and fusion with the lysosome. LC3/GABARAPs undergo two processing steps, the proteolytic cleavage of pro-LC3 and the de-lipidation of LC3-PE from autophagosomes, both executed by cysteine proteases of the ATG4 family.

We hypothesized that ATG4B activity is regulated by post-translational modifications. We identified that ULK1 can bind to and phosphorylate ATG4B, leading to phosphorylation of Serine 316 in proximity to the active substrate recognition site. Phosphorylation at this residue results in inhibition of its catalytic activity *in vitro* and *in vivo*. On the other hand, phosphatase PP2A-PP2R3B can remove this inhibitory phosphorylation. We propose that the opposing activities of ULK1-mediated phosphorylation and PP2A-mediated de-phosphorylation provide a phospho-switch that regulates the cellular activity of ATG4B to control LC3 processing and de-lipidation.

Next, we investigated the role of the four ATG4 isoforms (ATG4A-D) with regards to LC3/GABARAP processing. HeLa cells lacking ATG4B exhibit a severe but incomplete defect in LC3/GABARAP processing and autophagy. By further genetic depletion of ATG4 isoforms we uncover that ATG4A, ATG4C and ATGD all contribute to residual proteolytic activity, which is sufficient to enable lipidation of GABARAPL1 on autophagosomes. Furthermore, we demonstrate that protein conjugates tagged with LC3/GABARAP accumulate in ATG4 knockout cells, which constitutes a novel type of ubiquitin-like post-translational modification of proteins.

Biography

Robin Ketteler is Professor of Translational Cell Biology at University College London. After completion of his PhD at the Max-Planck Institute for Immunobiology in Freiburg, he trained as a Postdoc at Massachusetts General Hospital in Boston in the lab of Brian Seed. Since 2009, he is group leader at UCL, studying molecular mechanisms of autophagy and cell signaling. Robin also manages the UCL High-Content High-Throughput Screening facility.

Endocytosis of GM-CSF Receptor β is Essential for Signal Transduction Regulating Mesothelial-Macrophage Transition

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Abstract

During Freund's adjuvant induced inflammation rat mesenteric mesothelial cells transdifferentiate into mesenchymal, macrophage-like cells (EMT type II). They express macrophage markers, pro-inflammatory cytokines (TGF-β, TNFα, IL-6), and specific receptors. When primary mesenteric cultures were treated with GM-CSF and/or TGF-β (*in vitro*), similar phenotypic and molecular changes were observed. It seemed likely that GM-CSF receptor-ligand complex should be internalized to initiate this transition. To follow the intracellular route of GM-CSF receptor β, we co-localized this receptor subunit with various endocytic markers (Cav-1, EEA1, Rab7, Rab11a), and carried out detailed immunocytochemical, statistical and biochemical analyses. Since STAT5 is one of the main downstream element of GM-CSF signaling, we followed the expression level and distribution of the phosphorylated (active) transcription factor. Our results showed that in mesothelial cells GM-CSF receptor β is internalized by caveolae, delivered into early endosomes where the signaling events occur. When dynamin-dependent endocytosis of GM-CSFR β is inhibited by dynasore, the Jak2-mediated tyrosine phosphorylation of STAT5A is not occurred, confirming, that the internalization of receptor β is indispensable for signal transduction. At the early time of inflammation a significant receptor recycling can be found to the plasma membrane. Later (day 8) the receptor β is delivered into late endosomes. After late endosome-lysosome fusion, the receptor is degraded. Since there are no signal transmitters (receptors) on the plasma membrane, the regeneration of mesothelial cells can start. All of these data strongly support that the internalization of GM-CSF receptor β is required for signal transduction in mesothelial cells.

Biography

I was born in Hungary in 1990. I graduated from cell and molecular biology in Eötvös Lóránd University (2009-2015, Budapest). I have recently obtained my PhD degree as cell biologist in Semmelweis University (2015-2019, Budapest). I am working in the Department of Anatomy, Histology and Embryology in Semmelweis University where I research and teach. My main scientic fields are the cell and molecular biology, biochemistry and genetic.

Kinesin-1 Regulates Antigen Cross-presentation through the Scission of Tubulations from Early Endosomes in Dendritic Cells

Gaël Ménasché[1*], Meriem Belabed[1], François-Xavier Mauvais[2], Sophia Maschalidi[1], Mathieu Kurowska[1], Jian-Dong Huang[3], Peter van Endert[2] and Fernando E. Sepulveda[1]

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[2]Université de Paris, INSERM, U1151, Paris, France
[3]School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Abstract

Dendritic cells (DCs) constitute a specialized population of immune cells that among other functions, present exogenous

antigen (Ag) on major histocompatibility complex (MHC) class I molecules to initiate CD8+ T cell responses against pathogens and tumors. Although crosspresentation depends critically on the trafficking of Ag-containing intracellular vesicular compartments, the molecular machinery that regulates vesicular transport is incompletely understood. Here, we demonstrate that mice lacking Kif5b (the heavy chain of kinesin-1) in their DCs exhibit a major impairment in cross-presentation and thus a poor *in vivo* anti-tumour response. We found that kinesin-1 critically regulates antigen cross-presentation in DCs, by controlling Ag degradation, the endosomal pH, and MHC-I recycling. Mechanistically, kinesin-1 appears to regulate early endosome maturation by allowing the scission of endosomal tubulations - an essential step in the maturation of vesicles into recycling endosomes or late endosomes. Our results highlight kinesin-1's newly recognized role as a molecular checkpoint that modulates the balance between antigen degradation and cross-presentation.

Biography

Gaël Ménasché received her Ph.D. from the University Pierre et Marie Curie, Paris (France) in 2004. Then, she performed her postdoctoral training in the Gary Koretzky's lab in Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia (USA). In 2007, she joined INSERM at Imagine institute in Paris. Gaël Ménasché's research has been focusing on molecular mechanisms underlying genetic immune disorders affecting immune homeostasis and the molecular dissection of the cytotoxic activity in lymphocytes through the study of primary hemophagocytic syndromes. This work has led to the identification of several key molecules regulating the trafficking and the docking steps of lytic granules. Based on this expertise, Gael Ménasché has progressively focused her research on regulated secretion processes and vesicular trafficking in immune cells.

Matrix Metalloproteinases in Age-Related Macular Degeneration

Luis García Onrubia

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Abstract

Age-related macular degeneration (AMD) is the leading cause of central vision loss among the elderly in developed countries. AMD is a multifactorial and progressive retinal disease affecting millions of people worldwide. Although the pathogenesis of AMD has not yet been completely unveiled, recent studies have showed that disorders in the regulation of the extracellular matrix (ECM) play an important role in its etiopathogenesis. The dynamic metabolism of the ECM is closely regulated by matrix metalloproteinases (MMPs) and the tissue inhibitors of metalloproteinases (TIMPs). This presentation is focused on what we know so far about the possible role of MMPs and TIMPs in AMD development, and how its knowledge could be of benefit to the management of AMD patients, as a better insight into the pathological role of MMP/TIMP complexes may lead to the development of new strategies for AMD treatment and prevention. This presentation is based on our last published paper in the " The internation Journal of Molecular Sciences" which is entitled "Matrix Metalloproteinases in Age-Related Macular Degeneration (AMD)".

Rescuing Chromatin Bridges from Breaking in Cytokinesis

George Zachos* and Eleni Petsalaki

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Abstract

Chromatin bridges are strands of incompletely segregated chromatin connecting the anaphase poles or daughter nuclei and have been linked with chromosomal instability in human tumors and tumorigenesis in mouse models. In the presence of chromatin bridges in cytokinesis, human cells delay completion of cytokinesis (abscission) and retain accumulations of polymerized actin (actin patches) at the base of the intercellular canal to prevent chromosome breakage. Here, we

describe a novel MRN-ATM-Chk2 signaling pathway that promotes localization of the chromosomal passenger complex (CPC) to the midbody to impose the abscission checkpoint in human cells. We also show that the DNA damage kinase Chk1 phosphorylates the actin remodeling kinase Src at serine-51 to fully induce Src catalytic activity and promote actin patch formation in cytokinesis with chromatin bridges. These results identify mechanisms that protect genome integrity by preventing chromatin bridge breakage in cytokinesis.

Biography

George Zachos completed his PhD at the University of Crete in 1997. He then received postdoctoral training in the Beatson Institute for Cancer Research, Glasgow, U.K. before moving, in 2008, to the Department of Biology, University of Crete, Heraklion, Greece as an Assistant Professor in Cell Biology. In 2015, he became Associate Professor and continues to hold this position today. Discoveries from the Zachos lab have identified mechanisms that regulate the fidelity of chromosome segregation in mitotic cell division in higher eukaryotic cells. He has published 37 papers in leading scientific journals and his work has received >2,000 citations.

Physical Basis of Receptor Tyrosine Kinase Signaling

Kalina Hristova

Materials Science and Engineering and Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD

Abstract

I will discuss the transition model of receptor tyrosine kinase (RTK) activation, which is derived from biophysical investigations of RTK interactions and signaling. The model postulates that (1) RTKs can interact laterally to form dimers even in the absence of ligand, (2) different unliganded RTK dimers have different stabilities, (3) ligand binding stabilizes the RTK dimers, and (4) ligand binding causes structural changes in the RTK dimer. The model is grounded in the principles of physical chemistry and provides a framework to understand RTK activity and to make predictions in quantitative terms. It can guide basic research aimed at uncovering the mechanism of RTK activation and, in the long run, can empower the search for modulators of RTK function.

Biography

Kalina Hristova received her Ph.D. degree in Mechanical Engineering and Materials Science from Duke University, USA. She is a Professor of Materials Science and Engineering at the Institute for NanoBioTechnology at Johns Hopkins University. Dr. Hristova is the recipient of the 2007 Margaret Oakley Dayhoff award from the Biophysical Society. She was elected Fellow of the American Physical Society in 2016, and Fellow of the American Institute for Medical and Biological Engineering in 2018. The main focus of the research in her laboratory is the physical principles that underlie membrane protein folding and signal transduction across biological membranes.

How Vesicles Find their Target

Peter Novick* and Xia Li

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Abstract

To test the role of Rabs in defining the directionality of transport we have redirected the GEF for an exocytic Rab onto endocytic vesicles. Vps9 is a GEF for the Rab5 homolog, Ypt51. It is recruited to endocytic vesicles through the interaction of its CUE domain with ubiquitinated endocytic cargo. Sec2 is a GEF for the exocytic Rab Sec4 and it is normally recruited to exocytic secretory vesicles. We have fused the GEF domain of Sec2 to the CUE domain of Vps9. While a variant with a CUE mutation that blocks ubiquitin binding remains cytosolic, Sec21-160-GFP-CUE is recruited to puncta, often at bud

tips. These colocalize with Sec4, as well as exocyst subunits Sec8 and Sec15. Partial co-localization (30%) is also observed with an endosome marker, Vps8. Thus, we have formed a compartment with mixed identity, having markers for both post-Golgi exocytic vesicles and endosomes. To address the role of Rabs in controlling the directionality of transport we have followed the internalization of a methionine permease, Mup1. Mup1 resides at the plasma membrane in the absence of methionine, but is rapidly internalized upon methionine addition, first to endosomes and then to the vacuole. In cells expressing Sec21-160-GFP-CUE, the delivery of Mup1 to the vacuole is delayed with transient co-localization with Sec21-160-GFP-CUE. We can directly visualize the dynamics through time lapse microscopy as Mup1 is internalized, delivered to Sec2-CUE puncta and to the vacuole. These results are consistent with redirection of endocytic vesicles through the ectopic acquisition of an exocytic Rab.

