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Meeting Abstracts

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# 7th International Conference on Cell and Experimental Biology (CEB-2026)

## Plenary Presentations

### Normalizing the Tumor Microenvironment to Improve Cancer Treatment: Bench to Bedside and Back

**Rakesh K. Jain**[1,2*]

[1]*Department of Radiation Oncology, Harvard Medical School, Boston, USA*
[2]*Edwin L. Steele Laboratories, Massachusetts General Hospital, Boston, USA*

#### Abstract

Immune checkpoint blockers (ICBs) have revolutionized the treatment of multiple malignancies. However, <20% of patients currently benefit from these therapies. Similarly, chimeric antigen receptor T-cells (CAR-T) have revolutionized the treatment of hematological malignancies but have not been effective against solid tumors. We have demonstrated that this limited efficacy is due to abnormal tumor microenvironment (TME). The dysfunctional tumor vessels not only inhibit infiltration of immune cells (nascent, reactivated with ICBs, or adoptively transferred, such as CAR-T cells) into tumors, but also create a hypoxic and acidic microenvironment that suppresses the function of immune cells after they accrue in tumors. In 2001, I proposed that judicious use of anti-angiogenic agents – that were originally developed to starve tumors – can "normalize" tumor vessels and improve the delivery and efficacy of concurrent therapies. Our preclinical finding that vascular normalization can improve immunotherapy was confirmed by others in phase III trials on combining antiangiogenic therapy with ICBs for lung, kidney, liver, and endometrial, led to the U.S. Food and Drug Administration approvals of seven such combinations for these cancers, and formed the foundation of novel bifunctional antibodies that target both vascular endothelial growth factor and PD1/L1. Additionally, we discovered that anti-hypertensive drugs capable of "normalizing" the tumor matrix and stromal cells can re-program the TME to an immunostimulatory milieu and improve the delivery and efficacy of cancer therapies. A phase II trial led by my clinical collaborators provided evidence in support of this concept for improving the treatment outcome for patients with pancreatic ductal.

### New Insights into the Biology and Treatment of Pancreatic Cancer

**Raghu Kalluri**[*]

*Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, USA*

#### Abstract

Pancreatic cancer remains a treatment-refractory disease. Oncogenic Kras drives not only tumorigenesis but actively reshapes the stromal and immune microenvironment to sustain disease progression and therapeutic resistance. This talk will examine how Kras suppression can restore tissue homeostasis and reprogram tumor immunity; why new Kras inhibitors show promise yet remain limited by toxicity and acquired resistance; and why immune engagement is essential for durable disease control. It will highlight new therapeutic strategies to inhibit Kras signaling with greater precision, minimizing off-target effects, and opening the door to preventive or early-intervention use.

# Keynote Presentations

## Regulation of Pyruvate Dehydrogenase Complex: Dancing to Different Drums in Cancer

**Mulchand S. Patel***

*School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, USA*

### Abstract

Mammalian pyruvate dehydrogenase complex (PDC), a gatekeeper of glucose oxidation, is composed of three catalytic enzymes: pyruvate dehydrogenase (PDH), dihydrolipoamide acetyltransferase, and dihydrolipoamide dehydrogenase. PDC activity in normal cells is controlled by the reversible phosphorylation of three serine residues in the alpha subunit of PDH by dedicated pyruvate dehydrogenase kinases 1 - 4 (PDK 1 - 4) and pyruvate dehydrogenase phosphatase 1 - 2 (PDP 1 - 2). Mechanisms governing the regulation of PDC are markedly different in cancer cells, involving novel post-translational modifications of the PDC proteins and transcriptional mechanisms. Aerobic glycolysis (the Warburg effect) appears to be a key metabolic switch with attenuation of mitochondrial oxidative metabolism in some types of tumors. In this switch, novel tyrosine phosphorylation of the specific residues in the alpha subunit of PDH, PDK1, and PDP1 proteins by oncogenic kinases cause alterations in their catalytic rates, resulting in reduction of PDC activity. Also, increased gene expression of PDK1 enhances inactivation of PDH by serine phosphorylation. In some cancer cells, to enhance the mitochondrial oxidative metabolism to meet increased energy requirement, PDC is maintained in its active state by employing yet another novel mechanism involving activated protein kinase-mediated phosphorylation of two different serine residues. Interestingly, impairment in PDC function as a major supplier of mitochondrial acetyl coenzyme A to the nuclear pool of acetyl coenzyme A is circumvented by the translocation of the PDC to the nucleus for histone acetylation. These unique cancer-specific PDC regulatory mechanisms represent an incredible advancement in our understanding of reprogramming of cellular metabolism in cancer cells.

# Oral Presentations

## Endoplasmic Reticulum-plasma Membrane Fusion at Contact Sites

**Markus Babst***

*Department of Biology, Center for Cell and Genome Science, University of Utah, Salt Lake City, USA*

### Abstract

As a consequence of hypoosmotic shock, yeast cells swell rapidly and increase the surface area by ~20% in 20s. Approximately 35% of this surface increase is mediated by the endoplasmic reticulum (ER) plasma membrane contact sites, specifically the tricalbins, which are required for the delivery of both lipids and the glycosylphosphatidylinositol-anchored protein corticotropin-releasing hormone 2 from the cortical ER to the plasma membrane. Therefore, we propose a new function for the tricalbins: mediating the fusion of the ER to the plasma membrane at contact sites. This proposed fusion is triggered by calcium influx via the stretch-gated calcium channel and is supported by the anoctamin Ist2. Similarly, we found that human tissue culture cells rapidly expand the cell surface area as a result of hypoosmotic conditions. This surface increase is accompanied by ER swelling and the influx of extracellular content into the ER, suggesting that ER-plasma membrane fusion is a conserved mechanism to prevent cell lysis during hypoosmotic shock.

## The Spatial Expression of the Developmental Patterning Cue Wnt5 is Negatively Regulated by Ion Channel Activity

**Cynthia A. Bradham***

*Department of Cell and Molecular Biology, Boston University, Boston, USA*

### Abstract

Defining pattern formation mechanisms during embryonic development is important for understanding the etiology of birth defects and to inform tissue engineering approaches. In this study, we used tricaine, a voltage-gated sodium channel (VGSC) inhibitor, to show that VGSC activity is required for normal skeletal patterning in *Lytechinus variegatus* (sea urchin larvae). We demonstrate that tricaine-mediated patterning defects are rescued by an anesthetic-insensitive version of the VGSC LvScn5a. Expression of this channel is enriched in the ventrolateral ectoderm, where it spatially overlaps with posterolaterally expressed Wnt5. We show that VGSC activity is required to spatially restrict Wnt5 expression to this ectodermal region that is adjacent and instructive to clusters of primary mesenchymal cells that initiate secretion of the larval skeleton as triradiate. Tricaine-me-

diated Wnt5 spatial expansion correlates with the formation of ectopic primary mesenchyme cells clusters and triradiate. These defects are rescued by Wnt5 knockdown, indicating that the spatial expansion of Wnt5 is responsible for the patterning defects induced by VGSC inhibition. These results demonstrate a previously unreported connection between bioelectrical status and the spatial control of patterning cue expression during embryonic pattern formation.

## Lighting Up Cellular Stress: Ultra-small Fluorescent Molecular Tools for Visualizing Oxidative Damage

**Ozlem Dilek***

*Department of Chemistry and Biochemistry, Institute for Advanced Biomedical Research, Manassas, George Mason University, USA*

### Abstract

Oxidative stress plays a central role in cancer progression, particularly in breast cancer, where aberrant accumulation of reactive oxygen species drives cellular damage and therapeutic resistance. Yet, real-time visualization of this oxidative landscape with molecular precision remains a significant challenge. Existing fluorescent probes and antibody-based tools frequently suffer from poor photostability, slow kinetics, size limitations, and inadequate stokes shifts, restricting their effectiveness in complex biological systems. To address these limitations, we developed a click chemistry-based platform for designing ultra-small fluorescent molecular probes that selectively detect carbonyl species and metal ions — two hallmark indicators of oxidative damage. Our probes exhibit broad spectral tunability, fast reaction kinetics, and high signal-to-noise ratios. Spectroscopic analysis and confocal microscopy confirmed their sensitivity and specificity across multiple cancer cell lines and tissues compared to normal counterparts. Notably, target binding induced a significant red shift in absorption and emission maxima alongside a large stokes shift, while one probe demonstrated selective metal ion chelation, triggering a "turn-on" fluorescence response ideal for dynamic biological imaging. These small-molecule probes represent a powerful and versatile platform for visualizing oxidative stress in real time, with promising implications for early cancer diagnostics and the development of oxidative stress-targeted therapeutics.

## Quality Control of Transmembrane Protein Insertion at the Endoplasmic Reticulum

**Donald J. Tipper***

*Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Cambridge, USA*

### Abstract

In 2023, I proposed that Spf1 and Ste24 and their analogs, found in all eukaryotes, comprise a quality control system that inspects nascent trans-membrane proteins, transiently engulfing them and using their inner surface negative charges to identify and retain those with positive outside peptides. These become substrates for helix dislocation (Spf1) or zinc metalloprotease fragmentation (Ste24). This quality control system is a major contributor to turnover of defective proteins.

## Fine-tuning of Chromatin Looping by Balancing Cohesin Dynamics

**Yoori Kim[1*], Jinwoo Kim[1], Chan Im[2] and Jae-Hyung Jeon[2]**

*[1]Daegu Gyeongbuk Institute of Science and Technology, South Korea*
*[2]Pohang University of Science and Technology, South Korea*

### Abstract

Chromosome organization is essential for genome segregation, gene regulation, and recombination. Structural maintenance of chromosomes (SMC) complexes, including cohesin, organize the genome by extruding deoxyribonucleic acid (DNA) loops. Cohesin also mediates sister chromatid cohesion, and its activity is regulated by several cofactors including nipped-B-like, wings apart-like protein (WAPL), and precocious dissociation of sisters 5, as well as post-translational modifications. Importantly, acetylation of the SMC3 subunit by establishment of sister chromatid cohesion n-acetyltransferase is known to stabilize cohesin on chromatin by antagonizing WAPL-mediated release, promoting cohesion and potentially modulating loop extrusion. Despite this, the impact of SMC3 acetylation on the dynamic behavior of cohesin during loop extrusion remains poorly understood. In this study, we combine single-molecule imaging and polymer modeling to explore how cohesin acetylation and its regulatory partners influence DNA loop dynamics. Our findings highlight a potential mechanism by which the reversible acetylation of cohesin fine-tunes higher-order chromatin structure, providing new insights into the interplay between biochemical regulation and 3-dimensional genome organization.

## Structural Basis of Kir7.1 Blockade: Linking Appetite Circuits and Retinal Function

**Alys Peisley[1*], Ciria C. Hernandez[1*], Naima S. Dahir[1*], Laura Koepping[1], Ashleigh Raczkowski[1], Min Su[1], Masoud Ghamari-Langroudi[2], Xinrui Ji[1], Luis E. Gimenez[1] and Roger D. Cone[1,3,4]**

*[1]Life Sciences Institute, University of Michigan, Ann Arbor, USA*
*[2]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, USA*
*[3]Department of Molecular and Integrative Physiology, School of Medicine, University of Michigan, Ann Arbor, USA*
*[4]Department of Molecular, Cellular and Developmental Biology, College of Literature, Science, and the Arts, University of Michigan, Ann Arbor, USA*

### Abstract

Kir7.1 is an inward rectifier potassium channel that acts as a critical effector of melanocortin-4 receptor (MC4R) signaling in hypothalamic circuits regulating appetite and energy expenditure. *In vivo* selective Kir7.1 blockade with ML418 depolarizes and activates paraventricular nucleus MC4R neurons, suppresses food intake, and produces acute weight loss comparable to the MC4R agonist setmelanotide. Kir7.1 also serves essential roles in extra-hypothalamic tissues. In retinal pigment epithelial cells, electrolyte homeostasis is required, and genetic loss-of-function causes inherited retinal degenerations such as Leber congenital amaurosis and snowflake vitreoretinopathy. This tissue specificity is particularly important as emerging pharmacovigilance signals now link glucagon-like peptide-1 receptor agonist obesity medications to serious ocular events, emphasizing the need for eye-safe central mechanisms of weight loss. Using single-particle cryo-electron microscopy, we determined the first structures of human Kir7.1 in an apo state and bound to the Kir7.1 selective blocker ML418, revealing how a retinitis pigmentosa–associated R162Q variant stabilizes an "open-like" docked architecture supported by defined lipid contacts, whereas ML418 binding in the central cavity disrupts a coupled transmembrane domain 1/transmembrane domain 2 interaction network, drives cytosolic-domain translation and rotation, and constricts the helix-bundle crossing into a non-conducting state. Electrophysiology shows that R162Q is a gain-of-function allele with altered ML418 sensitivity, indicating a shifted gating equilibrium. Together, these data define the molecular mechanism of Kir7.1 blockade and provide a framework for designing MC4R–Kir7.1 directed obesity therapies that achieve central appetite suppression while minimizing on-target retinal risk.

## Targeting Nuclear Kinase ULK3 in Skin Squamous Cell Carcinoma with Small-molecule Inhibitors

**Sandro Goruppi[*]**

*Chemical Genetics Program, Cutaneous Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, USA*

### Abstract

Ultraviolet-damaged skin frequently develops field cancerization (FC), a condition in which malignant keratinocytes and cancer-associated fibroblasts (CAFs) cooperate to drive squamous cell carcinoma (SCC) initiation, progression, and recurrence. Strategies targeting shared key epithelial–stromal regulatory pathways underlying tumor development remain largely unexplored. Unc-51-like kinase 3 (ULK3) is a nuclear kinase that we showed is required for SCC cell self-renewal and tumorigenic potential in the epithelial compartment, as well as for converting human dermal fibroblasts into tumor-promoting CAFs in the stroma. From a screen of 65,000 small molecules, we identified two lead compounds, A13 and C4, that effectively suppress ULK3 activity without interfering with ULK1/2. Both inhibitors recapitulate the biochemical and epigenetic effects of ULK3 genetic depletion, suppressing SCC cell proliferation with no cell toxicity. In stromal human dermal fibroblasts, both compounds downmodulate CAF-effector and activation-related signatures. Functionally, A13 and C4 significantly reduced SCC sphere formation and anchorage-independent multicellular spheroid growth, and disrupted tumor–stroma interactions by limiting SCC invasion in CAF/SCC spheroids and SCC cell expansion in CAF co-cultures. *In vivo* topical ULK3 inhibition restrained SCC expansion in an orthotopic mouse model. These findings establish first-in-class, dual-compartment ULK3 inhibitors as a promising therapeutic strategy for skin SCC and FC.

## Mechanisms Linking Diet and Metabolism to Cancer Development and Progression

**John Blenis[*]**

*Department of Cancer Research and Pharmacology, Weill Cornell Medicine, New York, USA*

### Abstract

The essential polyunsaturated fatty acid omega-6 linoleic acid (LA) is significantly elevated in the unhealthy, obesogenic "Western diet" with a ratio of LA to the omega-3 alpha-LA (ALA) of ~ 10 - 30:1. In contrast, the healthier "Mediterranean diet"

has a LA:ALA ratio closer to 1:1. We have discovered that LA binding to its receptor, fatty acid-binding protein 5 (FABP5), promotes triple negative breast (TNBC) cancer progression but not hormone responsive breast cancer (HR-BC) growth. This is due in part to the activation of mechanistic target of rapamycin complex 1 signaling in TNBC but not HR-BC which is directly linked to FABP5 expression levels. To understand this relationship *in vivo*, we have used diets high in LA vs diets high in ALA.

## Carcinogenesis and Combination Therapy: An Epigenetic Perspective

**Sibaji Sarkar[1-3*]**

[1]*Department of Biotechnology, Quincy College, Quincy, USA*
[2]*Department of Biology, MassBay Community College, Wellesley, USA*
[3]*Department of Biology, Roxbury Community College, Boston, USA*

### Abstract

Epigenetics play significant role in reversibly turning on and off important genes during development and for diverse functions in somatic cells. Epigenetic regulation involves histone modifications and DNA CpG methylation. Different types of RNAs add to this regulation. Histone modifications, such as activating histone 3 lysine 4 methylation, and histone 3 lysine 27 trimethylation inhibitory, including other histone modifications regulate gene expression in concert with alterations in DNA methylation. Our system biology analysis revealed that DNA methyl transferase1, the enzyme which maintains CpG residue methylation is allosterically activated in cancer cells. Recent studies suggest similar regulation in histone methylation/acetylation. Histone modifications and DNA methylation are associated and regulated by each other. We hypothesized that every cell type possesses an epigenetic signature, which we call, "epigenetic switch", a combination of specific histone modifications and DNA methylation. Alteration of this switch may cause different health problems including production of cancer progenitor cells. Traditional therapies do not kill cancer progenitor cells and drug-resistant cancer cells, causing cancer relapse. Interestingly, combination therapy, including epigenetic drugs, was more effective against breast and ovarian cancer cells. Our analytical study suggested that breast and ovarian cancers possibly have similar epigenetic origin. Other studies have shown that combination therapy including epigenetic drugs reduced cancer relapse, sensitized drug-resistant cancer cells, and killed cancer stem cells supporting our hypothesis that epigenetic regulation is involved in the production of cancer progenitor cells.

## Decoding the Early Glial Signature of Breast Cancer Brain Colonization

**Masakazu Kamata[1*], Naoki Hama[1], Alysha Ho[1], Gargi Chitre[1], Hunter Dickens[1] and Yoshiko Nagaoka-Kamata[2]**

[1]*Department of Microbiology, School of Medicine, University of Alabama at Birmingham, Birmingham, USA*
[2]*Department of Pathology, School of Medicine, University of Alabama at Birmingham, Birmingham, USA*

### Abstract

**Background:** Early detection of breast cancer brain metastasis is a major clinical challenge, as lesions often remain hidden until blood-brain barrier (BBB) disruption. We hypothesized that glial cell responses within the tumor microenvironment could serve as early diagnostic indicators before BBB compromise. In this study, we developed an *in vivo* system to investigate these tumor–glia interactions.

**Methods:** Breast cancer cells were injected via the tail vein. Brain metastasis and tumor development were analyzed using 3 dimensions light-sheet and confocal microscopy after tissue clearing. Glial cells were characterized via immunofluorescence (Ionized calcium-binding adaptor molecule 1 (Iba1), transmembrane protein 119, cluster of differentiation 68 ( CD68), Glial fibrillary acidic protein). BBB integrity was assessed with a 70 kDa dextran tracer. Lesion volume, glial distribution, and BBB permeability were quantified through 3D reconstruction and fluorescence analysis.

**Results:** Within four days, small metastatic deposits were primarily detected in the cerebral cortex, with no confirmed hemorrhagic BBB disruption. During these early stages, microglial activation was observed, characterized by a transition of Iba1-positive activated microglia from a ramified to an amoeboid morphology, reduced process complexity, and increased CD68 expression, even in the absence of dextran leakage. Astrocytes also accumulated in peritumoral regions, forming an early astrocytic boundary around nascent metastatic foci. As metastatic progression continued, pronounced BBB disruption associated with hemorrhagic leakage developed. These findings indicate that microglial activation and astrocytic accumulation occur during the window preceding overt BBB breakdown.

**Conclusions:** Glial activation precedes BBB disruption during early breast cancer brain colonization. These early responses may serve as biomarkers for metastatic initiation before clinical barrier breakdown.

## Identification of Isoform Switching Events Linked with Esophageal Adenocarcinoma Patient Survival Informs Novel Prognostic and Therapeutic Targets

**Yun Zhang, Jean-Jack Riethoven, Jennifer L. Clarke, Kiran H. Lagisetty, Jules Lin, Rishindra M. Reddy, Andrew C. Chang, Divid Odell and Laura A. Kresty***

*Department of Surgery, University of Michigan, Ann Arbor, USA*

### Abstract

**Background:** Esophageal adenocarcinoma (EAC) represents a growing health problem characterized by rising incidence and poor prognosis due to late-stage diagnosis coupled with therapeutic resistance. Moreover, EAC is a cancer with a high mutational burden but lacks highly prevalent oncogenic drivers that can be successfully targeted. Herein, we investigated whether newly identified isoform switching events, defined as differential usage of gene transcripts between conditions, may reveal new therapeutic targets and inform the molecular mechanisms contributing to EAC resistance.

**Methods:** We conducted RNA-sequencing followed by isoform switching analysis using IsoformSwitchAnalyzeR on Barrett's low-grade dysplasia (BE.LGD) compared to Barrett's with high-grade dysplasia (BE.HGD) + EAC tissues alone or in combination with tumor protein p53 (TP53) mutation. Patients were stratified into tertiles based on isoform fraction levels, followed by overall and cancer-free survival analysis using the Cox proportional-hazards model. To assess whether mortality-linked isoforms influence cancer cell growth, viability, migration, and response to chemotherapy (Paclitaxel and Carboplatin). Isoform-specific siRNA knockdown experiments targeting histocompatibility minor 13 (HM13) and tubulin tyrosine ligase like 12 (TTLL12) were performed in OE19 and OE33 EAC cell lines, with search tool for the retrieval of interacting (STRING) protein prediction interaction analysis and western blots conducted to assess pathway and protein alterations.

**Results:** Twenty-one isoform switched genes were significantly linked with all-cause mortality in BE.LGD versus BE.HGD + EAC and similarly 20 were significantly associated with cancer-specific patient mortality with 14 shared between the two groups. With inclusion of TP53 mutation status, 13 isoform switched genes were significantly linked with cancer-specific mortality. HM13, DXZ4 associated non-coding transcript 2,, cranio facial development protein 1, KRAS, SIMM6 were among the most significantly altered. Knockdown of the pro-cancer isoform of HM13 or TTLL12 significantly inhibited EAC cell migration and induced EAC cell death. Knockdown of HM13 or TTLL12 combined with chemotherapeutic treatment exhibited synergistic effects in EAC cell lines. Protein-interaction prediction using STRING and immunoblot analysis suggest different mechanisms leading to the inhibition of cell viability and migration. TTLL12 is linked with the activation of chaperon-medicated autophagy (lysosome-associated membrane protein 2A and HSC70) and protein translation, whereas HM13 is linked with unfolded protein response (Inositol-requiring enzyme 1-alphaa, PERK, and activating transcription factor 6) and may be linked with immunotherapy outcome.

**Conclusions:** Our research identified isoform switching events significantly linked to all-cause mortality as well as cancer specific mortality among EAC patients with and without TP53 mutations. Knockdown of the pro-cancer isoform of HM13 or TTLL12 significantly inhibited EAC cell migration and induced EAC cell death with synergistic effects observed with chemotherapeutic agent treatment supporting a role for specific isoform switches in therapeutic sensitization and in this case via the endoplasmic reticulum associated degradation and endoplasmic reticulum unfolded protein response pathways as well as through chaperone mediated autophagy. Modulation of specific proteins following isoform specific knockdown further supports an important role for specific isoform switches in therapeutic sensitization. In conclusion, isoform switches may inform molecular mechanisms contributing to poor patient outcomes and reveal new potential therapeutic targets for EAC.

## Decoding Mechanisms in Splicing Factor Mutated Cancer and Therapeutic Targeting

**Mohammad A. Rahman***

*Department of Biochemistry and Molecular Biology, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, USA*

### Abstract

Recurrent oncogenic mutations in splicing factors are most frequently identified in serine-rich splicing factor 2, splicing factor 3b subunit 1 and U2 small nuclear RNA auxiliary factor 1. These mutations usually change their RNA-binding preferences and promote genome-wide splicing alterations affecting important gene functions. Among hundreds of altered splicing events, only a few are functionally correlated with tumorigenesis (cancer drivers). Several mRNA isoforms promoted by the different splicing factor mutants comprise a premature termination codon (PTC). These PTC-containing mRNAs are degraded by nonsense-mediated mRNA decay (NMD); therefore, the cellular protein levels are downregulated. This combined regulation is called alternative splicing-coupled-NMD (AS-NMD), which plays a critical role in gene expression regulation linked with tumorigenesis. However, the precise mechanisms of splicing factor mutations in AS-NMD remained poorly understood.

AS-NMD is an intricate mechanism involving complex regulations of pre-mRNA splicing in the nucleus, mRNA decay in the cytoplasm, and a series of dynamic and coordinated choreography among RNA-binding proteins. We developed efficient molecular approaches to study the regulation of targeted genes (cancer drivers) in the entire RNA processing pathway, from splicing to decay. Using minigene reporter constructs, we selectively capture pre-RNA- and mRNA-bound protein complexes, resolve them by quantitative mass spectrometry and western blotting, and study functional coordination in the AS-NMD pathway. Furthermore, we developed molecular strategies to correct aberrant AS-NMD for therapeutic targeting. Decoding precise mechanisms of AS-NMD could potentially identify critical or/and common molecular determinants(s) in all splicing factors mutated for therapeutic targeting.

## Targeting the Aryl Hydrocarbon Receptor Suppresses the Growth of Chemoresistant Triple Negative Breast Cancer

**Anna Bianchi-Smiraglia***

*Department of Cell Stress Biology, Roswell Park Comprehensive Cancer Center, New York, USA*

### Abstract

Chemotherapy-resistance is one of the most significantly unmet clinical needs in patients with triple negative breast cancer (TNBC). Understanding the molecular pathways that impart resistance to therapy would benefit patients significantly. We recently reported on the role of the guanosine triphosphate (GTP) rate-limiting biosynthetic enzyme inosine monophosphate dehydrogenase 2 in the establishment and support of chemoresistance in TNBC and identified transcription factor aryl hydrocarbon receptor (AhR) as a negative modulator of the STING-mediated type I interferons expression in TNBC. More recently, we have revealed that AhR regulates GTP synthesis in chemoresistant TNBC settings and that its inhibition can significantly hamper the growth of chemoresistant tumors in syngeneic mouse model. Thus, AhR could represent a novel therapeutic target in recurrent/relapsing chemoresistant TNBC.

## Evolution and Targeting of Chromosome 4P Loss in Cancer

**Elena Kuzmin***

*Department of Biology, Concordia University, Canada*

### Abstract

Basal breast cancer subtype is enriched for triple-negative breast cancer (TNBC) and exhibits a recurrent large chromosomal deletion in chromosome 4p (chr4p). Chr4p loss is associated with poor survival, evolves early in tumorigenesis and confers on cells a proliferative state. Here, we map the integrated metabolic complex genetic interaction network of chr4p in basal breast cancer to identify targetable vulnerabilities. The analysis of differential gene expressions of patient derived xenografts and cancer cell models and DepMap pooled genome-wide clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9, screens revealed that chr4p loss is associated with changes in cellular energetics and reduction/oxidation balance. Functional assays revealed that chr4p loss is associated with metabolic rewiring sensitizing it for mitochondrial perturbation. We also began to apply this analytic and experimental strategy to analyze the complex genetic interaction network of chr4p loss across multiple solid cancers. Ultimately, this study sheds light on targeted therapies for cancers harboring large chromosomal deletions.

## The Role of BRD7 in Mediating Unfolded Protein Response Signaling

**Sang W. Park***

*Harvard Medical School, Boston, USA*

### Abstract

Endoplasmic reticulum (ER) stress leads to activation of a complex signaling pathway called the unfolded protein response (UPR), and chronic activation of the UPR has been shown to play a role in metabolic disorders, including insulin resistance and type 2 diabetes. While bromodomain-containing protein 7 (BRD7) has been implicated in metabolic regulation and insulin signaling, its role in coordinating UPR signaling remains poorly defined. We employed a combination of *in vitro* and *in vivo* approaches to access the UPR pathway. The three main branches of canonical UPR markers were examined, which include protein kinase R-like ER kinase, inositol-requiring enzyme 1, and activating transcription factor 6. We also analyzed downstream effector proteins and transcriptional activities. BRD7 upregulation promoted adaptive UPR signaling and improved cellular resilience to ER stress. Mechanistically, BRD7 modulated transcriptional programs involved in protein folding. These findings identify BRD7 as a critical regulator of UPR signaling and metabolic adaptation. BRD7 integrates ER stress responses with

metabolic pathways, suggesting its potential as a therapeutic target for metabolic diseases characterized by chronic ER stress.