Biography

Peter Novick is a Distinguished Professor in the Department of Cellular and Molecular Medicine and holder of the George Palade endowed chair. He is a member of the National Academy of Sciences, the American Academy of Arts and Sciences and the American Association for the Advancement of Science.

Expected Ratio of Types of Founders' mtDNA to Surrounding Populations' mtDNA

Joseph Livni[1*] and Karl Skorecki[2]

[1]Independent Researcher, Woburn, MA
[2]Azrieli Faculty of Medicine at Bar Ilan University, Israel

Abstract

By definition, the size of the founding generation of founder populations is small. For example two independent and dissimilar genetic research studies of Ashkenazi Jews arrived at a founder generation size of approximately 150 families. The findings of one of these studies show that only 1/3 of the first generation mtDNA signatures survived. Naturally, founder populations living in the midst of neighbouring populations absorbed surrounding mtDNA types. This investigation will show that even negligible admixture ratios produce an unusually high ratio of surrounding mtDNA to founder mtDNA.

Not surprisingly, another investigation indeed reported that the a large proportion of current Ashkenazi Jews carry mtDNA of remote European ancestry; based on this finding the investigators concluded that the female founders were Europeans; the investigators do agree with that previous studies indicating that the Y-chromosome analyses correctly established that the male founders were Middle Easterners.

The main reason that triggered the suggestion of European maternal origin was the aforementioned high European mtDNA proportion. Further calculations of the results explain why even if both genders were Middle-Easterners one should obtain the same high European mtDNA proportion. A common origin of both genders is more credible than a model consisting of 150 families of Middle-Eastern fathers and European mothers.

Therefore the current study continued to assess the likelihood of Middle Eastern founder fathers and European founder mothers of current Ashkenazi Jews. The results show that this scenario is practically impossible.

Biography

Joseph Livni B. Sc. 1972, Technion, Israel, M. Sc. 1986, University of Tel-Aviv Israel. 1972-2010 Mathematical modelling in Aerospace Industry, IAI Israel and Bombardier, Montreal, Canada; 2010-2019 Scientific Research Omega-n, Aviation, Science & Art Inc. Montreal, Canada; 2019 independent researcher Woburn, MA, USA. Relevant research interest: lineage extinction theory, founder populations. Relevant publication: Livni H, Livni J: Interpretation of findings of founder population genetics studies applying lineage extinction theory. Physica A: Statistical Mechanics and its Applications 2016, 462:641-653.

Unlocking the Potential of Stem Cells to Model Airway Diseases

Hongmei Mou

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Abstract

Stem cells possess the ability to self-renew and to give rise to multiple types of differentiated progenies, presenting an opportunity to durably repopulate damaged tissues. Airway basal cells, characterized by the expression of p63 and cytokeratin 5, and named for their position on the basement membrane of the airway, function as bona fide stem cells for the airway epithelium. There are two potential resources of patient-specific and disease-specific human airway stem cell populations to produce sufficient functional airway epithelium. The first one is generated from induced pluripotent stem cells (iPSC). The second one is the primary stem cells cultured from human airway. Here, I will address the advantages and limitations of these two cell resources and also discuss their potential applications in stem cell biology study, airway disease modeling and the future regenerative medicine.

References

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[3] Lu J, et al., (2020). Rho/SMAD/mTOR triple inhibition enables long-term expansion of human neonatal tracheal aspirate-derived airway basal cell-like cells. Pediatr Res.

Cell Membrane Transmits High-Level Integrin Tensions for Rear De-Adhesion During Rapid Cell Migration

Xuefeng Wang[1,2*], Yuanchang Zhao[1], Yongliang Wang[1] and Anwesha Sarkar[1]

[1]Department of Physics and Astronomy, Iowa State University, Ames, IA
[2]Molecular, Cellular, and Developmental Biology interdepartmental program, Iowa State University, Ames, IA

Abstract

Integrin-transmitted cellular forces play critical roles in the migration of many eukaryotic cells. However, these forces have not been calibrated or mapped at the molecular tension level in migrating cells. The range, distribution, force source and biological function of integrin molecular tensions during cell migration remain unclear. Here we developed integrative tension sensor (ITS) which converts molecular tensions to fluorescent signals, therefore enabling cellular force mapping by fluorescence imaging with high resolution and sensitivity. Using ITS, we calibrated and mapped integrin tensions in fish epidermal keratocytes, the classic cell model for migration study, with piconewton (pN) sensitivity and 0.4 μm resolution. We discovered that keratocytes generate high-level integrin tensions in a range of 50~100 pN exclusively at the cell rear margin to rupture integrin-ligand bonds and detach rear adhesion sites during cell migration. We further demonstrated that these tensions are transmitted by cell membrane instead of actomyosin which is the common force source for integrin tensions in less motile cells, revealing that cell membrane can supersede actomyosin to produce high-level integrin tensions to mediate rear de-adhesion and facilitate cell migration.

Biography

Xuefeng Wang is an assistant professor in Biophysics at Iowa State University (ISU), where his lab studies cell mechanobiology with molecular tension sensors that visualize and map cellular forces by fluorescence. Before joining ISU, Xuefeng obtained Ph.D. in physics at Purdue University and had postdoctoral training at University of Illinois at Urbana-Champaign. There he received training in optics, biophysics and single molecule imaging. In past research, he invented picometrology which

calibrates ultrathin film with picometer sensitivity. He also developed tension gauge tether, a linker that quantitatively and globally knocks down molecular tensions transmitted by mechanosensitive receptors on cell membrane.

RNA Binding Protein SRSF3 is Required for Cardiac Integrity Preservation

Mannix Auger-Messier[1*], Audrey-Ann Dumont[1], Lauralyne Dumont[1], Delong Zhou[2], Hugo Giguère[1], Denis Blondin[3] and Michelle Scott[2]

[1]Department of Medicine, Université de Sherbrooke, Québec, Canada
[2]Department of Biochemistry and Functional Genomics, Université de Sherbrooke, Québec, Canada
[3]Département de Pharmacologie et Physiologie from the Centre de Recherche du CHUS, Université de Sherbrooke, Québec, Canada

Abstract

Background and hypothesis - The APEX2-based proximity assay is a powerful method to resolve the spatiotemporally dynamic interactome of protein such as kinases in living cells. With this exquisite approach, we recently demonstrated that mitogen-activated protein kinase p38alpha closely interacts with the RNA binding protein Srsf3 in primary cultured neonatal cardiomyocytes. Considering how important the Srsf1 and Srsf2, other SR proteins with non-redundant functions, are for the maintenance of cardiac integrity, we further investigated the *in vivo* role of Srsf3 in developing cardiomyocytes.

Experimental design and results – The conditional invalidation of Srsf3 gene in cardiomyocytes during development with the β-MHC-Cre transgenic mouse led to slightly reduced mendelian ratio at birth and poor survival within the first 1 month. Echocardiographic measurements from 15-day-old mice revealed the potent reduction of cardiac systolic function in homozygous Srsf3-floxed/Cre mice. Analysis of RNA-Seq results indicated that the mRNAs abundance and splicing events detected from neonatal cardiomyocyte-specific Srsf3-deficient hearts were modified differently than those previously reported following Srsf3 conditional KO from cardiomyocytes at the adulthood. Interestingly, comprehensive analysis of the differentially regulated mRNAs from neonatal hearts with the web-based portal Metascape indicated that the oxidative phosphorylation was amongst the most highly enriched biological pathway to be affected. This finding was further corroborated by mitochondrial DNA content determination, Western blot analysis, and quantitative functional mitochondrial measurements.

Conclusion – Taken together, these results indicate that the loss of SRSF3 in cardiomyocytes affects mitochondrial integrity, an important finding that might impact other crucial physiological processes beyond the cardiac research field.

Biography

Professor Auger-Messier is a molecular cardiovascular biologist and pharmacologist at the Université de Sherbrooke. His research program aims to delineate cell signaling mechanisms participating in cardiac physiology and disease processes. His laboratory exploits a wide range of approaches from molecular pharmacology to physiopathology studies of the heart in genetically modified mouse models. Ongoing studies in his laboratory focus on elucidating the mechanisms of action, amongst others, of p38 MAPK and APJ receptor signaling in the heart. The Auger-Messier laboratory is funded by the Natural Sciences and Engineering Research Council of Canada and the Canadian Institutes of Health Research.

Evolution of Life on Earth: tRNA, Aminoacyl-tRNA Synthetase and Genetic Code Evolution

Zachary Burton[1*] and Lei Lei[2]

[1]Department of Biochemistry and Molecular Biology, Michigan State University, E. Lansing, MI
[2]Department of Biology, University of New England, Biddeford, ME

Abstract

A selection and history are apparent for the placements of every amino acid in the standard genetic code. The genetic

code evolved by parallel tracks of chaotic and highly ordered processes. Liquid-liquid phase separation (hydrogels), a chaotic process, constructs membraneless compartments within cells, resulting in regulated hydration and sequestration and concentration of reaction components. Hydrogels relate to chaotic amyloid fiber production. At the inception of genetic code evolution, polyglycine and GADV polymers generated hydrogels and amyloids that supported diverse protocell chemistries. tRNA evolved by ligation of 3-31-nt minihelices of highly regular (repeats and inverted repeats) and known sequence, followed by 9-nt internal deletions. Aminoacyl-tRNA synthetases (aaRS; i.e. GlyRS-IIA) diverged from a GlyRS-IIA root. Although aaRS class I and II protein folds are distinct, GlyRS-IIA is a sequence homolog of ValRS-IA and IleRS-IA. The pattern of divergence of aaRS enzymes gives the pattern of genetic code evolution. The genetic code evolved along genetic code columns (2nd anticodon position) and filled by genetic code rows (3rd anticodon position). Models are apparent for evolution of stop codons, 6-codon sectors (Leu, Ser, Arg) and third column innovation (Glu, Asp, Lys, Asn, Gln, His, STOP, Tyr). EF-Tu evolution correlates with expansion of the code from an 8 aa bottleneck to the standard code, by suppression of wobbling at the 3rd anticodon position. EF-Tu was necessary to fill row 1 of the genetic code (3rd anticodon position A).

Biography

Burton received his Ph.D. from UCLA, in the laboratory of David Eisenberg, in 1980. He was a postdoc with Richard Burgess at the University of Wisconsin, Madison from 1980-1983. He did a second postdoc with Jack Greenblatt at the University of Toronto, from 1983 to 1987. He took a faculty position at Michigan State University, Department of Biochemistry and Molecular Biology, where he taught and did research for 30 years. He is now retired. Research work was on transcriptional mechanisms and ancient evolution of transcription and translation systems.

Early Growth Response 1 (Egr1) Coordinates Metabolic and Circadian Regulation of Adipose Functions

Konstantin Kandror

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Abstract

Adipose tissue plays the central role in metabolic health and homeostasis. In particular, it has been established that abnormal metabolic or circadian regulation of such fat-specific responses as lipolysis and/or leptin production lead to metabolic disease. We have found that transcription factor Egr1 plays the central role in the regulation of both lipolysis and leptin production. Egr1 is strongly but transiently induced in adipocytes by insulin and nutrients and directly interacts with the promoter of the rate-limiting lipolytic enzyme, ATGL, and with the leptin promoter suppressing the former and activating the latter. This leads to down-regulation of lipolysis and up-regulation of leptin expression. Expression Egr1 in adipocytes is not only regulated by nutrients and insulin but also, undergoes cell autonomous oscillations both *in vitro* and *in vivo* and may be responsible for the circadian changes in lipolysis and leptin expression.