## Targeting RNA Modifications in Pediatric Leukemia

**Qingfei Jiang***

*Division of Regenerative Medicine, Department of Medicine, Moores Cancer Center, University of California, San Diego, USA*

### Abstract

25% of pediatric T cell lymphoblastic leukemia (T-ALL) cases show resistance to chemotherapeutic agents and relapse months or years after remission. One out of four children with relapsed T-ALL will succumb to leukemia within 5 years. Our research focuses on an endonuclease called adenosine deaminase acting on RNA 1 (ADAR1), which catalyzes the transition of adenosine (A) to inosine (I) in precursor double-stranded RNA (dsRNA). The most well documented functional role of ADAR1 is to "hide" self-dsRNA dsRNA from cytosolic dsRNA sensors. In normal cells, dsRNAs generated by viral infection or endogenous sources are rigidly detected by cellular dsRNA sensors to trigger innate immune response. However, these pathways are borrowed by many cancer cells to prevent overdrive of inflammation signals and cell death. Our study indicates that a striking percentage of T-ALL patients exhibit high level of ADAR1 and hyper-RNA editing, which is associated with significantly worse clinical outcome. Indeed, inhibition of ADAR1 impairs self-renewal of leukemia initiating cells and prevent disease propagation. We further determined that although RNA editing activity of ADAR1 contributes to disease progression, RNA-editing independent activity may play more critical role in a portion of T-ALL patients. Our findings indicate that deregulated RNA editing is a critical process that fuels leukemia generation, which has important implications for T-ALL chemoresistance and therapeutic outcome.

## Understanding LINC00355 Functional Regulation of Proteins and MicroRNAs in Estrogen Receptor Late Relapse Breast Cancer

**Richa Mishra[1], Jessica Camacho[1], Brianna Duhart[1], Dhanusha Duriyan[1], Prasanth Thunuguntla[1], Kyla Gelev[1], Debbie C. Crans[2], Cynthia X. Ma[1,3] and Jessica Silva-Fisher [1,3*]**

*[1]Washington University School of Medicine, St. Louis, USA*
*[2]Colorado State University, USA*
*[3]Siteman Cancer Center McDonnell Genome Institute, USA*

### Abstract

Late-relapsed breast cancer, defined as recurrence occurring five or more years after initial treatment, remains a major clinical challenge, with a five-year survival rate of only 32% for estrogen receptor-positive (ER) patients. Despite advances in endocrine therapy, late-relapse disease remains incurable, underscoring the need to define molecular mechanisms that sustain long-term tumor persistence. Although more than 30,000 long non-coding RNAs (lncRNAs) are annotated in the human genome, only a small fraction has been functionally characterized. Our group was the first to identify deregulated lncRNAs in late-relapse ER breast cancer, identifying LINC00355 as the most highly upregulated transcript. LINC00355 has emerged as a regulator of tumor biology across multiple cancer types through both protein-binding and RNA-mediated mechanisms. In ER late-relapse breast cancer, we demonstrate that LINC00355 directly binds the tumor suppressor MENIN, promoting cell-cycle progression. Functional studies in long-term estrogen-deprived models revealed that LINC00355 drives cellular proliferation and invasion, while targeted knockdown using locked nucleic acid antisense oligonucleotides significantly reduced proliferation. To define LINC00355-associated small RNA regulatory networks, we performed small RNA sequencing and identified 588 significantly differentially expressed small RNAs, including microRNAs (miRNAs), piwi-interacting RNAs, and small nucleolar RNAs. miRNA pathway analysis using DNA intelligent analysis-miRPath revealed significant enrichment of Molecular Signatures Database Hallmark pathways, including MTORC1 signaling, G2/M checkpoint regulation, and transforming growth factor-signaling, with top enriched kyoto encyclopedia of genes and genomes pathways encompassing proteoglycans in cancer, pathways in cancer, and axon guidance. Together, these findings position LINC00355 as a central hub integrating protein and miRNA regulatory networks that promote late-relapse ER breast cancer progression.

## Loss of FAM60A Disrupts Hippo Signaling and Promotes YAP1 Activation in Breast Cancer

**Sayem Miah***

*Department of Biochemistry and Molecular Biology, Winthrop P. Rockefeller Cancer Institute (WPR-CI), College of Medicine, University of Arkansas for Medical Sciences (UAMS), Little Rock, USA*

## Abstract

FAM60A (SINHCAF) is a subunit of the Sin3/ histone deacetylases (HDAC) complex implicated in chromatin remodeling, yet its broader functional role remains unclear. Here, we define a mechanistic link between FAM60A and Hippo pathway regulation. FAM60A is associated with HDAC1 and is required for stable integration of the Sin3/HDAC complex. Disruption of HDAC1 abolishes the FAM60A–SIN3A interaction, establishing HDAC1-dependent complex assembly. Loss of FAM60A induces widespread transcriptional changes, including suppression of WWC3, a key scaffold for LATS1/2 activation. This results in reduced yes-associated protein 1 (YAP1) phosphorylation, increased nuclear YAP1 accumulation, and altered cell-cycle progression with G1 enrichment and enhanced resistance to metabolic stress. Restoration of FAM60A or WWC3 rescues LATS1/2 activity, reinstates YAP1 phosphorylation, and normalizes cellular phenotypes. These findings identify FAM60A as a critical regulator linking chromatin remodeling to Hippo signaling through control of WWC3 expression and define a FAM60A–HDAC1–WWC3 axis that modulates YAP-dependent cellular programs.

# UV-driven Epitranscriptomic Remodeling in Keratinocytes and Cutaneous Squamous Cell Carcinoma

**Stefano Sol***

*Department of Dermatology, Cutaneous Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, USA*

## Abstract

While tumor progression is classically viewed as an inexorable path toward invasion and metastasis, certain cancers enforce intrinsic mechanisms of self-restraint. The molecular and epitranscriptomic regulators underlying such tumor restraint remain poorly understood. Here, we identify an environmentally induced epitranscriptomic circuit in cutaneous squamous cell carcinoma (cSCC) that actively suppresses invasive progression. Integrative epitranscriptomic profiling, combining direct poly(A) RNA nanopore sequencing across normal skin, sun-exposed skin, and cSCC, reveals extensive remodeling of 5-methylcytosine (m5C) landscapes, with preferential enrichment at transcripts associated with cellular plasticity. Mechanistically, ultra-violet (UV) coordinately upregulates the m5C methyltransferase NSUN2 and the m5C reader Y-box binding protein 1 (YBX1),, which stabilizes anti-invasive mRNAs — including neuropilin-1, nuclear receptor binding SET domain protein 2, forkhead box K1, NSD2, and FOXK1 — through binding to structured, m5C-modified regions. Disruption of this program uncouples proliferation from invasion, promoting metastatic dissemination *in vivo*. Consistent with clinical relevance, loss of YBX1 or its target transcripts correlates with aggressive disease in patients. Together, our findings define a context-dependent, epitranscriptomic mechanism of tumor restraint that challenges prevailing linear models of cancer progression. This UV-driven m5C–NSUN2–YBX1 axis represents a new layer of cell signaling in skin cancer and exposes molecular vulnerabilities with potential for therapeutic exploitation.

# Diabetic Retinopathy is a Ceramidopathy Reversible by Anti-ceramide Immunotherapy

**Richard Kolesnick[1]* and Julia V Busik[2]**

*[1]Laboratory of Signal Transduction, Memorial Sloan Kettering Cancer Center, New York, USA*
*[2]Department of Biochemistry & Physiology, The University of Oklahoma, Oklahoma City, USA*

## Abstract

**Background:** Diabetic retinopathy is a microvascular disease that causes blindness. Using acid sphingomyelinase knockout mice, we reported that ceramide generation is critical for diabetic retinopathy development. Here, in patients with proliferative diabetic retinopathy, we identify vitreous ceramide imbalance with pathologic long-chain C16-ceramides increasing and protective very long-chain C26-ceramides decreasing. C16-ceramides generate proinflammatory/pro-apoptotic ceramide-rich platforms on endothelial surfaces.

**Methods:** To geo-localize ceramide-rich platforms, we invented a three-dimensional confocal assay and showed that retinopathy-producing cytokines tumor necrosis factor and interleukin-1 induce ceramide-rich platform formation on retinal endothelial cells within seconds, with volumes increasing 2-logs, yielding apoptotic death.

**Results:** Anti-ceramide antibodies abolish these events. Furthermore, intravitreal and systemic anti-ceramide antibodies protect from diabetic retinopathy in standardized rodent ischemia reperfusion and streptozotocin models.

**Conclusion:** These data support (1) retinal endothelial ceramide as a diabetic retinopathy treatment target, (2) early-stage therapy of non-proliferative diabetic retinopathy to prevent progression, and (3) systemic diabetic retinopathy treatment; and they characterize diabetic retinopathy as a "ceramidopathy" reversible by anti-ceramide immunotherapy.

## Detection of a Novel Xanthone Compound from *Garcinia cowa* Leaf Extract Demonstrating Promising Anti-lung Cancer Effects

**Santanu Paul***

*Department of Botany, University of Calcutta, Kolkata, West Bengal, India*

### Abstract

The leaf methanolic extract of *Garcinia cowa* (Clusiaceae) was previously shown by us to exert strong anti-proliferative effects on A549 cells. Now, we have isolated and characterized the bioactive lead fraction responsible for this effect using bioactivity-guided fractionation. Liquid chromatography–mass spectrometry analysis of the active fraction identified cowaxanthones H, cowaxanthone, mangostin, and guttiferone F, with mangostin, a polyphenolic xanthone, representing the most abundant component based on chromatographic area. Commercially sourced mangostin was further assessed for its anticancer efficacy in human A549 lung cancer cells, alongside evaluation of its cytotoxicity in normal cells. Treatment with mangostin resulted in a marked reduction in cell proliferation, increased apoptosis, and cell cycle arrest at the G0/G1 phase in A549 cells. Apoptotic effects were confirmed by AO-EtBr staining and nuclear fragmentation observed through 4′,6-diamidino-2-phenylindole staining. Mechanically, mangostin-induced apoptosis was associated with elevated intracellular reactive oxygen species levels, mitochondrial membrane depolarization, and upregulation of caspase-3 and caspase-9 expressions. Notably, mangostin exhibited minimal cytotoxicity in normal cells. These findings highlight mangostin's potent and selective anti-cancer properties, suggesting its promise as a potential therapeutic agent for lung cancer treatment.

## Autoimmune Diseases of the Basement Membrane Zone: Mechanisms and Clinical Implications

**Dewan Raja***

*Department of Clinical Anatomy, AUC School of Medicine, Netherland Antilles*

### Abstract

The basement membrane zone (BMZ) of the skin is a vital structural and functional interface between the epidermis and dermis. Autoimmune subepidermal blistering diseases that target the BMZ include bullous pemphigoid, mucous membrane pemphigoid, linear IgA disease (LAD), and epidermolysis bullosa acquisita. These conditions are defined by autoantibodies directed against BMZ components such as BP180 (type XVII collagen), BP230, laminin-332, type VII collagen, and LAD antigen-1. Binding of these autoantibodies initiates complement activation, inflammatory cell recruitment, and subepidermal blister formation. Each disease has distinct clinical presentations and immunopathologic features. Bullous pemphigoids typically affect the elderly with widespread tense bullae, while mucous membrane pemphigoid involves mucosal scarring. LAD may mimic dermatitis herpetiformis and occurs in both pediatric and adult populations. Diagnosis relies on direct immunofluorescence microscopy, which demonstrates linear IgG, IgA, or C3 deposition along the BMZ, supported by serologic detection of circulating autoantibodies. Advances in understanding BMZ autoimmunity have led to emerging targeted therapies, including B-cell depleting agents (e.g., rituximab), Interleukin (IL)-4/IL-13 blockers (e.g., dupilumab), and novel immunomodulators that minimize systemic immunosuppression. Despite therapeutic progress, challenges persist in managing severe and relapsing cases. This presentation will explore the molecular mechanisms, clinical features, diagnostic techniques, and current and future treatment strategies for autoimmune BMZ diseases. Emphasis will be placed on how insights into autoimmunity at the dermal-epidermal junction inform precision approaches in dermatologic care.

## MicroRNA-219 is Important for Glutamatergic Synaptic Plasticity in Drosophila

**Kumar Aavula*, Hansine Heggeness and David Van Vactor**

*Harvard Medical School, Boston, USA*

### Abstract

MicroRNAs (miRNAs) are known to be critical regulators of nervous system development, neural circuit function and plasticity that coordinately regulate the levels of many target effector proteins. This regulatory system is also highly relevant to neurological and neurodegenerative disease. To identify miRNAs involved in activity-induced synaptic remodeling, we performed a targeted functional screen by expressing miRNA-specific sponges in motor neurons and subjecting larvae to a well-established high potassium (K) space depolarization protocol developed by colleagues in the Budnik laboratory. This simple and elegant paradigm, consisting of cycles of high K stimulation followed by rest periods, induces the formation of immature synaptic boutons at the larval glutamatergic neuromuscular junction within tens of minutes. Nascent "ghost" boutons are defined by their transient lack of postsynaptic density cytomatrix and presynaptic active zones and serve as a reliable initial feature of acute

synaptic structural plasticity. Our screen revealed that knockdown of multiple developmentally important miRNAs - miR-13a, miR-14, miR-34, and miR-219 -significantly impaired ghost bouton formation in response to high K stimulation. Deeper analysis of the highly conserved miR-219 and a key target protein reveal an activity-dependent mechanism to coordinately control multiple aspects of synaptic cell biology.