A FAK/HDAC5 Signaling Network Controls Osteocyte Mechanotransduction

Marc N. Wein* and Tadatoshi Sato

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Abstract

Osteocytes, cells ensconced within mineralized bone matrix, are the primary skeletal mechanosensors. Osteocytes sense mechanical cues by changes in fluid flow shear stress (FFSS) across their dendritic projections. Loading-induced reductions of osteocytic Sclerostin (encoded by Sost) expression stimulates new bone formation. However, the molecular steps linking mechanotransduction and Sost suppression remain unknown. Here, we report that class IIa histone deacetylases

(HDAC4 and HDAC5) are required for loading-induced Sost suppression and bone formation. FFSS signaling drives class IIa HDAC nuclear translocation through a signaling pathway involving direct HDAC5 tyrosine 642 phosphorylation by focal adhesion kinase (FAK), a HDAC5 post-translational modification that controls its subcellular localization. Osteocyte cell adhesion supports FAK tyrosine phosphorylation, and FFSS triggers FAK dephosphorylation. Pharmacologic FAK catalytic inhibition reduces Sost mRNA expression *in vitro* and *in vivo*. These studies demonstrate a role for HDAC5 as a transducer of matrix-derived cues to regulate cell type-specific gene expression.

Biography

Marc Wein is an Assistant Professor of Medicine at Harvard Medical School and an Associate Member of the Broad Institute. Marc received his B.S. and M.S. from Yale University, M.D. and Ph.D. from Harvard Medical School, and was trained in Internal Medicine and Endocrinology at Massachusetts General Hospital where he currently runs his research laboratory and clinical practice. He has received research funding and awards from the NIH, American Society of Clinical Investigation, Harrington Discovery Institute, Endocrine Society, Stepping Strong Center for Trauma Innovation, and American Society of Bone and Mineral Research.

AP-2b/KCTD1 are Critical Regulators of Distal Nephron Differentiation and Function

Alexander G. Marneros

Harvard Medical School, Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, MA

Abstract

Human genetic studies identified mutations in the transcriptional regulator KCTD1 in Scalp-Ear-Nipple (SEN) syndrome, which manifests with multiple ectodermal abnormalities, but the physiological and developmental functions of KCTD1 remain unknown. By generating KCTD1 mutant mice we identified a critical role of KCTD1 for distal nephron development. We find that the transcription factor AP-2b is required for the formation of early-stage distal convoluted tubules (DCT) of the distal nephron. Subsequently, AP-2b induces the expression of KCTD1 in the distal nephron to promote terminal differentiation of early stage DCTs into fully differentiated DCTs. Lack of KCTD1 leads to immature DCTs, which leads to a severe salt-losing tubulopathy. Moreover, AP2-b/KCTD1 activity is required to maintain DCTs in their terminal differentiation state in the adult. The terminal differentiation defect of DCTs due to KCTD1 deficiency leads with age progression to b-catenin hyperactivation in DCTs and renal fibrosis, which can be ameliorated by targeting b-catenin. Notably, a subset of SEN syndrome patients with KCTD1 mutations also develop progressive renal fibrosis and kidney failure, resembling the abnormalities observed in KCTD1 mutant mice. Collectively, these findings demonstrate that the AP-2b/KCTD1 axis is critical for the development and postnatal maintenance of distal nephron functions.

Biography

Alexander G. Marneros is an Associate Professor of Dermatology at Harvard Medical School and a principal investigator at the Cutaneous Biology Research Center of Massachusetts General Hospital in Boston.

Tubulogenic Growth by Ribosomal Regulation in the Embryo

Rajprasad Loganathan*, Michael B. Wells and Deborah J. Andrew

Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD

Abstract

The basic architecture of a multitude of metazoan organs is tubular. Tubular architecture forms the basis for fluid secretion, absorption, storage, exchange, and transport. The functional efficacy of tubular tissues is largely determined by factors affecting their characteristic sizes and shapes. Although morphogenetic studies in the embryo have provided profound

insights into the molecular mechanisms of tube shape determination, little is known on how the size of tubular tissues is determined. Using the Drosophila embryonic salivary gland as a model organ for tubulogenesis, we have uncovered the dynamics of a mechanism pivoting on the transcriptional regulation of ribosomal protein genes to boost early (embryonic) tubulogenic growth. Ribbon, a BTB-domain containing transcription factor, boosts the expression of ribosomal protein genes to drive non-proliferative growth of the embryonic salivary gland. Interestingly, the tubulogenic growth-boosting mechanism of Ribbon is tissue-specific as it targets non-ribosomal genes in the embryonic trachea, yet another model tubular organ. In the salivary gland, however, which assembles some of the largest cells in the embryo, Ribbon likely interacts with known ribosomal protein gene transcription activators to coordinately upregulate ribosomal protein gene transcription, thus, allowing cell volume gain and the resultant tube elongation.

Biography

Raj Loganathan studies embryonic tubulogenesis in the Department of Cell Biology at the Johns Hopkins School of Medicine. He is a research associate in the Andrew lab. He attended the Madras Medical College for undergraduate training and the University of Kansas for graduate training. He received postdoctoral training at the Johns Hopkins School of Medicine.

Dysregulation of Cell Type-Specific Membrane Protein Complexes in the Pathogenesis of Ichthyosis and Epidermodysplasia Verruciformis

Chuanjin Wu[1*], Mark C. Udey[2] and Lawrence E. Samelson[3]

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Abstract

Mutations of matriptase or its inhibitor HAI-1 have been associated with ichthyosis. Biallelic mutations in either TMC6 or TMC8 are detected in 60-70% cases of epidermodysplasia verruciformis (EV), which results from abnormal susceptivity to b-HPV. How genetic alterations lead to these skin diseases is unknown or controversial. We identified that the membrane protein homologs EpCAM and TROP2 are both novel substrates of matriptase. We determined that co-expressed EpCAM and TROP2 play a redundant role in stabilizing their associated claudins in keratinocytes. Simultaneous inhibition of HAI-1 and HAI-2 led to nearly complete cleavage of EpCAM and TROP2, dissociation of EpCAM/TROP2 and claudins, and robust downregulation of these proteins in keratinocytes. We have identified a pathway in keratinocytes that involves HAI/matriptase regulation of membrane complex formation and stability of EpCAM/TROP2 and claudins. Our study may provide molecular basis for explaining why mutations in matriptase and HAI-1 cause ichthyosis and offer clues for a better understanding of these proteins in cancer. In contrast to matriptase, transmembrane homologs TMC6 and TMC8 are most abundant in lymphocytes. Biochemical studies demonstrated that TMC6, TMC8 and CIB1 heterotrimerize. TMC6-TMC8-CIB1 trimer formation stabilized each component in T cells. TMC6 and TMC8 levels were drastically lower and markedly less active in regulating CIB1 in keratinocytes. The identification of TMC6-TMC8-CIB1 complexes and their mutual regulation may provide molecular basis to explaining the identical disease presentations of patients harboring TMC6, TMC8 or CIB1 mutations and help to resolve the controversy regarding whether TMC6/TMC8 mutation-associated EV originates from keratinocyte or lymphocyte defects.

Biography

Chuanjin Wu is currently a Staff Scientist in Laboratory of Immune Cell Biology, National Cancer Institute, NIH. Prior to this, he had been a Staff Scientist in NCI Dermatology Branch and Laboratory of Cellular and Molecular Biology since 2009. Dr. Wu is interested in studying molecular and cellular bases for diseases, including those caused by genetic alterations. He has been investigaing immune regulation- and tumorigenesis-related signal transduction, protein ubiquitination and epithelial biology and found that NEMO sensing of linear and K63-linked polyubiquitin chains is critical for IKK and NF- κB activation. His present work is focused on T cell receptor signaling.

The Role of Gastrin and the ECL Cell in Gastric Carcinogenesis. Implications for Prophylaxis and Treatment

Helge Waldum

Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Norway

Abstract

Although the prevalence of gastric cancer is in decline, due to high mortality gastric cancer is still an important type of cancer. Gastric cancer was early associated to reduced gastric acidity, later to gastritis, and after the recognition of Helicobacter pylori as the principal cause of gastritis.

Helicobacter pylori was accepted as the major cause of gastric cancer. The three above mentioned conditions give reduced gastric acidity and secondary increase in gastrin. Gastrin is the most important regulator of gastric acidity by stimulating the ECL cell to histamine release and proliferation. Histamine in turn stimulates the acid secretion.

Hypergastrinemia leads to ECL cell hyperplasia and in long term to neoplasia in all species examined, including man. By using methods with improved sensitivity, it became evident that many of the gastric cancer cells express neuroendocrine, and more specifically ECL cell markers, indicating that the cancers should be reclassified from adenocarcinomas to neuroendocrine carcinomas. Helicobacter pylori predisposes to gastric cancer only after having induced oxyntic atrophic gastritis suggesting that the carcinogenic effect is due to hypergastrinemia. Thus, gastric acidity should be reduced as little as possible using the less potent inhibitors of acid secretion, and persons with Helicobacter pylori infection should be treated early before development of oxyntic atrophy.

Biography

Helge Waldum MD at Oslo University, second-best results ever (reported to the King). Speciality in Internal Medicine and Gastroenterology and Hepatology 1980. Theses: "Studies on Group I pepsinogens and secretin" Tromsø 1980 and : "La cellule ECL, une cellule clé dans la muqueuse gastric acide" Paris 1993. Head of Department of Gastroenterology and Hepatology, Trondheim University Hospital for 20 years. Supervised 20 candidates for PhD. More than 400 publications. Recently published a book: "The influence of the pharmaceutical industry on medicine, as exemplified by proton pump inhibitors". Ten years as Editor (eight as Editor-in-Chief) in Scandinavian Journal of Gastroenterology.

Epithelial to Mesenchymal Transition (EMT) in Head and Neck Cancer

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Abstract

Epithelial to mesenchymal transition (EMT) is a reversible process of a phenotype change in epithelial cells to gain an additional mesenchymal phenotype, or for complete transformation from epithelial to mesenchymal path. EMT is a major contributor of therapy resistance and recurrent disease in head and neck cancer. EMT is a complex regulatory process, which we divide into two major components: first: a tumor cell internal component, which might be a stress management mechanism of epithelial tumor cells responding to a hostile microenvironment, metabolic stress, hypoxia, chemotherapeutic treatments or ionizing radiation. The second component is the effect of growth factors produced by the

tumor microenvironment as TGF-beta1 or IL-6, which can induce EMT in the border of cancer cell nests. We also observed a gradient reduction of EMT-related transcription factor Slug from the border of cancer cell nests towards the center of the nest. At the same time, an epithelial related transcription factor increased in the opposite direction. In our laboratory, we developed methods to identify EMT cells and to quantify their epithelial and mesenchymal gene expression in cancer tissue and in an experimental work. We defined EMT-related signal transduction mechanisms that contain targetable elements, which will be instrumental of therapy development.

Biography

My name is Julia Ingruber (Date of Birth: 23.02.1991), I live in Austria/Tyrol and work at the Medical University Innsbruck at the department of Otorhinolaryngology in the Molecular-Biology Tumor Group Lab. I join the PhD Programm in Biology, Division of Molecular Biology at the Leopold-Franzens-University Innsbruck.

Occupational Experience: Scientific Assistant in Dept. Otorhinolaryngology-Molecular Oncology, Medical University Innsbruck; Practices at the department of Biomedical Aging Research and Molecular Biology; Lecture in Biology, BFI Tyrol;

Main scientific interests: Clinical Cancer Research, Epithelial-mesenchymal Transition, Tumor Immunology, Tumor Microenvironment, Inflammatory cytokines, Head and Neck Oncology, Tumor markers, Fibroblasts and Mesenchymal Cells, Cancer Stem Cells.