## Exosome Mediated Suppression of Liver Regeneration Exposes a Fundamental Revision to the Chalone Concept

**Mina McGinn, Christopher Rabender, Ross Mikkelsen and Vasily Yakovlev***

*Department of Radiation Oncology, Massey Cancer Center, Virginia Commonwealth University, Richmond, USA*

### Abstract

Despite decades of investigation into liver regeneration, key regulatory mechanisms governing the precise initiation and termination of hepatocyte proliferation remain poorly defined. This unresolved question represents a classical chalone concept – tissue-specific, endogenous inhibitors that maintain organ size homeostasis through feedback control of cell proliferation. Substantial evidence supports the existence of a putative inhibitor of liver cell proliferation (ILCP), yet its molecular identity and downstream targets remain unknown. Emerging data suggests extracellular vesicles (EVs) may fulfill this role. EVs are nanoscale (30 - 150 nm) membrane-bound particles secreted by most cell types that carry proteins and RNAs, including microRNAs (miRNAs), and mediate intercellular communication by transferring bioactive cargo to recipient cells. Using complementary *in vivo* and *in vitro* models, we demonstrated that hepatocyte-derived EVs (HD-EVs) act as a functional ILCP. Circulating EV levels correlate with total liver mass, consistent with a feedback-based mechanism. Following partial hepatectomy, dynamic changes in HD-EV abundance tightly regulate hepatocyte proliferation, ensuring accurate restoration of liver size to its physiological set point. We further identified HD-EV associated miRNAs as candidate effectors, suggesting ILCP activity is mediated, in part, through miRNA dependent regulation of hepatocyte gene expression. These findings provide a molecular framework for the modern revision of the chalone paradigm. We hypothesize that disruption of this HD-EV mediated feedback represents an early event in hepatocarcinogenesis, whereby loss of ILCP function contributes to hepatocellular carcinoma development. Ongoing studies aim to define ILCP associated miRNAs and their targets, establishing a novel mechanism of liver regeneration with significant therapeutic potential.

## Autism-linked Activity-dependent Neuroprotective Protein and Cortical Development

**Kazuhito Toyooka***

*Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, USA*

### Abstract

Activity-dependent neuroprotective protein (ADNP) is an essential protein for cortical development, neuronal morphogenesis, and circuit development, which is linked to autism. Mutations and deficiencies in this gene result in ADNP syndrome, which is a neurodevelopmental disorder linked to autism spectrum disorder (ASD) and characterized by complex clinical symptoms, including intellectual disability and general developmental delays. The previous studies mainly focused on the effects of davunetide, an 8-amino acid snippet derived from ADNP. Our study focused on the crucial role of full-length ADNP in cortical development. We used *in vivo* and *in vitro* knockdown (KD) models and revealed that ADNP strictly regulates neuronal morphogenesis. ADNP has a negative impact on neurite formation, resulting in an increase in neurite initiation, basal dendrite formation, and interhemispheric axon length after KD. ADNP has both nuclear transcription factors and cytoplasmic microtubule regulator functions. Its cytoplasmic distribution is regulated by binding to 14-3-3 proteins, which allows ADNP to move and maintain in the cytoplasm, where it works as a microtubule regulator. Furthermore, ADNP is essential for proper synaptogenesis; its deficiency leads to decreased dendritic spine density, impaired maturation, and disrupted corticocortical connections. Functionally, using a calcium indicator, we found altered calcium signaling, particularly within the female cortex. These results indicate that ADNP is crucial for the proper formation of cortical circuits, and its malfunction could be the cause of the neurological deficits observed in ASD.

## Revealing Glial Diversity and Molecular Clocks across Human Hippocampal Postnatal Lifespan and in Alzheimer's Disease using Single-cell RNA-seq Technology

**Yijing Su***

*Department of Oral Medicine, University of Pennsylvania, Pennsylvania, USA*

## Abstract

The molecular diversity of glial cells in the human hippocampus and their temporal dynamics over the lifespan remain largely unknown. Here we performed single-nucleus RNA sequencing to generate a transcriptome atlas of the human hippocampus across the postnatal lifespan. Detailed analyses of astrocytes, oligodendrocyte lineages, and microglia identified subpopulations with distinct molecular signatures and revealed their association with specific physiological functions, age-dependent changes in abundance, and disease relevance. We further characterized spatiotemporal heterogeneity of glial fibrillary acidic protein-enriched astrocyte subpopulations in the hippocampal formation using immunohistology. Leveraging glia subpopulation classifications as a reference map, we revealed diversity of glial cells differentiated from human pluripotent stem cells and identified dysregulated genes and pathological processes in specific glia subpopulations in Alzheimer's disease (AD). Together, our study significantly extends our understanding of human glial cell diversity, population dynamics across the postnatal lifespan, and dysregulation in AD, and provides a reference atlas for stem cell-based glia differentiation.

# Epigenetic Mechanisms of Liver Aging

**Nishat Sarker, Na Yang and Payel Sen***

*Laboratory of Genetics and Genomics, National Institute on Aging, National Institutes of Health, Baltimore, USA*

## Abstract

The liver exhibits striking spatial organization and profound age- and sex-dependent functional changes, yet the regulatory mechanisms remain incompletely understood. Prior single-cell studies have described age-related loss of hepatocyte zonation, immune remodeling, and metabolic decline, but lack integrated transcription factor mapping, and systematic sex comparisons. Therefore, we generated a lifespan- and sex-resolved single-cell multi-omics atlas of mouse liver integrating ribonucleic acid and assay for transposase-accessible chromatin modalities. By profiling five age groups, our data reveal continuous and non-linear regulatory dynamics that cannot be inferred from binary young-old contrasts. This resource identifies cell-type-, zone-, age-, and sex-resolved transcription factor regulation, oscillatory metabolic programs, emergence of distinct senotypes, and extensive chromatin plasticity. We further uncover a transient, lineage-inappropriate gene expression pattern in female hepatocytes with age. Together, our study defines the spatiotemporal regulatory logic of hepatic aging in both sexes.

# Identification of GULF, a Functionally Conserved Human Long Noncoding Ribonucleic Acid Motif as a Potential Glucose and Lipid-lowering Therapeutic

**Haiming Cao***

*National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, USA*

## Abstract

Long noncoding ribonucleic acid (lncRNAs) are increasingly linked to metabolic disease, but their therapeutic development has been limited by poor sequence conservation across species. To address this challenge, we developed a discovery framework based on functionally conserved lncRNAs, which retain biological function despite limited sequence similarity, and applied it to identify human lncRNA targets for metabolic disorders. Using expression quantitative trait locus mapping together with functional conservation analyses, we identified a human lncRNA and its mouse functional counterpart, termed glucose and lipid lowering (GULL). Although these transcripts share little sequence similarity, both improved glucose and lipid metabolism in obese mice, with greater benefit after sustained activation. Mechanistically, human and mouse GULL bind CREB-regulated transcription coactivator 2 (CRTC2) and regulate metabolic gene programs by modulating CAMP response element-binding-dependent gluconeogenic pathways and sterol regulatory element-binding protein 1-driven lipogenic pathways. To define a therapeutically tractable element, we mapped the CRTC2-binding region and identified a short functional motif, termed GULF. A standalone human GULF RNA oligomer, resembling clinically used oligonucleotide therapeutics, was sufficient to lower blood glucose and circulate lipids in obese mice. Together, these findings establish a strategy for identifying therapeutically relevant human lncRNAs through functional conservation and highlight GULF as a promising RNA therapeutic candidate for glucose and lipid lowering.

# Cell Based Therapeutic Approaches for Airway Regeneration

**Golnaz Karoubi[1,2]***

[1]*Latner Thoracic Research Laboratories, University Health Network, Toronto, Canada*
[2]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada*

## Abstract

Autologous cell-based therapies hold tremendous promise for lung regenerative medicine, but their clinical application is limited by the availability of suitable progenitor cell sources that can expand sufficiently while maintaining lineage specificity and functional capacity. We have developed an "interrupted reprogramming" strategy to address this fundamental challenge by generating induced progenitor-like (iPL) cells from patients' own terminally differentiated lung epithelial cells. This approach uses carefully timed, transient expression of pluripotency factors (Oct4, Sox2, Klf4, and c-Myc; OSKM) to unlock proliferative capacity while preserving tissue-specific differentiation potential. In murine proof-of-concept studies, we demonstrated the versatility of this autologous cell expansion strategy across distinct lung epithelial populations. Club cell-derived iPL cells functioned as bronchiolar progenitors, generating mature Club cells, mucin-producing goblet cells, and cystic fibrosis transmembrane conductance regulator (CFTR)-expressing ciliated epithelium under clonogenic conditions, with demonstrated capacity to repopulate CFTR-deficient epithelium *in vivo*—a critical requirement for cystic fibrosis cell therapy. Alveolar epithelial type II (ATII) cell-derived iPL cells overcame the limited clonogenic capacity of primary ATII cells, expanded in a controlled manner while maintaining alveolar lineage commitment, and ameliorated bleomycin-induced pulmonary fibrosis when transplanted into injured lungs. Importantly, by precisely controlling OSKM expression duration, iPL cells avoid full reprogramming to pluripotency while retaining lineage memory, allowing efficient return to their original functional phenotype. A major limitation of autologous cell therapies is age-related decline in donor cell quality, particularly relevant for idiopathic pulmonary fibrosis where aging and repetitive ATII cell injury drive pathogenesis in elderly patients. We are translating our interrupted reprogramming approach to human ATII cells to address this challenge. We have established novel isolation strategies and 3-dimensional alveolosphere cultures that preserve the pro-surfactant protein C positive phenotype. Comparative studies reveal that aged (≥65 years) versus young (≤35 years) human ATII cells show increased senescence markers and reduced proliferative potential. We hypothesize that transient OSKM expression will induce epigenetic remodeling to generate rejuvenated ATII-iPL cells with restored functionality. This interrupted reprogramming platform addresses key limitations in autologous cell therapy—limited cell numbers, loss of progenitor capacity, and age-related dysfunction—establishing a practical strategy for generating functional therapeutic cells from patients' own tissue across multiple lung diseases including cystic fibrosis, pulmonary fibrosis, and age-related lung dysfunction.

# A New Epigenetic Layer within the Genome: DNA-embedded Ribonucleotides Regulate DNA

**Deepali L. Kundnani[1], Yeunsoo Lee[1], Tyler Warner[1], Taehwan Yang[1], Tejasvi Channagiri[2], Francisco Martinez-Figueroa[2], Ryan Eckerty[1], Penghao Xu[1], Zachary F. Pursell[3], Nataša Jonoska[2] and Francesca Storici[1*]**

*[1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, USA*
*[2]Department of Mathematics and Statistics, University of South Florida, Tampa, USA*
*[3]Department of Biochemistry and Molecular Biology, Tulane Cancer Center, Tulane University School of Medicine, New Orleans, USA*

## Abstract

Ribonucleoside monophosphates (rNMPs) represent the most prevalent non-canonical nucleotides incorporated into genomic DNA; however, their genome-wide distribution and functional roles in human cells are still not well characterized. Here, we generate high-resolution maps of approximately one million rNMPs per genome across diverse human cell types, revealing a non-random nuclear "ribome" enriched in C/G-rich sequences, epigenetic features, and telomeric regions. We identify discrete ribonucleotide-enriched zones (REZs) that preferentially localize near transcription start sites (TSSs), overlap with cytosine-phosphate-guanine islands and R-loops, and scale with gene expression, indicating a close relationship between rNMP incorporation, transcriptional activity, and local DNA topology. Loss of RNase H2, the primary ribonucleotide excision repair enzyme, leads to elevated rNMP abundance and pronounced strand-biased accumulation near transcription start sites, particularly for riboguanosine monophosphates. These patterns are consistent with transcription-coupled and strand-selective rNMP processing. Increased rNMP enrichment within REZs in RNase H2-deficient cells further indicates that these regions are sites of active rNMP turnover under normal conditions. Cleavage of embedded rNMPs by RNase H2 introduces transient nicks in the DNA backbone that alter local DNA topology and relieve torsional stress. Strand-specific rNMP distributions observed in the absence of RNase H2, together with attenuation of this strand bias upon depletion of topoisomerase I (Top1), are consistent with a potential auxiliary role for Top1 in mitigating torsional stress in highly transcribed regions. Together, these findings establish DNA-embedded rNMPs as a transcription-associated epigenetic feature that contributes to genome topology control and gene regulation in human cells.

## PARP Inhibition Sensitizes H3K27M Diffuse Midline Glioma to Radiotherapy and Natural Killer Cell-mediated Immunity

**Qiang Zhang[1*], Yupei Guo[1], Zian Li[1], Leslie A. Parsels[1], Zhuwen Wang[1], Joshua D. Parsels[1], Anushka Dalvi[1], Stephanie The[1], Sriram Venneti[2], Daniel R. Wahl[1], Michael D. Green[1], Theodore S. Lawrence[1], Carl Koschmann[3] and Meredith A. Morgan[1]**

*[1]Department of Radiation Oncology, Rogel Cancer Center, University of Michigan, USA*
*[2]Department of Pathology, Rogel Cancer Center, University of Michigan, USA*
*[3]Department of Pediatrics, Rogel Cancer Center, University of Michigan, USA*

### Abstract

Radiotherapy (RT) is the primary treatment for diffuse midline glioma (DMG), a lethal pediatric malignancy defined by histone H3 lysine 27-to-methionine (H3K27M) mutation. Based on the loss of H3K27 trimethylation producing broad epigenomic alterations, we hypothesized that H3K27M causes a functional double-strand break repair defect that could be leveraged therapeutically with poly (ADP-ribose) polymerase (PARP) inhibitor and RT for selective radiosensitization and antitumor immune responses. Here we show that H3K27M mutation caused a homologous recombination repair defect characterized by impaired RT-induced K63-linked polyubiquitination of histone H1 and inhibition of homologous recombination repair protein recruitment. H3K27M DMG cells were selectively radiosensitized by olaparib in comparison to isogenic controls, and this effect translated to efficacy in H3K27M orthotopic brainstem tumors. Olaparib and RT induced an innate immune response and induction of natural killer (NK) cell (NKG2D) activating ligands leading to increased NK cell-mediated lysis of DMG tumor cells. In immunocompetent syngeneic orthotopic DMG tumors, either olaparib or AZD9574 in combination with RT enhanced intratumoral NK cell infiltration and activity in association with NK cell-mediated therapeutic responses and favorable activity of AZD9574. The HRR deficiency in H3K27M DMG can be therapeutically leveraged with PARP inhibitors to radiosensitize and induce an NK cell-mediated antitumor immune response selectively in H3K27M DMG, supporting the clinical investigation of best-in-class PARP inhibitors with RT in DMG patients.

## Epigenetic Regulation of Sex-divergent Aortic Remodeling

**Yasir Alsiraj[*]**

*Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, USA*

### Abstract

Aortic aneurysms are sexually dimorphic diseases, with males exhibiting greater prevalence and severity than females. While hormonal influences have been extensively investigated, emerging evidence suggests that gene dosage effects of X-linked epigenetic regulators may contribute to sex-specific vascular phenotypes. We previously demonstrated that an XX sex chromosome complement confers protection against AngII-induced aortopathy. Among X-linked genes escaping inactivation, the histone demethylase Kdm5c is expressed at higher levels in XX compared to XO aortas, suggesting a potential gene dosage–dependent mechanism. To directly test this hypothesis, we generated female mice globally hemizygous for Kdm5c (Kdm5c+/) and compared them to wild-type (Kdm5c+/+) controls in a hypercholesterolemic AngII infusion model. Kdm5c+/- females exhibited increased baseline aortic lumen diameters and developed significantly greater ascending and abdominal aortic dilation following AngII infusion. Endpoint analyses demonstrated increased maximal external diameters, aortic weights, and arch area in hemizygous mice, indicating exacerbated remodeling and pathology. These findings demonstrate that reduced gene dosage of the epigenetic modifier Kdm5c promotes susceptibility to aortic remodeling, identifying epigenetic regulation as a mechanism contributing to sex-divergent vascular disease. Ongoing studies aim to define downstream chromatin and transcriptional targets mediating these effects.

## Neurotrophin 4 Plays a Dual Role in Breast Cancer in Mammary Tumorigenesis and Metastatic Progression

**Ran Sun[1,2*], Jin He[1], Qin Xiang[1], Yixiao Feng[1], Yijia Gong[1], Yijiao Ning[1], Chaoqun Deng[1], Kexin Sun[1], Mingjun Zhang[3], Zhaobo Cheng[4], Xin Le[1], Qi Xiong[1], Fengsheng Dai[1], Yongzhong Wu[4] and Tingxiu Xiang[1,4]**

*[1]Department of Oncology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China*
*[2]Department of Oncology, Jiulongpo People's Hospital, Chongqing, China*
*[3]Department of Laboratory Medicine, Jiulongpo People's Hospital, Chongqing, China*

[4]Chongqing Key Laboratory of Translational Research for Cancer Metastasis and Individualized Treatment, Chongqing University Cancer Hospital, Chongqing, China

## Abstract

Breast cancer metastasis can happen even when the primary tumor is relatively small. But the mechanism for such early metastasis is poorly understood. Herein, we report that neurotrophin 4 (NTF4) plays a dual role in breast cancer proliferation and metastasis. Clinical data showed high levels of NTF4, especially in the early stage, to be associated with poor clinical outcomes, supporting the notion that metastasis, rather than primary cancer, was the major determinant of breast cancer mortality for patients. NTF4 promoted epithelial-mesenchymal transition, cell motility, and invasiveness of breast cancer cells *in vitro* and *in vivo*. Interestingly, NTF4 inhibited cell proliferation while promoting cellular apoptosis *in vitro* and inhibited xenograft tumorigenicity *in vivo*. Mechanistically, NTF4 elicited its pro-metastatic effects by activating PRKDC/AKT and ANXA1/NF- B pathways to stabilize SNAIL protein, therefore decreasing the level of E-cadherin. Conversely, NTF4 increased ANXA1 phosphorylation and sumoylation and the interaction with importin, leading to nuclear import and retention of ANXA1, which in turn activates the caspase-3 apoptosis cascade. Our findings identified an unexpected dual role for NTF4 in breast cancer which contributes to early metastasis of the disease. Therefore, NTF4 may serve as a prognostic marker and a potential therapeutic target for breast cancer.

## Cisplatin Resistance is Mediated by Both Up and Down-regulation of Serine Arginine Protein Kinase1 in Different Cancers

**Duygu Duzgun and Sebastian Oltean***

*Department of Clinical and Biomedical Sciences, University of Exeter Medical School, Exeter, UK*

## Abstract

**Introduction:** Abnormal pre-mRNA alternative splicing (AS) is critical in shaping tumor chemoresistance by modulating the function of genes involved in resistance mechanisms. The serine arginine protein kinase 1 (SRPK1) is a major regulator of AS by phosphorylating an important group of splice factors named SR-proteins. Our study aims to elucidate the molecular mechanisms by which SRPK1 promotes resistance to chemotherapeutic drugs.

**Methods:** Two cell lines resistant to cisplatin (CDDP) (breast cancer MDA-MB-231-CDDP-R and colon cancer HCT-116-CDDP-R) were established by derivation from their parental using the pulse selection method. The CDDP resistance and the expression of SRPK1 of cells were verified by the IC50 measurements, MTT assay and western blotting (WB). The molecular mechanisms were determined using WB, siRNA, immunoprecipitation, the JC-10 apoptosis assay, and RT-PCR.

**Results:** The MTT assay revealed that resistant cell lines were about 4 - 5 times more resistant to chemotherapeutic agents than parental cell lines. SRPK1 protein was upregulated in MDA-MB-231-CDDP-R; however, its expression in HCT-116-CDDP-R cells was markedly lower than that in parental cells. Using siRNA to knockdown SRPK1 expression lead to the re-sensitization of the MDA-MB-231-CDDP-R to CDDP, but this effect was not observed in SRPK1-downregulated HCT-116-CDDP-R cells. Both WB and JC-10 assay demonstrated that co-treatment with CDDP and SRPK1 specific inhibitor, SPHINX31, remarkably upregulated pro-apoptotic markers (cleavage of caspase-8, caspase-3, and PARP) in MDA-MB-231-CDDP-R, compared with CDDP and SPHINX31 alone conditions. Notably, in SRPK1 down-regulated HCT-116-CDDP-R cells, transient transfection of SRPK1 increased the sensitivity of the cells to the cytotoxicity of CDDP. JC-10 assay results also indicated an increase in apoptosis upon CDDP or a combination treatment with SPHINX31 in HCT-116-CDDP-R cells transfected with SRPK1. Moreover, immunoprecipitation assay showed that the level of SRSF1, phosphorylated by SRPK1, was elevated in CDDP-treated MDA-MB-231-CDDP-R cells. Mechanistically, SRSF1 modulates various cancer-related splicing events, particularly the splicing of BCL2L1 and MCL-1. SPHINX31 dramatically promoted the pro-apoptotic Bcl-xS and Mcl-1S isoforms in MDA-MB-231-CDDP-R cells as compared to control.

**Conclusion:** Our research reveals a key role for SRPK1 in chemoresistance by modulation of apoptotic genes splicing, suggesting a potential therapeutic avenue for alleviating challenges posed by chemoresistance. Down-regulation of SRPK1 results in hypo-phosphorylation of splice factors like SRSF1 and up-regulation results in hyperphosphorylation; both hypo- and hyper-phosphorylation of splice factors have been shown to inactivate them.

## Extracellular Vesicle-mediated Communication in Health and Disease: Effectors, Biomarkers and Theranostics Tools

**Irina Matei***

*Department of Pediatrics, Drukier Institute for Children's Health, Weill Cornell Medicine, Meyer Cancer Center, New York, USA*

## Abstract

Extracellular vesicle (EV)-based communication is an evolutionarily conserved mechanism of intercellular communication, from bacteria to humans. Eukaryotic EVs are nanosized particles of endosomal origin that carry and deliver to target cells complex cargo including DNA, RNA, proteins, metabolites, etc., leading to functional changes and reprogramming of target cells. As long-range messengers, EVs elicit as well as report systemic alterations, and are responsible for many of the systemic effects of cancer as well as other pathologies such as autoimmune diseases. We have developed novel *in vivo* approaches to dissect the EV-mediated mechanisms driving these effects, including pre-metastatic niche formation, thrombosis and cachexia, and used these to guide biomarker discovery for early cancer detection and responses to treatments. The theranostic and biomarker potential of EVs in systemic diseases will be discussed, as will be the mechanisms underlying their functions in homeostasis and disease. Finally, we will discuss how these mechanistic insights can be exploited to design new therapies and improve cancer outcomes, especially for solid tumors, where treatment options for metastatic disease are limited.

# Cancer Prevention Efficacy of Embryonic Stem Cells

**Chi Li[1,2*] and Kavitha Yaddanapudi[3-5]**

[1]*Experimental Therapeutics Group, Brown Cancer Center, Department of Medicine, University of Louisville, Louisville, USA*
[2]*Department of Pharmacology and Toxicology, University of Louisville, Louisville, USA*
[3]*Department of Microbiology and Immunology, University of Louisville, Louisville, USA*
[4]*Immuno-Oncology Program, Brown Cancer Center, University of Louisville, Louisville, USA*
[5]*Division of Immunotherapy, Department of Surgery, University of Louisville, Louisville, USA*

## Abstract

Based on the antigenic similarity between tumor cells and embryonic stem cells (ESCs), we explored the potential of ESCs functioning as a cancer vaccine. We have discovered that a vaccine based on ES-D3, a murine ESC line, successfully blocked the outgrowth of an implantable lung cancer and lung tumors caused by a combination of a mutagen and chronic pulmonary inflammation. Since the capacity for self-renewal is one of the most specialized properties shared between ESCs and a subset of tumor cells, cancer stem cells, we investigated whether the undifferentiated state of murine ESCs is essential for the prophylactic effectiveness of ESC-based vaccine. The undifferentiated state of ES-D3 was essential for their anchorage-independent growth potential. Importantly, differentiation of ES-D3 cells decreased their efficacy in preventing the outgrowth of implanted lung tumors. Furthermore, the long-term cancer-preventive potential of this vaccine was also inhibited by the differentiation of these cells. To examine the antigenicity of the ESC-derived vaccine, we performed combined affinity chromatography shotgun immunoproteomic experiments to identify antigens specific to the whole-cell ES vaccine as well as to the ESC-derived exosome vaccine. Our data identified keratins 8, 16, and 17 as potential lung tumor-associated target antigens. In summary, these data suggest that the tumor-preventing efficacy of ESC-based vaccine is reliant on the differentiation properties of these stem cells.

# Can Acute Myeloid Leukemia Show Mast Cell Differentiation?

**Jie Xu***

*Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, USA*

## Abstract

Blasts in acute myeloid leukemia (AML) can show differentiation toward various lineages, including monocytic, erythroid, megakaryocytic, and plasmacytoid dendritic cell lineage. The incidence and clinicopathological features of mast cell (MC) differentiation in AML is largely unknown. Among 2,167 AML cases, we identified 21 (~1%) cases of AML with MC differentiation (AML-MC), defined as: (1) an increased immature MC population (>0.3%) by flow cytometry immunophenotyping; (2) cells with metachromatic granules observed on bone marrow aspirate smears; and (3) >1% MCs shown by tryptase immunohistochemistry in biopsy specimens. The median age of these patients was 68 years. The MCs consistently had low side scatters, consistent with immature and hypo granular forms, and were positive for CD38, CD123 and CD45 (dim), partially positive for CD34 in 81%, positive for CD25 in 33% of cases, and consistently negative for CD2. Tryptase immunohistochemistry showed interstitial MCs. Conventional chromosomal analysis showed a complex karyotype in 13 (68%) cases. Recurrent translocations were identified in 5 cases, including t(9;22)(q34.1;q11.2), inv(16)(p13.1q22), and t(8;21)(q22;q22.1). Fluorescence *in situ* hybridization showed TP53 deletion in 9 (43%) cases. Next generation sequencing showed TP53 mutations in 11 of 21 (52%) cases analyzed. All cases were negative for KIT mutation. Patients with AML-MC had a very poor outcome, with a median OS of 9.6 months. OS was significantly associated with older age (>65 years; $p = 0.005$). In conclusion, patients with AML-MC are characterized by older age, interstitial MCs, complex karyotype, TP53 alterations, and poor prognosis.