The Nuclear Translocation of MAPKs as a Therapeutic Target for Cancer and Inflammation

Rony Seger

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Abstract

A hallmark of MAPK signaling is their nuclear translocation upon stimulation, which is necessary for their physiological/pathological functions. We have identified two novel, distinct, regulated nuclear translocation mechanisms for ERK1/2 and JNK/p38, of which we made use of as a promising therapeutic approach. We developed a myristoylated, NTS-derived phosphomimetic peptide (EPE peptide), which blocked ERK1/2 nuclear translocation. In culture, the EPE peptide induced apoptosis of melanoma cells, inhibited the proliferation of other cancer cells but had no effect on immortalized cells. In xenograft models, the peptide was significantly more effective than BRAF inhibitors in preventing tumor recurrence of treatment-eradicated melanoma xenografts. We also developed p38-derived myristoylated peptide, termed PERY peptide, which inhibited the importin interaction with JNK1/2 and p38α/β and prevented their nuclear translocation. This peptide affected viability of several cancer-derived cell lines, and significantly reduced inflammation and intestinal damage in a mouse model of colitis. Moreover, the peptide inhibited inflammation-induced colorectal cancer in an AOM/DSS mouse model. Taken together, both the cancer and inflammatory models support the use of nuclear translocation of MAPKs as a novel drug target for signaling-related diseases.

Biography

Prof. Rony Seger became a group leader in the Weizmann Institute of Science in 1994 promoted to full professor full professor in 2007, and was the head of the department of Biological Regulation (2011-2017). His research group is interested in MAPK and AKT signaling, and in particular in the subcellular localization of their components. Recently, the group elucidated the distinct mechanisms of nuclear translocation of ERK and p38/JNK, which are used as anti-cancer and inflammation targets. Dr. Seger published more than 220 papers, supervised more than 80 research students and post-docs, and received many prizes and awards.

Deficiency of GABARAP but Not Its Paralogs Causes Enhanced EGF-Induced EGFR Degradation

Jochen Dobner[1*], Indra M. Simons[1,2], Kerstin Rufinatscha[3], Sebastian Hänsch[4], Melanie Schwarten[2], Oliver H. Weiergräber[2], Iman Abdollazahdeh[5], Thomas Gensch[5], Johannes G. Bode[3], Silke Hoffmann[2] and Dieter Willbold[1,2]

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[5]Institute of Biological Information Processing: Molecular and Cell Physiology (IBI-1), Forschungszentrum Jülich, Germany

Abstract

The γ-aminobutyric acid type A receptor-associated protein (GABARAP) and its paralogs GABARAPL1 and GABARAPL2 form a subfamily of human autophagy-related 8 (ATG8) proteins. Although they have been mainly characterized for their role during autophagy, they are associated with a plethora of membranes of both autophagic and non-autophagic origin. By acting as adaptors, tethers and adhesion factors, they shape membranes in both autophagy-related and autophagy-unrelated processes. We describe the role of GABARAP during intracellular trafficking of the epidermal growth factor (EGF) receptor (EGFR). We show that deficiency of GABARAP alone, but none of its paralogs, sufficiently results in accelerated degradation of ligand-activated EGFR in two independent cell types. This decrease in total EGFR levels over time is accompanied by reduction of EGF uptake, altered characteristics of EGF-containing vesicles, and translates into reduction of downstream MAPK signaling and target gene expression. By employing a knock-in cell line of endogenously fluorescence protein tagged GABARAP, we demonstrate transient comigration of GABARAP and EGF in living cells. Furthermore, GABARAP associates with EGFR during co-immunoprecipitation experiments and binds to synthetic peptides derived from the regulatory tail of the EGFR. In summary, our data strongly indicates a unique and novel role for GABARAP during intracellular EGFR trafficking.

Biography

Born in south-west Germany, Jochen Dobner studied biology (B Sc) and molecular cell and developmental biology (M Sc) at the Leopold-Franzens-University Innsbruck, Austria. After completing the master studies in the field of nutritional biochemistry, he performed his PhD project in the lab of Prof. Dieter Willbold as a stipendiary of the Molecules of Infection (MOI) graduate school. Now, as a member of the Collaborative Research Centre 1208 (CRC1208) at the Heinrich-Heine-University Düsseldorf, Germany, he intensifies his studies on deciphering the roles of the versatile GABARAP subfamily proteins in processes beyond autophagy.

Pro-survival Bcl-2 Proteins Suppress Beclin 1/Atg6-mediated Lethal Autophagy in Polyploid Cells

Jing Zhang[*], Shenqiu Zhang, Qiong Shi, Thaddeus D. Allen and Dun Yang

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Abstract

Inhibition of Aurora-B kinase is a synthetic lethal therapy for tumors that overexpress the MYC oncoprotein. It is currently unclear whether co-occurring oncogenic alterations might influence this synthetic lethality by conferring more or less potency in the killing of tumor cells. To identify such modifiers, isogenic cell lines were utilized to test a variety of cancer genes that have been previously demonstrated to promote survival under conditions of cellular stress, contribute to chemoresistance and/or suppress MYC-primed apoptosis. It was found that Bcl-2 and Bcl-xL, two antiapoptotic members of the Bcl-2 family, can partially suppress the synthetic lethality, but not multinucleation, elicited by a pan-aurora kinase inhibitor, VX-680. Suppression required localization of Bcl2 and Bcl-xL to the endoplasmic reticulum, and could be attributed to the inhibition of autophagy, specifically in multinucleated cells, rather than inhibition of apoptosis. The anti-autophagic

activity of Bcl-2 and Bcl-xL also markedly enhanced polyploid cell recovery in colony-forming assays, suggesting a route of escape from MYC-VX-680 synthetic lethality that may lead to drug resistance and tumor relapse in clinicals. These findings expand on previous conclusions that autophagic death of VX-680-induced polyploid cells is mediated by Atg6. Bcl-2 and Bcl-xL negatively modulate MYC-VX-680 synthetic lethality and it is the anti-autophagic activity of these two Bcl-2 family proteins, specifically in multinucleate cells, that contributes to resistance to Aurora kinase-targeting drugs.

Biography

Dr. Jing Zhang joined the J. Michael Bishop Institute of Cancer Research (MBICR) since its foundation in 2016, after her doctoral training at the University of Nottingham and post-doctoral training at the University of St Andrews in the UK. Her research at MBICR focuses on high-throughput/content screening for small molecule compounds that have synthetic lethal interactions with oncogenic alterations frequently found in human malignancies and investigating the molecular mechanism of the synthetic lethality. MBICR is a private organization with public purposes and strives to provide clinical benefits to cancer patients worldwide at an affordable cost.

Design, Bioproduction, and Tumor Delivery of Extracellular Vesicles Carrying Heterodimeric Interleukin-15

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[2]University Hospitals, Case Western Reserve University, Cleveland, OH

Abstract

Background: Eukaryotic cells secrete bioactive extracellular vesicles (EV) carrying lipids, proteins, and nucleic acids. We developed methodologies to engineer, bioproduce, and control delivery of EV carrying heterodimeric interleukin-15 (hetIL-15), a cytokine that activates anti-tumor immunity, as a platform for tumor-targeted immunotherapy.

Methods: A HEK293 cell clone expressing a novel form of hetIL-15 fused to the C1C2 domains of Lactadherin was grown either in conventional cell culture or a hollow-fiber bioreactor. EV were purified from conditioned culture media by ultracentrifugation or ultrafiltration and size-exclusion chromatography. We characterized the size, composition, and immunological effects of purified EV. Biodistribution and cell-specific uptake of purified EV in the presence of uptake receptor-blockers was assessed in mice and *in vitro*.

Results: hetIL-15/Lactadherin EV carried ~100-fold more cytokine compared to those expressing wild-type cytokine. Bioreactor cell culture increased EV yield by 40-fold over conventional cell culture. Compared to ultracentrifugation, purification of EV by ultrafiltration and size-exclusion chromatography dramatically reduced non-EV protein contamination, without sacrificing yield or bioactivity. EV *in vitro* and *in vivo* had affinity towards monocytic cells, with uptake mediated by scavenger receptors. Blockade of Scavenger receptor class A enabled accumulation of EV in tumors of mice.

Conclusions: We developed tools enabling bioproduction and purification of customized EV using cGMP-compatible technologies, along with delivery to tumors. These findings will facilitate further clinical development of EV therapeutics. Future work employing these technologies on primary cells with intrinsic therapeutic qualities may provide an alternative to traditional cell therapy, with potential advantages regarding safety, stability, and universal compatibility.

Biography

Dionysios (Dennis) Watson is a physician-scientist dedicated to developing novel therapeutics for immunotherapy of cancer. His PhD research on the translational development of heterodimeric interleukin-15 for HIV-1 and cancer was conducted at the National Cancer Institute, as part of a Graduate Partnership Program agreement with the University of Patras, Greece. He is currently a Medical Oncology fellow at University Hospitals Cleveland Medical Center (Case Western Reserve University). Dennis' current research focuses on identifying therapeutic opportunities in host-microbe interactions in cancer.

EphrinB2-Ror2 Interaction Regulates Neural Tube Closure

Jaeho Yoon* and Ira O. Daar

National Cancer Institute, NIH, Frederick, MD

Abstract

The regulation of cell shape and movement during primary neurulation is orchestrated by several distinct pathways of which the Eph/ephrin and the Wnt-Planar cell polarity signaling pathways play a critical role. Numerous studies have demonstrated that the knockdown of any one of the Wnt-PCP components causes abnormal cell polarity and migration during neural tube closure. In our previous study, we showed that the expression level of ephrinB2, a component of the Eph/ephrin signaling pathways, is important for F-actin formation, which is critical for apical constriction and neural tube closure. Nonetheless, little is known about the molecular mechanisms pertaining to ephrinB2 and its involvement in regulating apical constriction during neural tube closure. In this study, we performed an Immunoprecipitation-Mass Spectroscopy using ephrinB2-HA overexpressed embryos and found Wnt4, a non-canonical Wnt pathway protein, and Ror2, a co-receptor for Wnt-PCP signaling, to be novel binding partners of ephrinB2 during neural tube closure. Knockdown of ephrinB2 or Ror2 significantly decreased contractile actin bundle formations and caused neural tube closure defects that were subsequently rescued by the re-expression of the wild-type counterparts of ephrinB2 or Ror2 and not by the re-expression of the interacting mutants. By determining the crosstalk between Eph-ephrin and Wnt-PCP signaling, we shed light on the molecular mechanisms of genes that are involved in neural tube morphogenesis. Our study can advance the field in our understanding of neural tube defects, which is the second most common birth defect in humans.

Biography

Dr. Jaeho Yoon obtained his Ph.D. in developmental biology in the laboratory of Prof. Dr. Jaebong Kim at Hallym University in South Korea in 2012. Dr. Yoon studied the mechanisms of convergence and extension (CE) cell movements in Xenopus gastrula. For his postdoctoral studies, he joined the Cancer and Developmental Biology Laboratory at the National Cancer Institute at Frederick in Dr. Ira Daar's lab in Dec 2013 as a Visiting Fellow. He has served as a Staff Scientist in the laboratory since August 2019.

An IKKa-Nucleophosmin Axis Utilizes Inflammatory Signaling to Maintain Genome Integrity

Yinling Hu

Laboratory of Cancer Immunometabolism, Center for Cancer Research, National Cancer Institute, NIH, Frederick, MD 21701, USA

Abstract

The inflammatory microenvironment promotes skin tumorigenesis. However, the mechanisms of how cells protect themselves from inflammatory signals have yet to be revealed. Downregulation of IKKα promotes skin tumor progression from papillomas to squamous cell carcinomas, which is frequently accompanied by genomic instability, including aneuploid chromosomes and extra centrosomes. In this study, we found that IKKα promoted oligomerization of nucleophosmin (NPM), a negative centrosome duplication regulator, which further enhanced NPM and centrosome association, inhibited centrosome amplification, and maintained genome integrity. Levels of NPM hexamers and IKKα were conversely associated with skin tumor progression. Importantly, pro-inflammatory cytokine-induced IKKα activation promoted the formation of NPM oligomers and reduced centrosome numbers in mouse and human cells, whereas kinase-dead IKKα blocked this connection. Therefore, our findings suggest a previously unknown mechanism in which an IKKα-NPM axis may use the inflammatory signal to suppress centrosome amplification, promote genomic integrity, and prevent tumor progression.

Biography

Dr. Yinling Hu is a senior investigator, head of Inflammation and Tumorigenesis Section, at Laboratory of Cancer

Immunometabolism, in the National Cancer Institute (NCI), National Institute of Health. Her research interest focuses on inflammation and infection related skin and lung carcinogenesis. Dr. Hu obtained her Ph.D degree at the University of Melbourne, Australia and was trained as a postdoc fellow in Dr. Michael Karin laboratory, UCSD.

S6K1 and S6K2 Networking with the AXL Tyrosine Kinase in PTEN-deficient Glioblastoma

Pranjal Sarma

Department of Cancer Biology, VONTZ Center for Molecular Studies, University of Cincinnati, Cincinnati, OH

Abstract

Glioblastoma multiforme (GBM), the most lethal type of malignant brain cancer in adults, sustains frequent mutations and/or deletions in the tumor suppressor gene PTEN. In PTEN-deficient GBM, mTOR complex 1 (mTORC1) and the S6 kinases (S6Ks) mediate increased metabolism and apoptosis resistance. Previously we published that combining the LY-2584702 inhibitor of S6K1 with the BMS-777607 inhibitor of the AXL receptor tyrosine kinase (RTK) was selectively cytotoxic for PTEN-deficient GBM. Here we determined the impact of these inhibitors on the S6K1 and S6K2 signal transduction and tumor cell metabolism.

Genetic analysis of interactions between S6Ks and PTEN revealed that the S6K1 inhibitor LY2584702 was insufficient to prevent increased S6K substrate phosphorylation in PTEN-null GBM. Intriguingly, inactivation of S6K2 using sgRNA cooperated with LY2584702 to prevent increased S6K substrate phosphorylation, indicating that inactivation of S6K2 is required to overcome the resistance to single agent treatment. Similarly, persistent S6K signaling in BMS777607-treated GBM cells was significantly reduced when S6K2 was targeted. These results indicate that S6K2 integrates signal transduction inputs from both PTEN-regulated and AXL-regulated pathways. Metabolomic analysis revealed combination effects of S6K and AXL inhibitors in reducing nucleotide precursor metabolic flux. We therefore propose that combination inhibition of S6K and AXL signaling compromises S6K-dependent nucleotide synthesis in PTEN-deficient GBM

An HPV-Independent Mechanism of Cervical Carcinogenesis

Cheng Wang

Department of Obstetrics and Gynecology, Massachusetts General Hospital/Harvard Medical School, Boston, MA

Abstract

Cervical human papillomavirus (HPV) infections are common in women but only rarely cause cervical cancer, suggesting that unknown intrinsic factor(s) associated with individual genetic/genomic background may play a critical role in HPV persistent infection and cervical cancer development. Our recent studies provide convincing *in vitro* and *in vivo* evidence showing that disruption of the Hippo pathway and subsequent hyperactivation of YAP1 oncogene is a critical pathological event that determines individual susceptibility to HPV infection and cervical carcinogenesis. We found that hyperactivation of YAP1, the major effector of the Hippo/YAP signaling pathway, in mouse cervical epithelial cells was sufficient to induce malignant transformation of cervical epithelial cells and promote the development of invasive cervical cancer. The cervical epithelial cell-specific HPV16 E6/E7 and YAP1 double knock-in mouse model demonstrated that HPV synergized with hyperactivated YAP1 to promote the initiation and progression of cervical cancer. Our mechanistic studies indicated that hyperactivation of YAP1 in cervical epithelial cells facilitated HPV infection via increasing the putative HPV receptor molecules and disrupting the host cell's innate immunity. Results from this study challenge the HPV dogma of cervical cancer development, uncover a novel molecular mechanism of cervical carcinogenesis, and provide new targets for developing strategies to improve the prevention and treatment of cervical cancer.

Biography

Dr. Cheng Wang is a Principal Investigator in the Department of Gynecology & Gynecology, Massachusetts General

Hospital, and an Associate Professor at Harvard Medical School. Research in Dr. Wang's laboratory focuses on uncovering the cellular and molecular mechanisms underlying the development of cancers in female reproductive organs, aiming to facilitate the effective prevention, early diagnosis, and better treatment of these cancers.

Regulation of Macrophages by AEG-1: Implications in Cancer

Devanand Sarkar*

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Abstract

Chronic inflammation is a key driving event in Hepatocellular carcinoma (HCC). Liver-resident macrophages (Kupffer cells) play a vital role in establishing a pro-inflammatory, pro-tumorigenic environment. During initial tumorigenesis, damaged hepatocytes release cytokines, such as IL-1β, which stimulates Kupffer cells to activate NF- κB resulting in release of IL-6 that activates the oncogenic STAT3 signaling in the hepatocytes thereby promoting proliferation of transformed cells. Astrocyte elevated gene-1 (AEG-1)/metadehrin (MTDH) functions as a major oncogene for HCC. AEG-1 knockout mouse (AEG-1-/-) is completely resistant to experimental HCC and shows marked resistance to inflammation because AEG-1 is fundamentally required for activation of NF- κB, a key regulator of inflammation. Both AEG-1-/- hepatocytes and macrophages show inherent inability to activate NF- κB upon lipopolysaccharide (LPS) treatment. While global deficiency of AEG-1 in AEG-1-/- mice completely abrogated experimental HCC, hepatocyte-specific AEG-1 deficiency (AEG-1 $^{\Delta HEP}$) led to only an attenuation (and not complete abrogation) of HCC, while myeloid-specific AEG-1 deficiency (AEG-1 $^{\Delta MAC}$) led to complete abrogation of HCC indicating that AEG-1 in macrophages plays a key regulatory role in HCC. Notably, AEG-1-/- macrophages were resistant to either M1 or M2 differentiation with significant inhibition in migration, endothelial adhesion and efferocytosis activity, indicating that AEG-1 ablation renders macrophages functionally anergic. Thus AEG-1 plays a key regulatory role in both tumor cells and tumor-associated macrophages in hepatocarcinogenesis. AEG-1 targeting in both HCC cells and HCC-associated macrophages might be an efficient therapeutic strategy for HCC.

Biography

Devanand Sarkar, MBBS, PhD, is a Professor of Human and Molecular Genetics and Associate Director of Education and Training of Massey Cancer Center, Virginia Commonwealth University. His research interest includes molecular pathogenesis of hepatocellular carcinoma (HCC) and non-alcoholic steatohepatitis (NASH), mouse modeling and targeted gene and immunotherapy. His research is supported by grants from NCI, NIDDK and DOD.

Role of pSer784-VCP in DNA Damage Response and Cancer Chemotherapy Efficacy

Jieya Shao

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Abstract

Genotoxic chemotherapies are the mainstay treatment for many cancer types including triple-negative breast cancer. Recent years have seen the exciting development of more targeted genotoxic treatments exploiting intrinsic defects in the DNA damage response (DDR) abilities of cancer cells. Thus, deeper understanding of DDR mechanisms is critical for our ability to discover as well as create vulnerabilities in cancer cells that can sensitize them to genotoxic chemotherapies. A unifying element of most, if not all, DDR pathways is the spatiotemporal protein reorganization at DNA damage sites to allow dynamic assembly and disassembly of DNA repair factors and signaling molecules. This process is orchestrated by the evolutionarily conserved AAA+ ATPase named valosin-containing protein (VCP). Often called a protein segregase and aided by different cofactors, VCP physically extracts polyubiquitinated substrates from various organelles and subcellular structures to facilitate their turnover. However, due to such pleiotropic effects in global proteostasis, it remains

challenging to understand and target the DDR-specific functions of VCP. In recently published work, we functionally characterized a DNA-damage-induced phosphorylation event (Ser784) of VCP, which selectively enhances chromatin-associated protein degradation and is required for DNA repair, signaling, and cell survival upon treatment with diverse genotoxic agents. Clinically, high intra-tumor phospho-Ser784-VCP levels are significantly associated with poor outcome among chemotherapy-treated breast cancer patients. Thus, Ser784 phosphorylation is a clinically relevant DDR-specific enhancer of VCP function which can be further exploited to improve genotoxic chemotherapies.

Biography

Dr. Jieya Shao is an Assistant Professor at Washington University in St. Louis, USA. She received her PhD degree from Oklahoma State University and her postdoctoral training from University of California, San Francisco. She established her laboratory at Washington University in 2014. She is broadly interested in mechanism-based cancer research, with a particular focus on dissecting complex biology of multi-functional proteins, i.e. functional moonlighters, and identifying new ways to target their cancer-relevant activities.

HCF-1 Regulates De Novo Lipogenesis through a Nutrient-Sensitive Complex with ChREBP

Dong Wook Choi[1*], Elizabeth A. Lane[1], Luisa Garcia-Haro[1], Zebulon G. Levine[2], Meghan Tedoldi[1], Suzanne Walker[2] and Nika N. Danial[1]

[1]Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
[2]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA

Abstract

Carbohydrate response element binding protein (ChREBP) is a key transcriptional regulator of de novo lipogenesis (DNL) in response to carbohydrates and in hepatic steatosis. Mechanisms underlying nutrient modulation of ChREBP are under active investigation. Here we identify host cell factor 1 (HCF-1) as a previously unknown ChREBP-interacting protein that is enriched in liver biopsies of nonalcoholic steatohepatitis (NASH) patients. Biochemical and genetic studies show that HCF-1 is O-GlcNAcylated in response to glucose as a prerequisite for its binding to ChREBP and subsequent recruitment of OGT, ChREBP O-GlcNAcylation, and activation. The HCF-1:ChREBP complex resides at lipogenic gene promoters, where HCF-1 regulates H3K4 trimethylation to prime recruitment of the Jumonji C domain-containing histone demethylase PHF2 for epigenetic activation of these promoters. Overall, these findings define HCF-1's interaction with ChREBP as a previously unappreciated mechanism whereby glucose signals are both relayed to ChREBP and transmitted for epigenetic regulation of lipogenic genes.

Biography

Dong Wook Choi earned his B.S. (2008), M.S. (2010) and Ph.D. (2014) in biological sciences from Sungkyunkwan university, South Korea where he participated in studies that revealed previously unappreciated molecular connections between nutrient signaling pathways, autophagy, amino acid transport and DNA damage response. Since he joined the Danial lab at Dana Farber Cancer Institute, Harvard Medical School as a postdoctoral fellow, he has been focusing on molecular underpinnings of hepatic transcriptional responses to glucose stimulation.