## Targeting Immune Suppression in the Tumor Microenvironment of Pancreatic Cancer

**Yaqing Zhang[1,2*], Wei Yan[1], Rosa E. Menjivar[3], Katelyn L. Donahue[4], Kristee L. Brown[1], Ashley Velez-Delgado[5], Monica E. Bonilla[4], Nina G. Steele[5], Samantha B. Kemp[6], Eileen S. Carpenter[7], Faith R. Avritt[8], Sion Yang[8], William R. Burns III[1], Timothy L. Frankel[1,2], Filip Bednar[1,2] and Marina Pasca di Magliano[1-5]**

*[1]Department of Surgery, University of Michigan, Ann Arbor, USA*
*[2]Rogel Cancer Center, University of Michigan, Ann Arbor, USA*
*[3]Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, USA*
*[4]Cancer Biology Program, University of Michigan, Ann Arbor, USA*
*[5]Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, USA*
*[6]Molecular and Cellular Pathology Graduate Program, University of Michigan, Ann Arbor, USA*
*[7]Department of Internal Medicine, Division of Gastroenterology, University of Michigan, Ann Arbor, USA*
*[8]College of Literature, Science, and The Arts, University of Michigan, Ann Arbor, USA*

### Abstract

The tumor microenvironment of pancreatic ductal adenocarcinoma (PDA) includes abundant fibroblasts and infiltrating immune cells, the latter largely immunosuppressive. Immunotherapy approaches have been ineffective in PDA, pointing to the need for a better understanding of the mechanisms of immune suppression. To understand the contribution of regulatory T cells (Tregs) to the immunosuppressive microenvironment, we depleted Tregs into a mouse model of pancreatic cancer. Contrary to our expectations, Treg depletion failed to relieve immunosuppression, and led to accelerated tumor progression. Tregs depletion reprogramed the fibroblasts and increased immunosuppressive macrophages. Further, we sought to target tumor-associated macrophages (TAMs) in pancreatic cancer using a combination of genetically engineered mouse models and pharmaceutical approaches. TAMs with activated Notch signaling expressed higher levels of immunosuppressive mediators, suggesting that Notch signaling plays a role in macrophage polarization within the PDA microenvironment. In the orthotopic PDA mouse model, genetic inhibition of Notch in myeloid cells reduced tumor size and intratumoral macrophage infiltration. Neither pharmacological Notch inhibition with LY3039478, an orally active Notch and secretase inhibitor, nor anti-PD1 immune checkpoint blockade were effective in treating PDA *in vivo*, whereas combined inhibition of the Notch pathway and immune checkpoint resulted in significant reduction in tumor growth. Our data demonstrates that Notch activation within TAMs from murine PDAs is associated with an immunosuppressive (M2-like) phenotype. Targeting the Notch signaling pathway in combination with immune checkpoint blockade improves antitumor efficacy in pancreatic cancer.

## The World of RNA Granules - More Than Storage Sites

**Tomas Grousl***

*Laboratory of Cell Signalling, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*

### Abstract

A conserved evolutionary strategy of cells under stress conditions is to shut down protein synthesis and form RNA and protein granules. These granules are stress granules (SGs) and processing bodies (P-bodies), or aggregates of misfolded proteins. SGs and P-bodies, which harbor components of the translation machinery or mRNA decay factors, are believed to help the cell efficiently restart protein synthesis after stress relief. On the other hand, aggregates of misfolded proteins formed by small heat shock proteins alleviate the burden on the chaperone-mediated refolding machinery during stress conditions. Yeast has been instrumental in our understanding of SGs/P-bodies and the biology of protein aggregates. Although all the details of the functioning of these granules in cellular metabolism are not yet completely understood, their beneficial contribution to cellular proteostasis is undoubted.

## Actin Organization Controls Migration Signaling Networks and Cell Polarity

**Jonathan Kuhn***

*Department of Cell Biology, Johns Hopkins University, Baltimore, USA*

### Abstract

To migrate rapidly, many cells, including immune cells and invasive cancers, adopt polarized Laura Krestyamoeboid morphology. In this morphology, sustained forward motion requires the coordination of two actin networks: polymerizing branched actin at the front and contractile actomyosin at the back. Their assembly is guided by signaling molecules like activated Ras-GTPase and PI(3, 4, 5)P3 at the front and PI(4, 5)P2. Yet, while these molecules turn over within seconds, cells maintain processive

motion for minutes to hours. Understanding the principles that underline this network is key to explaining how cells can migrate long distances in complex environments. We recently showed that front and back actin networks locally regulate signaling branched actin activates Ras at the front, while actomyosin suppresses Ras at the rear. This feedback alone is sufficient to generate and maintain polarity. To understand the source and nature of cytoskeletal feedback on signaling, we engineered tools to manipulate and monitor actin mechanics. Expressing a series of synthetic actin crosslinkers dramatically polarizes cells and locally suppresses the activation of cell front markers like Ras. These effects on cell polarity and signaling are dependent on crosslinker properties and can be tuned by altering linker length. Additionally, we can directly map where and when actin networks come under strain using protein domains that bind specifically to compressed and tensed actin filaments. Interestingly, we determined that actin stress travels as a propagating wave across the cell membrane, closely correlated in time and space with branched actin nucleation and Ras activation. Together, our results uncover key biophysical differences in the actin populations at the cell front and back and demonstrate that these differences control signaling activity. This feedback may help explain how cells detect and negotiate obstacles in complicated tissue environments.

## A Novel Immortalization Method for Immortalizing Human Primary CD8+ T Cells by Inserting a Single Copy of Human Telomerase Reverse Transcriptase via CRISPR/Cas9

**Zhiyong He***

*Biomarker and Genomic Sciences Group, Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, USA*

### Abstract

Existing cell immortalization methods made the cells obtain oncogenesis phenotype and/or caused the cells gain and/or lose chromosomes. Immortalized normal human T cells lines provide critical *in vitro* models for basic research and therapeutic products development. We developed a novel method utilizing a clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9), system to replace the exon 2 of the cell cycle inhibitor gene CDKN2A (encoding p16 and p14 proteins) with a single copy of human telomerase reverse transcriptase (hTERT) to immortalize human primary CD8+ T cells (hCD8+T-TERT). By using Cas9 protein and low donor DNA copies/cell, we successfully immortalized hCD8+T cells with a single copy of hTERT transgene, which also avoided uncontrolled insertion of Cas9 gene and guide RNA vector. Human primary CD8+ cells from independent donors were immortalized and expanded more than 2.6 × 107 times. Characterization of one of the immortalized CD8+ T-TERT cell lines revealed that the cells retained most of the cell surface markers and normal karyotype. The CD8+ T-TERT cells also retained the dependence of interleukin-2 and CD3/CD28 activator for survival and expansion. We also successfully immortalized human primary mammary epithelial cells. In conclusion, we developed a novel method for immortalizing human primary cells and established immortalized CD8+ T cell line and human mammary epithelial cell lines. The immortalized cen8+ T cells had a phenotype consistent with T cells.

## Viral Vulnerability in Myhre Syndrome Airway Epithelium

**Hongmei Mou***

*Mucosal Immunology Research Faculty, Massachusetts General Hospital, Boston, USA*

### Abstract

Myhre syndrome is a rare genetic disorder caused by gain-of-function variants in SMAD4 and is clinically associated with recurrent rhinitis and increased susceptibility to respiratory infections. However, the epithelial-intrinsic mechanisms underlying this vulnerability remain poorly defined. Here, using patient-derived nasal airway epithelial cells, we demonstrate that respiratory syncytial virus (RSV) infection is markedly exacerbated in Myhre syndrome epithelium, characterized by enhanced viral spread, increased syncytium formation, and heightened epithelial injury. Mechanistically, we show that activation of transforming growth factor-1–SMAD4 signaling in nasal epithelial cells derived from healthy donors phenocopies the Myhre syndrome condition, leading to increased susceptibility to RSV infection. This identifies SMAD4 signaling as a pro-viral pathway in airway epithelium. Among potential downstream mediators, we identify a significant reduction in BPIFA1 expression in Myhre syndrome epithelial cells. BPIFA1, a key secreted host defense protein with antimicrobial and immunomodulatory functions, has emerging antiviral activity. We further demonstrate that SMAD pathway activation suppresses BPIFA1 expression, linking aberrant SMAD4 signaling to impaired epithelial innate immunity. To address this defect, we developed stabilized 22 – 24 amino acid BPIFA1-derived peptides that retain functional activity and resist enzymatic degradation. Treatment with these peptides significantly reduces RSV infection and epithelial damage in Myhre syndrome airway epithelial cultures. Collectively, our findings establish that gain-of-function SMAD4 signaling enhances viral vulnerability in airway epithelium, at least in part through suppression of BPIFA1. Impaired epithelial differentiation and defective host defense mechanisms likely contribute to recurrent respiratory disease in Myhre syndrome. Importantly, BPIFA1-derived peptides represent a promising therapeutic

strategy to restore antiviral defense, with potential applications extending beyond Myhre syndrome to broader viral respiratory infections.

## MYSM1 as a Drug-target for MYC-driven B Cell Lymphoma

**Anastasia Nijnik***

*Department of Physiology, McGill University, Montreal, Canada*

### Abstract

MYC is an oncogenic transcription factor that is over-expressed, amplified, or otherwise dysregulated in over 50% of all cancers. This includes over 10% of diffuse large B-cell lymphomas, where MYC translocations are associated with a poor therapy response and inferior prognosis for the patients. However, because MYC lacks ligand-binding or catalytic domains, it is a highly challenging drug target, and there is a wide interest in novel approaches to inhibit MYC oncogenic functions. MYSM1 is a chromatin-binding deubiquitinase (DUB) that promotes gene expression by catalytically removing the histone H2A Lysine 119 monoubiquitination epigenetic mark. In recent work, we demonstrated that MYSM1 acts in cooperation with MYC to sustain the expression of oncogenic transcriptional programs in hematopoietic cells, identifying MYSM1 as a potential therapeutic target for MYC-driven malignancies. We show for the first time that the loss of MYSM1 DUB catalytic activity, without the loss of MYSM1 protein expression, is sufficient to protect against MYC-driven lymphoma in murine models. We characterize the impact of MYSM1 loss-of-function on tumor cell physiology and on antitumor immunity, examining the tumor-intrinsic and the immune cell-mediated mechanisms involved in the protection against the disease. Leveraging human cancer genome databases, we provide first evidence linking MYSM1 loss-of-function to reduced fitness of human lymphoma cell lines in culture and to more favorable clinical outcomes in cancer patients. Overall, our studies support pharmacological inhibition of MYSM1 DUB catalytic function as a novel therapeutic strategy for MYC-driven lymphoma and potentially other cancers.

## Vesicle-mediated Gene Silencing: How Planarian Extracellular Vesicles Shape Regeneration

**Vidyanand Sasidharan*, Laura Ancellotti, Viraj Doddihal, Frederick Mann, Mary Cathleen McKinney, Joseph Varberg, Alison Fujii, Eric Ross, Fengyan Deng, Kexi Yi and Alejandro Sánchez Alvarado**

*Stowers Institute for Medical Research, Kansas City, USA*

### Abstract

Planarians exhibit robust regenerative capacity driven by pluripotent stem cells (neoblasts), yet the molecular signals that coordinate neoblast behavior during regeneration remain incompletely understood. Here, we identify extracellular vesicles (EVs) secreted by planarian stem cells *in vitro, in vivo,* and *ex vivo* as key carriers of double-stranded RNA (dsRNA)–derived small interfering RNAs (siRNAs). EVs were isolated from conditioned planarian buffer by differential centrifugation, ultrafiltration, and ultracentrifugation, characterized by electron microscopy and nanoparticle tracking analysis, and further purified by size-exclusion chromatography for proteomic and small RNA profiling. These analyses revealed condition-specific EV cargo and a prominent enrichment of Dicer-processed dsRNA-derived siRNAs, positioning EVs as selective RNA carriers during homeostasis and regeneration. Functionally, EVs from RNA interference (RNAi)–treated donors were sufficient to elicit gene-specific knockdown phenotypes when transplanted into intact animals or applied to neoblast-enriched cell cultures, demonstrating EV-mediated propagation of systemic RNAi. Mechanistic experiments uncovered argonaut protein (AGO3) as a molecular handler that loads dsRNA-derived siRNAs into EVs and enables their intercellular transport; perturbation of AGO3 expression abolished dsRNA-triggered silencing despite intact upstream RNAi machinery. Together, our data establishes dsRNA-derived siRNA cargo and AGO3-dependent loading as a dedicated EV-based pathway for long-range gene regulation, revealing a fundamental mechanism by which planarians coordinate stem cell behavior and tissue regeneration.

**Funding:** Stowers Institute and HHMI.

## Peri-mitochondrial Actin Filaments Inhibit Parkin Assembly via Disruption of ER-mitochondrial Contact

**Amrapali Ghosh[1*], Tak Shun Fung[2*], Maite R. Zavala[1], Zuzana Nichtova[1], Dhaval Kumar Shukal[1], Marco Tigano[1], Gyorgy Csordas[1], Henry N. Higgs[3] and Rajarshi Chakrabarti[1]**

*[1]Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia, USA*
*[2]Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, USA*
*[3]Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth College, Hanover, USA*

## Abstract

Mitochondrial damage represents a dramatic change in cellular homeostasis, necessitating metabolic adaptation and clearance of the damaged organelle. One rapid response to mitochondrial damage is peri-mitochondrial actin polymerization within 2 min, which we refer to as acute damage-induced actin (ADA). ADA is vital for a metabolic shift from oxidative phosphorylation to glycolysis upon mitochondrial dysfunction. In the current study, we investigated the effect of ADA on pink1/Parkin mediated mitochondrial quality control. We show that inhibition of proteins involved in the ADA pathway significantly accelerates Parkin recruitment onto depolarized mitochondria. Upon exploring the mechanism by which ADA resists Parkin recruitment onto depolarized mitochondria, we found that ADA disrupts endoplasmic reticulum (ER)–mitochondria contacts in an Arp2/3 complex-dependent manner. Interestingly, overexpression of ER–mitochondria tethers override the effect of ADA, allowing rapid recruitment of not only Parkin but also accelerates the entire mitophagy pathway by speeding up downstream LC3 recruitment after mitochondrial depolarization. Also, during chronic mitochondrial dysfunction, ADA-like filaments persist for days and disrupt close contacts between ER and mitochondria in an Arp2/3 dependent manner, ultimately delaying Parkin and LC3 recruitment. The blockage of Parkin and LC3 recruitment could be reversed rapidly by inhibiting ADA. All in all, we show that ADA acts as a protective mechanism, delaying mitophagy following acute damage, and deterring mitophagy during chronic mitochondrial damage.

# Regulation of the Formin Inverted Formin-2 by Actin Monomers and Calcium-calmodulin

**Miriam Lee[1*], Aiman Jalmukhambetova[1], T. Emme Burgin[2] and Henry N. Higgs[1]**

*[1]Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth College, Hanover, USA*
*[2]Thayer School of Engineering, Dartmouth College, Hanover, USA*

## Abstract

In response to elevated intracellular calcium, the formin inverted formin-2 (INF2) rapidly polymerizes approximately 20 - 30% of the total cellular actin pool within 30 s, indicating tight and robust regulation. INF2 activity is controlled by an autoinhibitory interaction between its N-terminal diaphanous inhibitory domain (DID) and C-terminal diaphanous autoregulatory domain (DAD). Dominant disease-associated mutations in the DID constitutively activate INF2, underscoring the importance of this regulatory interaction. However, binding of actin monomers to DAD competes with DID binding and disrupts autoinhibition, suggesting that INF2 can directly sense actin monomer availability. Using a novel cell-free assay, we show that INF2 inhibition does not require CAP proteins, contrary to previous models, but instead depends on actin buffering by monomer-binding proteins such as profilin or thymosin. INF2 activation requires calcium-bound calmodulin, which binds to the N-terminus, and we further demonstrate that the N-terminus contributes to INF2 regulation beyond calmodulin binding alone. Together, these findings reveal a concerted regulatory mechanism in which INF2 integrates intracellular calcium signals and actin monomer levels, highlighting an unexpected role for actin monomer-binding proteins in the specific regulation of an actin polymerization factor.

# Mechanistic Dissection of Genetic Drivers and Dysregulated Pathways in Hematologic Malignancies

**Uday Aditya Sarkar[1,2*] and Lalit Kumar[3]**

*[1]National Institute of Immunology, New Delhi, India*
*[2]Department of Medical Oncology, Dana-Farber Cancer Institute & Harvard Medical School, Boston, USA*
*[3]Department of Medical Oncology, All India Institute of Medical Sciences, New Delhi, India*

## Abstract

Genetic dependencies and dysregulated signaling pathways lie at the core of hematologic malignancies, shaping oncogenic programs that drive disease progression and therapy resistance. In multiple myeloma (MM), an incurable plasma cell malignancy, aberrant activation of NF-κB signaling is a major pathogenic hallmark. Both canonical (RelA/p50) and non-canonical (RelB/p52) NF-κB cascades contribute to MM biology, yet how they intersect remain poorly understood. We identify the tumor suppressor cylindromatosis (CYLD) as a key regulator of NF-κB crosstalk that reinforces RelB-dependent transcriptional programs promoting MM progression. Analysis of primary CD138 plasma cells and patient-derived MM cell lines, combined with transcriptomic profiling from the Multiple Myeloma Research Foundation datasets, revealed that elevated nuclear RelB correlates with increased RelB expression and poor clinical outcomes. Loss of CYLD, a negative NF-κB regulator, enhanced canonical NF-κB signaling and RelB activation, especially upon non-canonical pathway stimulation. Mechanistically, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9)–mediated CYLD deletion conferred survival and migratory advantages through RelB-driven induction of pro-survival genes (BCL2, BIRC2, BIRC3, TRAF1, c-FLIP) and chemokine receptors (CXCR4, CXCR7). These findings define a novel CYLD–RelB signaling

axis that integrates canonical and non-canonical NF-κB pathways to sustain MM cell survival and dissemination. Targeting this axis may offer a new therapeutic approach to disrupt NF-κB–mediated tumor promotion. Notably, CYLD-driven NF-κB dysregulation also emerges in splenic marginal zone lymphoma, underscoring its broader relevance across B-cell malignancies. In conclusion, our work suggests that NF-κB crosstalk mechanisms may provide prognostic evidence and therapeutic opportunities in the future.

## Guanosine Triphosphate Biosynthetic Pathway as a Novel Target in Chemo-resistant Triple Negative Breast Cancer

**Charles Manhardt***

*Department of Cell Stress Biology, Roswell Park Comprehensive Cancer Center, Buffalo, USA*

### Abstract

Triple negative breast cancer (TNBC) is one of the most aggressive subtypes of breast cancer, correlating with the highest rate of metastasis and worst survival rate. Patients with this subtype do not benefit from hormonal therapy but alternatively rely on surgery and chemotherapeutics like doxorubicin. Our lab has recently reported on the role of the guanosine triphosphate (GTP) rate-limiting biosynthetic enzyme inosine monophosphate dehydrogenase 2 in the establishment of chemoresistance in TNBC. We demonstrate that doxorubicin resistant lines, murine and human, upregulate GTP metabolism to support pro-tumorigenic functions but in doing so they become exquisitely sensitive to guanylates depletion, opening new potential avenues for therapeutic intervention. We tie this newfound reliance on guanylates to the establishment of pseudohypoxia in resistant cells. Understanding the molecular pathways supporting this guanylate-pseudohypoxia crosstalk in resistance could result in the identification of novel therapeutic targets and ultimately benefit patients, especially TNBC patients who present with chemo-resistant recurrence following chemotherapy.

## OCRL's Effects on the Polycystin 1/2 Signaling Complex

**Nicolae Iftimia[1*], Nikolay Gresko[2], Vanathy Rajendran[2] and Michael Caplan[2]**

*[1]Department of Neurosurgery, University of Massachusetts Chan Medical School, USA*
*[2]Department of Cellular and Molecular Physiology, Yale University, USA*

### Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disease, characterized by the development of fluid-filled cysts. ADPKD is caused by mutations in Pkd1/Pkd2 genes - encoding polycystin (PC) 1/2 proteins. Our goal was to investigate the underlying molecular mechanisms of ADPKD. We sought to determine whether interactions among components of the PC1/2 signaling complex are affected by PC1's activation state and whether these interactors modulate downstream signaling and polycystin localization. We performed a proteomics analysis of human embryonal kidney cells transfected with constitutively active (PC1ΔNTF) and inactive (PC1ΔNTFΔ-TA) PC1 constructs, truncated for the N-terminal fragment (NTF) and tethered agonist (TA), to discover potential novel interactors of PC1 belonging to its activity-dependent signaling complex. OCRL-1, a protein implicated in Lowe syndrome, was a top hit. Using confocal immunofluorescence microscopy, immunoblotting, immunoprecipitation studies, and fluorescent biosensor assays, we investigated the effects of chemically inhibiting OCRL's 5' lipid phosphatase activity upon the localization and function of the PC1/2 signaling complex. We find that OCRL modulates downstream signaling mediated by activated PC1; ΔNTFΔTA+PC2 and PC1+2 expressing cells exhibit substantial loss of ciliary construct localization; and neither OCRL's total level of expression nor binding interactions with PC1 are affected on pull-down experiments. Barbadin (arrestin inhibitor) overcame the effects of OCRL inhibition on ciliary PC1 localization. Our data suggest OCRL inhibition, which reduces the ciliary localization of ΔNTFΔTA and full-length PC1, induces removal from the cilia rather than preventing initial trafficking. Barbadin results indicate those effects are related to arrestin and PC1's activation state.

## Bridging Metabolic Dysregulation and Systemic Health: The Role of Postbiotics in Metabolic Syndrome

**Tanvi Sharma* and Naveen Kaushal**

*Department of Biophysics, Panjab University, Chandigarh, India*

### Abstract

Metabolic syndrome (MetS) is a cluster of metabolic disorders that significantly heightens the risk of cardiovascular disease

and hepatic complications. Dietary factors such as high-fat diets and gut dysbiosis have been implicated in MetS and associated systemic complications. Although diverse and multi-pronged therapeutic interventions and gut microbiota-based approaches (prebiotic/probiotic) exist, the limited therapeutic efficacy, non-specificity, and severe side effects restrict their use over long durations. In the current study, the therapeutic potential of postbiotics, specifically short-chain fatty acids (SCFAs), was evaluated in an experimental model of high-fat diet-induced MetS in Wistar rats. SCFA supplementation normalized key metabolic parameters, including reduced weight gain, enhanced insulin sensitivity, and better systolic blood pressure. This was achieved through the restoration of key lipid metabolic markers, including apolipoprotein B-100, apolipoprotein E, and low-density lipoprotein receptor, which conferred cardioprotective effects and improved the overall lipid profile. The study demonstrated that these therapeutic effects of SCFAs were mediated through the reduction of pro-inflammatory markers such as cyclooxygenase (COX)-1, COX-2, tumor necrosis factor, interleukin (IL)-1, IL-6, IL-12 and microsolar prostaglandin E synthase, as well as robust redox regulation in both the liver and heart. Furthermore, the study demonstrated that SCFA supplementation significantly protected against hepatic lipid accumulation and inflammation, which are key markers of MetS-induced liver damage. In conclusion, SCFA supplementation presents a promising multifaceted strategy for managing MetS, offering a potential novel dietary and therapeutic intervention for both hepatic and cardiac complications.

# Poster Presentations

## Garland Architecture of the Basement Membrane

**Elena Pokidysheva[1*], Sergei P. Boudko[1], Sergei Chetyrkin[1], Selene Colon[1], Gautam Bhave[1], Rachel Lennon[2] and Billy G. Hudson[1]**

*[1]Division of Nephrology, Department of Medicine, Vanderbilt University Medical Center, Nashville, USA*
*[2]Division of Cell Matrix Biology & Regenerative Medicine, University of Manchester, UK*

### Abstract

Diabetic nephropathy, Alport syndrome, and metastasizing cancer are examples of pathological conditions where the basement membrane is both structurally and functionally altered. Understanding the mechanisms that govern the assembly and supramolecular organization of the basement membrane in both health and disease is crucial for uncovering potential cures for these conditions. The classical components of the basement membrane include collagen IV, laminin, nidogen, and the proteoglycans perlecan and agrin. However, the interactions among these components that lead to the formation of a suprastructural organization remain largely unknown. In this study, we investigated the supramolecular organization and assembly of a model basement membrane. By employing transmission and scanning electron microscopy, along with enzymatic degradation and clustered regularly interspaced short palindromic repeats gene editing, we identified a unique suprastructural molecular complex termed the "garland" architecture of the basement membrane. Our findings revealed that the "garland" suprastructural complex functions as a collagen IV scaffold coated with proteoglycans, with agrin being an essential component that can compensate for the absence of perlecan. Notably, we observed that the assembly of the basement membrane occurs in distinct structural stages, with a delayed onset of proteoglycan production. Surprisingly, the laminin network is not necessary for the assembly of the "garland". In conclusion, the newly identified "garland" suprastructure may represent a fundamental organizational characteristic of basement membranes.

## Loss of FAM60A Disrupts Hippo Signaling and Promotes Triple Negative Breast Cancer Progression by Oncogenic YAP1 Activation

**Mojnu Miah[1*], Md Rafikul Islam[1], Sneha Khator[1], Akash Saha[1], Mohammad A Rahman[1], Michael P. Washburn[2] and Sayem Miah[1]**

*[1]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, USA*
*[2]Department of Cancer Biology, University of Kansas Medical Center, Kansas City, USA*

### Abstract

FAM60A (also known as SINHCAF) is a subunit of the Sin3/histone deacetylases (HDAC) complex with established roles in chromatin remodeling, yet its broader cellular functions remain largely undefined. Using immunological, biochemical, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9), genomic, and proteomic approaches, we mapped the FAM60A interaction network and its functional impact. We reveal that FAM60A binds directly to HDAC1 to recruit the Sin3/HDAC complex to SIN3A, acting as a cell cycle-regulated component that influences gene expression. At the same time, a dual-domain architecture mediates its additional associations with RNA and DNA-binding proteins. CRISPR/Cas9–mediated HDAC1 knockout abolishes the FAM60A–SIN3A interaction, confirming this depen-

dency. Loss of FAM60A triggers widespread transcriptional rewiring, including downregulation of WWC3, a scaffold protein for LATS1/2 kinase activation, thereby impairing activation of the Hippo signaling pathway. Consequently, YAP1 dephosphorylation and nuclear accumulation increased YAP1 target gene expression, shifted cell-cycle dynamics toward $G_1$ enrichment, conferred resistance to metabolic stress, and increased cell proliferation. Restoration of FAM60A or exogenous WWC3 reactivated Hippo "off" signaling, normalized cell-cycle distribution, and reversed stress resistance. In addition, yes-associated protein 1 (YAP1) depletion restored controlled cell growth, comparable to that of FAM60A wild-type cells. These findings establish FAM60A as a pivotal epigenetic tuner, linking histone deacetylation to Hippo pathway regulation and nominate the FAM60A–HDAC1–WWC3 axis as a potential therapeutic target to restore growth control in YAP-driven triple negative breast cancer.

## Bradykinin-induced Pulmonary Angioedema: A Curable Form of Acute Respiratory Distress Syndrome in COVID-19 Patients?

**Thomas T. Ma*, Xue-Song Sun, Yu-Jia Zhai and Douglas C. Eaton**

*Emory University, Atlanta, Georgia, USA*

### Abstract

Acute respiratory distress syndrome (ARDS) is the leading cause of death in COVID-19. Gene expression analysis of bronchoalveolar cells reveals that severe acute respiratory syndrome coronavirus 2 infection induces a severe bradykinin storm, suggesting a possible link between bradykinin-mediated vascular permeability and pulmonary angioedema. We hypothesize that bradykinin-induced pulmonary angioedema represents an early, potentially reversible stage of ARDS that can be blocked by the bradykinin $B_2$ receptor antagonist icatibant. Using intravital multiphoton microscopy in live mice, we found that intratracheal bradykinin administration causes lung capillaries to leak large molecules (albumin and dextran), reduces alveolar airspace, and increases lung wet weight—consistent with pulmonary angioedema. Because the renin–angiotensin system interacts with bradykinin signaling via the $AT_1R$ - $B_2R$ heterodimer, we investigated whether angiotensin II (Ang II) contributes to endothelial injury. Preliminary data show that Ang II induces lung endothelial cell death through the $AT_1R$-$B_2R$ heterodimer, and that this effect is blocked by [$Sar^1$,$Ile^4$,$Ile^8$]-Ang II (SII-Ang II). Because this heterodimer is mechanosensitive, it responds to increased mechanical stress from alveolar edema by the Ang II receptor antagonist. We propose that bradykinin-induced edema amplifies Ang II-mediated endothelial injury, leading to fatal ARDS. Future studies will determine whether intratracheal icatibant delivery or SII-Ang II treatment can prevent bradykinin- and Ang II–mediated ARDS, providing *in vivo* data to support ongoing and future clinical trials targeting $B_2R$ and $AT_1R$–$B_2R$ signaling in COVID-19 patients.