CaMKK2 – A Master Kinase with Roles in AMPK and AKT Signaling in Cancer

Arthur M. Edelman*, Shuhang Dai, Elisa Venturini, Angela M. Gocher, Loukia G. Karacosta Jungsook Cho-Lee, Dylan Clapp and Sanjana Shetty

Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, Buffalo, NY

Abstract

The elucidation of epinephrine-induced glycogenolysis (1920s-1990s) provided both mechanistic understanding of an important feature of the fight-or-flight reaction but also the first example of an extracellular signal being transduced by an intracellular waterfall (cascade) reaction. Subsequently, many such pathways were discovered, and often similarly conceptualized as linear cascades. Recently, a subset of protein kinases have been identified that have been termed, "master kinases". The term master kinase is used to specifically refer to kinase kinases that are upstream of multiple kinase targets. Thus they may be thought of as "mastering" their downstream kinases in more of an umbrella-like (rather than linear), fashion to regulate distinct physiological phenomena. This talk will describe, the master kinases LKB1 (Stk11), phosphoinositide-dependent kinase 1 (PDK1/PDPK1) and Calcium/Calmodulin (CaM)-dependent kinase kinase 2 (CaMKK2). LKB1 phosphorylates and activates the energy homeostasis regulator, AMPK and eleven kinases within the family related to AMPK. PDK1 activates the AGC group kinases, Akt, serum/glucocorticoid-induced protein kinase (SGK), p70 ribosomal S6 kinase (S6K), and protein kinase C (PKC). CaMKK2 phosphorylates and activates the downstream Ca2+-requiring, CaM kinases I and IV, and the non-Ca2+-requiring kinases, AMPK and Akt. A notable feature of these master kinases is that they appear to "compete" with each other for targeting of downstream kinases. LKB1 and CaMKK2 compete for regulation of AMPK. PDK1 and CaMKK2 compete in Akt regulation. The latter interaction will be focus of this talk. In addition, possible physiological rationale(s) for the existence and function of master kinases will be proposed.

Biography

Area: Molecular and cellular signaling in cancer; Training: PhD, Stanford University. Post-doc with Edwin Krebs, Nobel laureate for the regulation of biological processes by protein phosphorylation; Selected Contributions: Discovery of CaMKK2 (Edelman, A. M. et al. J. Biol. Chem., 271, 1996). Regulation of the androgen receptor in prostate cancer (Karacosta, L. G., et al., J. Biol. Chem., 287, 2012; and Karacosta, L.G., et al. The Prostate 76: 2016). Involvement of CaMKK2 in tumorigenesis/drug resistance through control of the Akt pathway in ovarian cancer cells (Gocher, A. M., et al., J. Biol. Chem., 292: 2017).

The Ins and Outs of Cancer Therapy: Modifying Endocytosis Reversibly in Clinical Applications

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Abstract

A safe and controlled manipulation of endocytosis *in vivo* may have disruptive therapeutic potential. We demonstrate that the anti-emetic/anti-psychotic prochlorperazine can be repurposed to reversibly inhibit the *in vivo* endocytosis of membrane proteins targeted by therapeutic monoclonal antibodies, as directly demonstrated by our human tumor ex-vivo assay. Temporary endocytosis inhibition results in enhanced target availability and improved efficiency of natural killer cell-mediated antibody-dependent cellular cytotoxicity (ADCC), a mediator of clinical responses induced by IgG1 antibodies, demonstrated here for cetuximab, traztuzumab and avelumab. Extensive analysis of downstream signalling pathways ruled out on-target toxicities. By overcoming the heterogeneity of drug target availability that frequently characterizes poorly responsive or resistant tumors, clinical application of reversible endocytosis inhibition may considerably improve the clinical benefit of ADCC-mediating therapeutic antibodies. In this presentation we expand the discussion to discuss our Phase IB safety trial outcomes and new targets for therapeutic intervention.

Biography

Fiona Simpson completed her PhD on cellular trafficking at the University of Cambridge, UK. She was then a Wellcome Trust Prize Post-doctoral fellow at The Scripps Research Institute, La Jolla, working on trafficking and endocytosis of RTKs. Fiona is currently a Fellow of the Queensland Head and Neck Cancer Centre. The Simpson lab research program is focused directly on the translation of research findings into new cancer therapies. Fiona was the lead scientific investigator on a Phase I proof of concept study of novel therapy in head and neck cancer (HREC/15/QPAH/48) and the Lead scientific Investigator on Phase IB dose escalation trail (CESTEM-1), now completed.

Inhibition of RNA Polymerase I Transcription Activates the DNA Damage Response and Demonstrates Therapeutic Efficacy in Ovarian Cancer

Elaine Sanij[1,2,3*], Katherine Hannan[4], Anthony Xuan[1], Natalie Brajanovski[1], Keefe Chan[1], Shunfei Yan[1], Anna Trigos[1], Matthew Wakefield[5], Ross Hannan[1,4,6,7], Clare Scott[5], Karen Sheppard[1,2,6] and Richard Pearson[1,2,6,7]

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Abstract

High-grade serous ovarian cancer (HGSOC) accounts for the majority of ovarian cancer and has a dismal prognosis. PARP inhibitors (PARPi) have revolutionized disease management of patients with homologous recombination (HR) DNA repair deficient HGSOC. However, acquired resistance to PARPi is a major challenge in the clinic.

The first-in-class drug CX-5461 that inhibits RNA polymerase I (Pol I) transcription of ribosomal RNA (rRNA) genes, has promising clinical activity in Phase I trials in patients with haematological malignancies (Peter MacCallum Cancer Centre) and solid cancers (Canada). We have recently shown that CX-5461 has a significant therapeutic benefit *in vivo* in a cisplatin- and PARPi-resistant HGSOC-patient derived xenograft (PDX) model (Sanij et al., Nature Communication 2020). Our data demonstrate CX-5461 and PARPi exhibit different spectrum of cytotoxicity due to their distinct modes of action in inducing a DNA damage response (DDR). CX-5461 activates the DDR at the rRNA genes leading to global replication stress involving MRE11-dependent degradation of DNA replication forks. CX-5461 co-operates with PARPi in exacerbating replication stress and enhances therapeutic efficacy against HR deficient HGSOC-PDX *in vivo*. Importantly, CX-5461 exhibits efficacy in patient-derived HGSOC cells with reduced sensitivity to PARPi involving replication fork

protection, a common mechanism of resistance to chemotherapy and PARPi.

Further, we have identified CX-5461-sensitivity gene expression signatures in primary and relapsed HGSOC. We propose CX-5461 is a promising therapy in combination with PARPi in HR-deficient HGSOC and also as a single agent for the treatment of relapsed disease.

Biography

Dr. Elaine Sanij is a Senior Research Fellow at the Peter MacCallum Cancer Centre and a Victorian Cancer Agency Mid-Career Research Fellow. Dr Sanij received her PhD from Monash University, Australia in 2003 and was awarded a Cancer Research UK Postdoctoral Fellowship to undertake postdoctoral studies at the London Research Institute, UK. Dr Sanij joined Peter Mac in 2006 and she is internationally recognised in the fields of RNA Polymerase I transcription and the DNA damage response. Her research is focused on developing innovative cancer therapeutics and novel combination therapies to address the significant clinical challenge of refractory ovarian cancer.

Unraveling the Mechanisms Controlling PI3K/AKT-driven Senescence in Cancer

Keefe T. Chan*, Jian Kang and Richard B. Pearson

Peter MacCallum Cancer Centre, Australia

Abstract

Dysregulation of the PI3K/AKT/mTORC1 signaling pathway is an early driver of one-third of human cancers, but sustained hyperactivation of the pathway in normal cells results in cellular senescence, a powerful tumor-suppressive mechanism that must be overcome to promote malignant transformation. We previously demonstrated that AKT-induced senescence (AIS) occurs due to robust activation of p53 and is independent of DNA damage in contrast to classic oncogenic RAS-induced senescence. We hypothesised there could be mediators besides p53 and thus performed a genome-wide RNAi screen for escape from AIS, identifying approximately 100 new regulators. We validated a subset of these novel mediators and identified their specificity for AIS. We highlight that neurofibromin 1 (NF1) is upregulated during AIS and its ability to suppress RAS/ERK signaling facilitates AIS maintenance. Given that AIS is also associated with profound metabolic changes, we investigated the role of cystathionine beta-synthase (CBS), an enzyme that regulates transsulfuration and transmethylation metabolic pathways, demonstrating its importance in regulating mitochondrial reactive oxygen species production required for AIS maintenance. Re-instatement of these pathways in cancer cells refractory to AIS could re-engage a senescence-like proliferative arrest. Together, our findings reveal novel mechanistic insights into the control of AIS and identify putative senescence regulators that can potentially be targeted to treat PI3K/AKT/mTORC1-driven cancers.

Biography

Dr. Keefe Chan obtained his PhD in 2010 in Molecular and Cellular Pharmacology at the University of Wisconsin-Madison examining integrin-mediated adhesions and their role in normal and cancer cell motility. He underwent postdoctoral training at the University of North Carolina Chapel Hill Lineberger Comprehensive Cancer Center in intravital and advanced microscopy to study cancer cell invasion using a genetically engineered murine model of melanoma. Since 2014 within the Cancer Signaling Laboratory at the Peter MacCallum Cancer Centre in Melbourne, Australia, Dr. Chan has been investigating the mechanisms governing oncogene-and therapy-induced senescence and how they can be exploited to treat cancer.

Transcriptomic Investigation of Molecular Mechanisms Mediating Adverse Effects of Prenatal Exposure to Oxidative Stress in a Neuronal Cell Model: Significance for Psychiatric Diseases

Behnaz Khavari[1,2*], Ebrahim Mahmoudi[1,2], Michael P. Geaghan[1,2] and Murray J. Cairns[1,2]

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Abstract

Perinatal environmental exposures, such as smoking and alcohol consumption during pregnancy, which are risk factors for the onset of neurodevelopmental disorders (NDDs) and psychiatric diseases, induce oxidative stress (OS) in cells. Although the role of OS in neurodegenerative diseases etiology is well studied, it remains ambiguous if it contributes to genomic dysregulations associated with NDDs. In our recently published study (https://doi.org/10.3390/ijms21239182), we used SH-SY5Y cell line model and total RNA-Sequencing to investigate transcriptomic changes in response to 10 µM H2O2-induced OS before or during neural differentiation.

Our results revealed differential expression of a substantially large number of genes, most of which involved in the biological processes neurogenesis and neuronal differentiation, and several schizophrenia (SZ)-associated signalling pathways, including axon guidance, PI3K-Act, and retinoic acid signalling. Intriguingly, development of circulatory system was also affected by both treatments, which might explain some of the risk for the observed increase in incidence of cardiovascular diseases among NDD patients. In addition, there existed a very noticeable increase, up to 400 times, in the immunity-related genes expression, which is interesting considering the well-studied involvement of the immune system activation in the etiology of psychiatric disorders.

In conclusion, we show that perinatal exposure to OS results in a widespread transcriptomic disruption of neurodevelopment, especially neuron differentiation, even before its initiation, and therefore, we suggest that exposure to OS-inducing environmental factors should be avoided from the very early months or even weeks of pregnancy. Further investigations, including animal models are required in the future to support this assumption.

Biography

Behnaz Khavari is a last-year PhD student of Medical Biochemistry in the University of Newcastle, Australia, under the supervision of Professor Murray Cairns. During her PhD, she has been applying several bioinformatics software and tools to analyse total and small RNA-Sequencing data obtained from cell culture experiments and schizophrenia patients-derived blood samples. In 2020, she published two papers; a review article about epigenetic and microRNA dysregulations in schizophrenia (PMID: 32764320) and a very recent research paper based on the results of the current study (https://doi.org/10.3390/ijms21239182). She is interested in following her career in analysing large datasets related to neurodevelopmental disorders.

Challenging Peripheral Arterial Disease: Growth Hormone (GH) Favors Neovascularization by Decreasing NOX4 Activity and Increasing VEGFR2/KDR

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[2]The Medical Center Foltra, Spain

Abstract

Peripheral Arterial disease (PAD) is an ischemic condition associated to high risk of limb loss and disability [1]. In patients with no revascularization option, several angiogenic approaches have been developed with inconsistent results, making the search for new targets still mandatory. Growth hormone (GH), with a pivotal role in vascular homeostasis, could be beneficial in ischemia, as it has been defended [2]. The GHAS trial, a randomized and placebo-controlled study, evaluated the safety and efficacy of GH as a bail out therapy in PAD. After two months of hormone administration, both wound healing (55.6% vs 12.5%, p=0.0098, OR=8.75) and rest pain (77.8% vs 25%, p=0.024, OR=10.5) significantly improved in GH group. The molecular analysis of muscle samples showed a significant decrease in mRNA levels of NOX4 (p=0.0250) and an increase in the receptor 2 of the vascular endothelial growth factor (VEGF-R2/KDR) (p=0.0413) only in GH group, while NOX4 remained high in placebo group, showing the persistence of the redox stress. We postulate that GH is capable of favoring neovascularization by decreasing redox unbalance, which leads to an increase in nitric oxide (NO) bioavailability, and by increasing VEGF-R2, a necessary actor for the angiogenic actions of VEGF. These findings open the door for new clinical studies with GH alone or in combination with other therapies. Dose and period of treatment have to be properly established. In conclusion, GH should be considered as a promising therapeutic agent to add for the approach of ischemic conditions.

References:

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Novel Signaling Hub of Insulin Receptor Dystrophin Glycoprotein Complex and Plakoglobin Regulates Muscle Size

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Abstract

Signaling through the insulin receptor governs central physiological functions related to cell growth and metabolism. Here we show by tandem native protein complex purification approach and super-resolution STED microscopy that insulin receptor activity requires association with the fundamental structural module in muscle, the dystrophin glycoprotein complex (DGC), and the desmosomal component plakoglobin (γ-catenin). The integrity of this high-molecular-mass assembly renders skeletal muscle susceptibility to insulin, because DGC-insulin receptor dissociation by plakoglobin downregulation reduces insulin signaling and causes atrophy. Furthermore, low insulin receptor activity in muscles from transgenic or fasted mice decreases plakoglobin-DGC-insulin receptor content on the plasma membrane, but not when plakoglobin is overexpressed. By masking β-dystroglycan LIR domains, plakoglobin prevents autophagic clearance of plakoglobin-DGC-insulin receptor co-assemblies and maintains their function. Our findings establish DGC as a signaling hub and provide a possible mechanism for the insulin resistance in Duchenne Muscular Dystrophy, and for the cardiomyopathies seen with plakoglobin mutations.

Biography

Shenhav Cohen is an Associate Professor at the Faculty of Biology at Technion-Institute of Technology in Israel. Her lab masters two areas in cell biology, the biochemistry of the ubiquitin-proteasome system (UPS) and muscle biology/physiology. A major research interest of the lab is understanding the molecular mechanisms of protein degradation, especially of the contractile myofibrillar apparatus, during muscle atrophy in aging or disease. In this context, the Cohen lab has made several important contributions that brought major breakthroughs and new concepts into the UPS and muscle fields.

Targeting the Water Channel Protein, Aquaporin-4, to Prevent Edema after Spinal Cord Injury

Zubair Ahmed

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Abstract

Spinal cord or brain injury leads to swelling (CNS edema) and affects millions of people every year. There are currently no pharmacological treatments available to prevent edema, meaning that symptom management is the only option. Edema after spinal cord injury occurs over several months, leading to the formation of fluid-filled cysts at the lesion site that damage spinal tissues which survived the original injury. The water channel protein, aquaporin-4, is expressed in astrocytes and mediates the flow of water across the blood-spinal cord and blood-brain barriers. We show that cell-surface abundance of aquaporin-4 is increased in response to hypoxia in a calmodulin-dependent manner. Calmodulin binds directly to the carboxy terminus of aquaporin-4 and causes a conformational change that drives aquaporin-4 cell-surface localization. We show that inhibition of calmodulin in a rat model of spinal cord injury, using an already licensed drug, trifluoperazine, inhibits aquaporin-4 re-localization to the blood-spinal cord barrier, ablates CNS edema and accelerates functional recovery. Our results suggest that targeting aquaporin-4-mediated cell surface re-localization is a potential therapeutic strategy in treating CNS edema.

Biography

Dr. Zubair Ahmed is currently an Associate Professor in Neuroscience at the University of Birmingham UK and Leads the Neuroscience and Ophthalmology Section. His research focusses on Neurotrauma and in particular optic nerve and spinal cord injury. He is trying to define the reasons why CNS neurons do not regenerate their axons after injury and devise therapeutic strategies to overcome these barriers.

Inflammatory Mechanisms Underlying Brain Dysfunction in Rett Syndrome

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[2]New York University School of Medicine, New York, NY

Abstract

The main two types of immune cells, i.e., myeloid and lymphoid cells, provide innate and adaptive immune defenses against infections and injury. Microglia are the most frequent longlived immune cells of the brain. Derived from yolk-sac progenitors, microglia populate the central nervous system (CNS) parenchyma during embryonic development and mature through intermediate stages, acquiring region-specific molecular phenotypes. In addition to their crucialrole in innate immunity, microglia have been shown to shape the establishment of neural circuits during normal postnatal development, and modulate learning-dependent synaptic plasticity in adult brain. In the CNS, other populations of myeloid cells share with microglia their origin from yolk sac progenitors and longevity, but locate outside of CNS parenchyma, forming a unique niche of meningeal and perivascular resident cells often referred to as CNS-associated macrophages. Furthermore, discrete populations of short-lived bone-marrow derived myeloid cells such as monocytes and monocyte-derived macrophages also contribute to innate immunity and locate at the brain-periphery interfaces (meninges, choroid plexus, and perivascular spaces). The role of these non-microglial myeloid cells in modulating brain function is unknown. My seminar will describe how peripheral myeloid cells contribute to synaptic and behavioral deficits in Rett syndrome (RTT), a postnatal neurodevelopmental disorder caused by mutations in the gene encoding MECP2. I will also discuss the role of P2X7Rs, which are purinergic receptors with pro-inflammatory functions in leptomeningeal macrophages, in modulating social behavior in MECP2 disorders.

References:

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Use of DREADD Technology to Identify Novel Targets for Anti-Diabetic Drugs

Jürgen Wess

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Abstract

G protein-coupled receptors (GPCRs) represent a family of cell surface receptors that consists of ~800 members in human. About 1/3 of drugs in current clinical use act one or more of these receptors. GPCRs are involved in the regulation of a large number of important metabolic functions. A particular GPCR usually preferentially couples to only one or two of the four major subfamilies of heterotrimeric G proteins, Gs, Gi, Gq, and G12. Since nearly all GPCRs are expressed by many different cell types, the *in vivo* metabolic roles of a specific GPCR expressed by a distinct cell type remain unclear. The development of designer GPCRs known as DREADDs (Designer Receptors Exclusively Activated by a Designer Drug) that selectively couple to Gs, Gi, Gq, or G12 has greatly facilitated studies in this area. During my talk, I will highlight several examples illustrating how the use of DREADD technology has elucidated the physiological and pathophysiological roles of distinct GPCR/G protein cascades in various metabolically important cell types. The novel information obtained through this line of research should guide the development of novel GPCR-based treatments for several metabolic diseases including type 2 diabetes and obesity.

Axonal Transport as an *In Vivo* Biomarker for Retinal Neuropathy

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Abstract

This study illustrates retinal axonal transport with a fluorescent nerve imaging tracer based on fast axonal transport in control and neuropathic eyes.

Methods: Neuropathy was induced in Norway brown rats eyes by injecting NMDA into the vitreous of one eye and PBS into the contralateral eye as a control. 48 Hours after NMDA injection, a fluorescently labeled neural imaging probe based on non-toxic, Tetanus Toxin C (TTc) was injected into the vitreous of both treated and control eyes. *In vivo* imaging of the distribution of TTc was performed using retinal ophthalmoscopy. Retinas were harvested 3 hours after TTc injections, and immune-histology performed using antibodies to retinal axons (RAs) and retinal ganglion cells (RGCs).

Results: In normal eyes, the *in vivo* uptake and transport of TTc in the RAs and RGCs could be observed with a retinal ophthalmoscope within 30 min and up to 3hrs of an intravitreal eye injection. TTc co-localizes with the RA neurofilament marker (SMI32) while a selective cytoplasmic marker for RGC (RBPMS), confirmed the presence TTc endovesicles in the RGC neuronal cell bodies. In contrast, retinopathic eyes showed a marked reduction in the RA TTc uptake. The total image fluorescence intensity for the normal versus retinopathic eyes was significantly different with retinal imaging data sets, corresponding to a 4.5-fold and 2.4-fold difference for *in vivo* and ex vivo respectively.

Conclusion: NMDA induced retinopathy decreases neuronal uptake and transport of TTc, indicating that abnormalities of neural uptake and transport are early events in the development of retinal neuropathy.

Biography

Dr. Le Roux's research focuses on developing clinically translatable neural imaging probes based on fast neural transport that can be used to develop clinically relevant imaging modalities for the assessment, prevention and treatment of chemotherapy and disease induced neuropathies. In this study we demonstrate the role of fast neural transport in an *in vivo* animal eye model of neuropathy using a neural imaging probe to demonstrate retinal axons. Other neural systems we investigate include the sciatic nerve, spinal cord, and genital nerve plexus.

The Role of Alzheimer's Disease Relevant Tau Modifications in Neurodegeneration and Mitochondrial Dysfunction

Sanjib Guha*, Gail Johnson and Keith Nehrke

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Abstract

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is the most common form of dementia. The pathological hallmarks of AD include the presence of extracellular senile plaques (SP) composed of β-amyloid protein (Aβ) and intraneuronal neurofibrillary tangles (NFTs) composed of the microtubule-associated protein Tau. Tau isolated from AD brain exhibits a number of abnormal post-translational modifications (PTMs), including increases in phosphorylation and acetylation at specific epitopes.

Method: Human 0N4R tau (wild type) was expressed in touch receptor neurons of the genetic model organism C. elegans through single-copy gene insertion. Defined mutations were then introduced into the single-copy tau transgene through CRISPR-Cas9 genome editing. These mutations included T231E and T231A, to mimic phosphorylation and phospho-ablation of a commonly observed pathological epitope, respectively, and K274/281Q, to mimic disease-associated lysine acetylation.

Result: Unlike existing tau overexpression models, C. elegans single-copy expression of tau did not elicit overt pathological phenotypes at baseline. However, strains expressing disease associated PTM-mimetics (T231E and K274/281Q) exhibited reduced touch sensation and neuronal morphological abnormalities that increased with age. Remarkably, the PTM-mimetics have significantly impaired mitolysosomal trafficking in precise neurons and lacked the ability to engage mitophagy in response to mitochondrial stress.

Conclusion: Using CRISPR-Cas9 genome editing tool we have successfully generated novel single copy Tau-PTM mimetics in C. elegans, which are able to phenocopy pathological AD related phenotypes such as loss of neuron function and neuronal degeneration without overexpressing Tau. The finding that disease-associated PTMs suppress compensatory responses to mitochondrial stress provides a new perspective into the pathogenic mechanisms underlying AD.

Biography

Dr. Guha was born and raised in Calcutta, India where he did his undergrad studies. Then in 2008, he moved to California where he completed his Masters. After that he moved to Singapore, where he worked as a research associate at NUS. Then he moved to Barcelona, where he did his Phd and after completion of that he again moved back to California, where his first postdoc was at the Buck Institute. And now, he is currently at University of Rochester, upstate New York. He works upon post-translational modifications of tau selectively impact neurodegeneration and causes mitochondrial dysfunction. This work has been recently accepted at Molecular Neurodegeneration journal, and the corresponding review was published at Molecular Neurobiology journal.

Using Neurotechnology and Artificial Intelligence to Treat Disease

Patrick D Ganzer[1*], Masoud S Loeian[1], Steve R Roof[2], Bunyen Teng[2], Luan Lin[3], Dave A Friedenberg[3], Ian W Baumgart[1], Eric C. Meyers[1], Keum S Chun[1], Adam Rich[3], William W Muir[2,4], Doug J Weber[1,6] and Robert L Hamlin[2,5]

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Abstract

Bioelectronic medicine is an emerging field of neurotechnology using peripheral nerve stimulation to treat disease. This presentation will broadly introduce bioelectronic medicine technology, and specifically outline how we are using it to treat episodes of myocardial ischemia. Myocardial ischemia is a cardiovascular pathology that is spontaneous, usually asymptomatic, and contributes to fatal cardiovascular consequences. Our bioelectronic medicine technology leverages artificial intelligence (AI), and works as follows: 1) We first train an artificial neural network (ANN) to reliably decode spontaneous events of myocardial ischemia (~94% accuracy; preclinical model). These events are induced by infusions of catecholamines that modulate the prime determinants of myocardial oxygen consumption. 2) Once myocardial ischemia is detected, the ANN responsively triggers closed-loop vagus nerve stimulation (VNS), providing on-demand bioelectronic medicine to restore myocardial oxygen balance. ANN controlled VNS specifically reduced pathological changes in heart rate, electrophysiological correlates of ischemic currents, and blood pressure. 3) Interestingly, preprogrammed open-loop VNS cannot react to spontaneous events of myocardial ischemia, and provides almost no benefit. Disruption of efferent vagal fibers also blocked the beneficial effects of ANN controlled VNS. These results show that VNS timing and nerve fibers engaged are both critical for the beneficial effects of ANN controlled bioelectronic medicine. Overall, we anticipate new innovations in the emerging field of artificially intelligent medicines, where AI systems can optimize and help deliver therapy for treating disease and dysfunction.

Biography

Dr. Patrick Ganzer received his B.S. in Neuroscience from King's College in 2008 (Summa Cum Laude), completed his Ph.D. in Biomedical Engineering from Drexel University in 2013, and finished his post-doctoral fellowship at the University of Texas at Dallas in 2017. He is now a Principal Research Scientist at Battelle Memorial Institute, the world's largest non-profit research organization located in Columbus, Ohio. Dr. Ganzer's research focuses on neurotechnology, applied artificial intelligence, and bioelectronic medicines. His team's work is now being translated to multiple clinical trial applications to treat individuals with disease and disability.

STING-dependent Type-1 Interferon Restrains Schistosome Immunopathology Via Down-Regulation of the CD209A Lectin Receptor

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Abstract

Infection with the helminth parasite Schistosoma mansoni causes morbidity and mortality via a pathogenic host CD4 T cell-mediated immune response directed against parasite egg antigens. We now demonstrate that stimulation of dendritic cells (DCs) with schistosome eggs induces robust IFNβ production in a manner dependent on the cyclic GMP-AMP synthase (cGAS)/Stimulator of Interferon genes (STING) cytosolic DNA sensing pathway, resulting in the suppression

of proinflammatory IL-1β and IL-23 production and in Th17 cell activation. Consistent with these results, low-pathology BL/6 mice lacking STING exhibited markedly enhanced hepatic granulomatous inflammation associated with significantly increased Th17 and diminished Th2 cytokine responses. Mechanistically, IFNβ acts by suppressing DC expression and function of CD209a, a C-type lectin receptor associated with severe schistosome immunopathology. Importantly, there was an increased baseline CD209a expression in unstimulated DCs from STING-/- mice, suggesting a role for constitutive IFN signaling. Our findings provide the first demonstration that innate, cGAS/STING-dependent sensing of parasite DNA represents a novel pathway inducing type I IFN production, which protects the host from excessive inflammation and immunopathology in schistosomiasis.

A Human *In Vitro* Model for Type-1 Diabetes Unravels Gene Editing Targets for Immune Protection of Stem Cell-Derived Beta Cells

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Abstract

Type 1 diabetes (T1D) is an autoimmune disorder leading to the destruction of insulin-producing β-cells in the pancreas. Despite recent scientific advances, questions remain regarding the initial trigger and the downstream mechanisms of disease progression. Human induced pluripotent stem cells (hiPSCs) provide new opportunities for cell replacement therapy of T1D. Therapeutic quantities of human stem cell-derived β-cells (SC-β) can be attained *in vitro* following a stepwise differentiation protocol. Yet, preventing immune rejection of grafted cells, without the use of life-long immunosuppressants, remains a major challenge. Using T1D patients' hiPSC derived β-cells (iPSC-β), we developed a human *in vitro* platform in an autologous setting that recapitulates aspects of the effector/target interactions in an autoimmune response. A donor-matched β-cell-specific response was observed by co-cultures with perihelial blood mononuclear cells (PBMCs) derived from the same donors' blood. We performed a droplet based single-cell RNA sequencing (scRNA-seq) of T1D iPSC-β co-cultured with their autologous PBMCs. scRNA-seq data analysis of co-cultured cell populations identified upregulated genes that contribute to the inflammatory microenvironment of a T1D pancreatic islet. Subsequent co-culture experiments have shown that CRISPR-depletion of such genes in SC-β, can reduce activation of T-cells and increase β-cell survival. These results provide insights into the nature of immune destruction of β-cells during T1D and suggest a path to prevent it by cell replacement approaches.

Generation and Characterization of Isoform-Specific p63g-/- Mice

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Abstract

The transcription factor p63 produces multiple isoforms with different amino- and carboxy-termini by the use of two different promoters and at least three distinct splicing at the 3'-end. We have shown previously that DNp63a, the predominant isoform in epithelia, plays an essential role in maintaining the proliferative potential of epithelial stem cells. In contrast, accumulating evidence suggest that the p63g isoforms act as anti-proliferative proteins. This assumption has led to a prevailing hypothesis in the field that the balance between DNp63a and p63g plays a critical role in maintaining the homeostasis of epithelia and that relative reduction in the p63g activity leads to malignant transformation. However, while the specific roles of D Np63 a have been well characterized both *in vitro* and *in vivo*, the studies addressing the relative roles of the p63g isoforms *in vivo* have been lacking. In this study, we have created a novel mouse model in which exon 10', the specific 3'-terminal exon of the p63g gene, has been deleted by homologous recombination. Our data show that unlike DNp63 knockout mice or p63g knockout mice, p63g-/- mice develop normally and show no gross abnormalities during embryogenesis. In addition, we find no evidence of tumor formation in p63g-/- mice up to 2 years of age. These data indicate that the p63g isoforms are dispensable for the development of epithelia and do not act as a classical tumor suppressor.

Biography

Dr. Filipa Pinto is a Senior Research Scientist in Senoo lab at Boston University Henry M. Goldman School of Dental Medicine. She started her career in Physics at University of Porto in Portugal and later shifted to the Biomedical field. Her Ph.D. thesis at Harvard Medical School focused on elucidating p63's role in maintaining the self-renewal capacity of epithelial stem cells. She then joined Dr. Gearhart's lab at University of Pennsylvania where she specialized in somatic nuclear reprogramming. Currently, Dr. Pinto studies how p63 isoforms control homeostasis and diseases of epithelia using novel mouse models.

Role of the Ubiquitin Ligase ITCH in Clathrin-Mediated Endocytosis of the Epidermal Growth Factor Receptor

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Abstract

Once activated by ligand, epidermal growth factor receptor (EGFR) is endocytosed in clathrincoated pits. ITCH is an E3 ubiquitin ligase that interacts with and ubiquitinates several proteins involved in clathrin-mediated endocytosis (CME) including endophilin. To further investigate the function of ITCH in EGFR endocytosis, the internalization of fluorescent EGF was measured inITCH-/- HeLa cells. In the absence of ITCH, there was a significant decrease in the CME of EGF. Rescue experiments using wild-type ITCH confirmed the importance of the protein for normal EGF uptake. ITCH point mutations that disrupt the interaction with endophilin failed to rescue the defects in EGFR uptake, as did a non-catalytic form of ITCH. Our study describes an important pathway regulating EGFR trafficking and reveals for the first time that the protein ITCH is required for CME of EGFR

Angiogenic Factors Serve as Regulators and Predictors of Immune Reconstitution After Umbilical Cord Blood Transplantation in Adults

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Abstract

Umbilical cord blood (UCB) has expanded the application of hematopoietic stem cell transplantation to patients who lack suitable HLA matched adult donors. Angiogenic cytokines have a regulatory role in reconstitution of hematopoiesis after bone marrow injury or transplantation, likely because hematopoietic and endothelial cells share a common progenitor and express membrane receptors (Flt-1, Flk-1, Tie 2) for these cytokines. HSCT compromises thymopoiesis by injury of the thymic microenvironment, particularly thymic epithelial cells. Vascular endothelial growth factor (VEGF) has been shown to play a key role in thymic recovery following experimental HSCT. We hypothesized that angiogenic factors might also be involved in T cell reconstitution after UCBT. We examined whether angiogenic cytokines, VEGF and angiopoietin-1 (ANG-1), or markers of endothelial injury, thrombomodulin (TM) and angiopoietin-2 (ANG-2), associate with thymic regeneration and recovery of T cell subsets in adult recipients of UCBT. We found that plasma levels of ANG-1 significantly correlated with reconstitution of naïve T cell subsets CD4+CD45RA+ and CD8+CD45RA+, whereas VEGF displayed a positive correlation with the non-naïve CD4+CD45RO+ T cells and T regulatory cells. TM and ANG-2 had a strong inverse correlation with naïve T cells and TRECs, which serve as indicators of thymic recovery. The angiogenic capacity of each patient's plasma was determined by *in vitro* angiogenesis assay and positively correlated with VEGF levels and reconstitution of CD4+ T cell subsets. These findings suggest that circulating angiogenic factors may be involved in posttransplant immune reconstitution and might serve as prognostic markers for clinical outcomes after UCBT.

Biography

I graduated Medical School of the Kapodistrian University of Athens in 2018 and I joint as a Postdoctoral Research Fellow the laboratory of Dr. Vassiliki Boussiotis at Beth Israel Deaconess Medical Center, Harvard Medical School. My research studies investigate mechanisms of anti-tumor immunity and cancer immunotherapy as well as reconstitution of immunity after allogeneic hematopoietic stem cell transplantation.

Comparison of Acetate Overflow between E. coli BL21 and E. coli K12 Strains using Different Carbon and Nitrogen Sources

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Abstract

E. coli is a microorganism largely used as a biotechnological model and is employed to produce high valuable compounds [1]. E. coli is capable to grow using diverse carbon and nitrogen sources, and them are relevant factors for the metabolism fluxes [2]. Furthermore, different E. coli strains have been employed, E. coli K12 is the most studied strain, but other strains such as E. coli BL21 achieve higher biomass yields, lack some proteases and produce less acetate. Acetate can be excreted and then reincorporated under some culture conditions, is related with protein lysine acetylation and entails a reduction in production of compounds, so involves a drawback for industrial processes [3, 4].

In this study we are going to deepen in the knowledge of differences between K and B strains regarding acetate metabolism and lysine acetylation. Thereby, E. coli K12 and BL21 wild-type and five gene deletion strains involved in lysine acetylation (ΔpatZ and ΔcobB) and acetate metabolism (Δpta, ΔackA and Δacs) were grown in TB7 medium or

MM9 medium (nitrogen source based in peptides or inorganic ammonium, respectively), supplemented with glucose or glycerol. Under these conditions growth rate, acetate concentration, and lysine acetylation level have been evaluated. The results revealed a higher acetate accumulation in K12 strains than in BL21, and in TB7 supplemented with glucose in both strains. Moreover, ΔackA and Δpta strains showed the lowest values of acetate accumulation. These results showed the great importance of choosing the correct conditions before carrying out a bioprocess in E. coli.

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