## Regulation of Protein Kinase C delta with Diacylglycerol Lactone-based RWD-5-188D Inhibitor and its Effect on Colorectal Cancer Growth, Migration, and Proliferation

**Michael Sun[1*], Michael R. Dores[2] and Robert Denton[3]**

[1]*Great Neck South High School, USA*
[2]*Department of Biology, Hofstra University, USA*
[3]*Department of Science and Allied Health, Medgar Evers College, City University of New York, USA*

### Abstract

Colorectal cancer cell (CRC) is one of the most prevalent and lethal cancers worldwide, with incidence and mortality rates predicted to increase dramatically in the next few years. Protein kinase C delta (PKC-δ) has emerged as a potential therapeutic target in CRC due to its involvement in tumor progression and development. However, previous studies display contrasting results regarding its role in cancerous cell growth, migration, and proliferation. In this study, we investigated the effect of RWD-5-188D, a diacylglycerol (DAG) lactone inhibitor of PKC-δ, on the growth, migration, and proliferation of CRC. HCT-116 CRC were exposed to varying concentrations of RWD-5-188D (0 - 60 μM). Mixed effect analysis indicates that increased concentrations (≥40 μM) effectively suppressed the proliferation of cells and induced cell death. Cells treated with lower to moderate concentrations (20 - 30 μM) possessed reduced proliferation rates compared to the control sample (dimethyl sulfoxide). Variations in growth and migration of individual cells were also investigated by a K-fold regression model targeting cell mean area and total distance. Strikingly, higher concentrations correlated directionally with greater suppression of cell growth and migration, with the model having excellent predictive power (normalized RMSE ~10%). In summary, our findings indicate that RWD-5-188D exhibits dose-dependent inhibitory activities against CRC proliferation, growth, and migration. This dual-method analysis strategy elucidates the temporal dynamics of proliferation and the quantifiable alterations of cellular morphology and motility. Our results justify the therapeutic efficacy of DAG lactone-based PKC-δ inhibitors and open further research into their application in CRC therapy.

## Defining the Role of FAM60A in RNA Processing and Splicing

**Akash Saha[1,2*], Mojnu Miah[1,2], Md Rafikul Islam[1,2], Preeti Nagar[1,2], Sneha Khator[1,2], M Asa-duzzaman[1,2], Mohammad Alinoor Rahman[1,2] and Sayem Miah[1,2]**

*[1]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, USA*
*[2]Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, USA*

### Abstract

FAM60A, also known as SINHCAF, is an integral subunit of the Sin3/histone deacetylases (HDAC) chromatin remodeling complex, a well-known transcriptional co-repressor. Beyond its role within multi-protein machinery, FAM60A has emerged as a multifaceted regulator implicated in diverse cellular processes, including mammalian development, cell cycle progression, maintenance of pluripotency, and the pathogenesis of diseases such as cancer. We previously reported that FAM60A's dual-domain architecture enables additional interactions with RNA and DNA-binding proteins, expanding its functional repertoire. Here, we employed an integrative multi-omics approach and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9), to extend the understanding of the functional dynamics of FAM60A. Our affinity purification-mass-spectrometry analysis of FAM60A revealed an enriched set of proteins associated with the intracellular ribonucleoprotein complex, spliceosomal snRNP complex, spliceosomal complex, and mRNA stability complex. The post-nuclease treatment diminishes RNA-binding protein interactions with FAM60A, confirming FAM60A's direct engagement with RNA. Consequently, FAM60A-associated transcripts code for regulators of RNA processing and splicing. Further CRISPR/Cas9–mediated FAM60A knockout triggers significant alterations in numerous splicing events. Collectively, these findings define a role for FAM60A in RNA processing and splicing through RNA-dependent interactions with spliceosomal and ribonucleoprotein complexes.

## Positive Mitochondrial DNA Dynamics in Human Oocytes

**Daniil Mirzoyan[1*], Melissa Franco[1], Zoë Fleischmann[1], Sofia Annis[1], Auden Cote-L'Heureux[1], Dylan Aidlen[1], Benjamin Vyshedskiy[2], Jacob Bandell[1], Konstantin Popadin[3-5], Dori C. Woods[1], Jonathan L. Tilly[1] and Konstantin Khrapko[1*]**

*[1]Department of Biology, Northeastern University, Boston, USA*
*[2]Cambridge University, Cambridge, UK*
*[3]School of Life Sciences, École Polytechnique Fédérale de Lausanne, Switzerland*
*[4]Swiss Institute of Bioinformatics, Lausanne, Switzerland*
*[5]Center for Mitochondrial Functional Genomics, Immanuel Kant Baltic Federal University, Kaliningrad, Russia*

### Abstract

Mitochondrial DNA (mtDNA) mutations play a central role in the development of human diseases, but the mechanisms regulating their inheritance remain unclear. Classical models suggest that purifying selection removes deleterious coding variants during oogenesis. To test this hypothesis, we re-analyzed high-accuracy sequencing data from individual oocytes using cumulative curve analysis, weighted synonymy, and Monte Carlo tests. We observed consistently low synonym (~20%) in oocytes, contradicting the dominant purifying selection. Instead, cumulative curve analysis and modeling revealed positive intracellular selection of non-coding and non-conservative mutations, which were disproportionately enriched at higher mutation frequencies. Notably, subsets of coding mutations, particularly RNA-coding variants, also showed signs of positive selection at mutation frequencies above 1%. The apparent depletion of protein-coding variants was not due to direct removal, but rather to competitive dynamics favoring alternative classes of mutations. These results demonstrate that mtDNA evolution in oocytes is governed by multiple, subset-specific modes of positive selection. This model challenges the long-standing assumption of a purifying filter in the female germline and proposes a revised framework for understanding mtDNA inheritance and the emergence of pathogenic variants.

## A Proteolysis-targeting Chimera R-919 Degrader Suppresses the Oncogenic Functions of Breast Tumor Kinase

**Sneha Khator[1*], Md Asaduzzaman[1], Baku Acharya[2], Brendan Frett[2] and Sayem Miah[1]**

*[1]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, USA*
*[2]Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, USA*

## Abstract

Metastatic triple-negative breast cancer (TNBC) remains a highly aggressive disease with limited therapeutic options and a five-year survival rate of approximately 12%. Protein tyrosine kinase 6/breast tumor kinase (PTK6/BRK), a non-receptor tyrosine kinase overexpressed across breast cancer subtypes, promotes tumor growth, survival, and metastasis and is associated with poor clinical outcomes. Notably, BRK exhibits both kinase-dependent and kinase-independent oncogenic functions, limiting the efficacy of conventional kinase inhibitors. Here, we evaluate a BRK-targeting proteolysis-targeting chimera (PROTAC), R-919, as a strategy to eliminate BRK protein and suppress oncogenic signaling. TNBC cell lines, including MDA-MB-231and MDA-MB-468, were treated with increasing concentrations of R-919 for 24 h, and BRK protein levels were assessed by western blot analysis. R-919 induced robust, proteasome-mediated degradation of BRK with nanomolar potency across multiple breast cancer models, while sparing non-transformed cells. BRK degradation suppressed cancer cell growth and viability and induced apoptosis, accompanied by increased expression of pro-apoptotic proteins. In contrast, BRK kinase inhibition failed to fully recapitulate these effects, although both approaches impaired cell migration. These findings highlight BRK-directed PROTACs as a promising therapeutic strategy for targeting both BRK's kinase-dependent and kinase-independent functions in metastatic TNBC.

# Growth Differentiation Factor 15 and a Distinct Phenotype of Congenital Disorders of Glycosylation

**Gursev Dhaunsi***

*College of Medicine, Kuwait University, Kuwait*

## Abstract

**Objectives:** To identify and record distinct neurological or extra-neurological features of congenital disorders of glycosylation (CDG) with regards to clinical phenotype variation and their association with serum levels of growth differentiation factor 15 (GDF 15) in pediatric population of Kuwait.

**Subjects and Method:** A total of 263 patients aged 16 days to 13 years, suspected of having CDG due to developmental delay, hypotonia, psychomotor retardation and seizures, and 59 healthy control subjects were recruited. A CDG diagnosis was confirmed using isoelectric focusing of serum sialo-transferrin and serum GDF 15 was assayed by enzyme-linked immunosorbent assay.

**Results:** Of the 263 patients, only 11 (4.2%) had CDG with 7 patients having type-I and 4 type-II. Consanguinity was remarkably high (50%) and all CDG patients had psychomotor retardation with more than 50% having seizures. Inverted nipples and abnormal fat distribution were notably rare and found in only 1 type-II patient. Asthma was observed in 2 type-I patients. Other atypical features included presence of pes planus and cryptorchidism, and absence of renal cysts in CDG type-I patients. Both CDG type-I and type-II patients notably lack heart anomalies. Serum GDF 15 levels were markedly elevated in all CDG type-II and only 29 percent of type-I patients, however all patients with abnormal GDF 15 levels had common clinical features of developmental delay, hypotonia and psychomotor retardation.

**Conclusion:** This study adds some distinctly atypical features to the clinical spectrum of CDG and suggests an association of GDF 15 with CDG pathogenesis.

# Structural Insights into Phosphorylated SMAD4 Interaction with NuRD Complex which Promotes Metastatic Breast Cancer

**Md. Asaduzzaman*, Mojnu Miah, Sneha Khator, Akash Saha and Sayem Miah**

*Winthrop P. Rockefeller Cancer Institute, Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, USA*

## Abstract

Metastatic breast cancer remains a leading cause of cancer-related mortality among women worldwide, driven in part by dysregulated signaling pathways that promote invasion and dissemination. Breast tumor kinase/protein tyrosine kinase 6 (BRK/PTK6) is frequently overexpressed in breast cancer cell lines, where elevated expression correlates with enhanced metastatic potential and poor clinical prognosis. Our previous studies demonstrated that BRK modulates the transforming growth factor-signaling axis through site-specific phosphorylation of SMAD4 at tyrosine residues Y353 and Y412. Phosphorylation at Y412 promotes SMAD4 ubiquitination and subsequent proteasomal degradation, thereby attenuating canonical signaling. In contrast, phosphorylation at Y353 does not induce degradation but instead redirects SMAD4 function by promoting its interaction with the nucleosome remodeling and deacetylase (NuRD) complex. In the present study, we employed phosphomimic mu-

tants to validate the phosphorylation-dependent interaction between SMAD4 and NuRD complex components. Biochemical analyses confirm enhanced association of SMAD4 with NuRD proteins under Y353 phosphomimic conditions. Complementary computational structural modeling reveals that Y353 phosphorylation induces a significant conformational rearrangement in SMAD4, resulting in (i) disruption of its canonical DNA-binding interface and (ii) reorientation of key residues within the binding pocket. This structural shift impairs SMAD4's ability to form transcriptionally active complexes and bind DNA, while simultaneously generating a favorable interface for interaction several core subunits of NuRD complex. Collectively, these findings support a model in which BRK-mediated phosphorylation at Y353 redirects SMAD4 from a transcriptional regulator toward association with a chromatin remodeling complex, thereby contributing to altered gene regulation in breast cancer. This phosphorylation-dependent switch provides new insight into how aberrant kinase signaling contributes to metastatic progression and highlights BRK–SMAD4–NuRD axis as a potential therapeutic target in breast cancer.

## From Polymerization to Remodeling: Inverted Formin-2 Driven Actin Assembly and Filament Regulation by Transgelin-2

**Miriam Lee[1], Aiman Jalmukhambetova[1], T. Emme Burgin[2] and Henry N. Higgs[1*]**

*[1]Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth College, Hanover, USA*
*[2]Thayer School of Engineering, Dartmouth College, Hanover, USA*

### Abstract

In response to elevated intracellular calcium, the inverted formin-2 (INF2) rapidly polymerizes approximately 20 - 30% of the total cellular actin pool within 30 s, indicating tight regulation. INF2 activity is controlled by an autoinhibitory interaction between its N-terminal diaphanous inhibitory domain (DID) and C-terminal diaphanous autoregulatory domain (DAD). Dominant disease-associated mutations in the DID constitutively activate INF2, underscoring the importance of this interaction. Binding of actin monomers to the DAD competes with DID binding and disrupts autoinhibition, suggesting that INF2 directly senses actin monomer availability. Using a novel cell-free assay, we show that INF2 inhibition does not require CAP proteins, contrary to previous models, but instead depends on actin buffering by monomer-binding proteins such as profilin or thymosin. INF2 activation requires calcium-bound calmodulin binding to the N-terminus, and we further demonstrate that the N-terminus contributes to INF2 regulation beyond calmodulin binding alone. Together, these findings reveal a concerted regulatory mechanism in which INF2 integrates intracellular calcium signals and actin monomer levels, highlighting an unexpected role for actin monomer-binding proteins in regulating an actin polymerization factor. In the second part of this study, we identify transgelin-2 (TG2), a small actin-binding protein, as an INF2-interacting partner. Although TG2 expressions are altered in poor-prognosis cancers, no direct link to actin polymerization has been described. We find that TG2 interacts with INF2 via actin filaments, and TG2 depletion leads to prolonged calcium-induced actin assembly. Biochemical and cellular analyses indicate that TG2 binds actin filaments weakly but promotes recruitment of actin-binding proteins such as tropomyosin and cofilin, facilitating actin remodeling and stabilization. These findings reveal a new role for TG2 in actin dynamics and demonstrate that precise INF2 regulation is essential for cytoskeletal organization and mitochondrial homeostasis.

## Plasma and Neuron-derived Extracellular Vesicle Proteomics Reveal Immune Signatures of Intracerebral Hemorrhage Outcome

**Blaine Baker[1,2*], David Wang[2,3], Nikita Mohapatra[2,3], Dua Hassan[2,3], Heather Hull[4], Bradley Ander[4], Boryana Stamova[4] and Aijun Wang[2,3]**

*[1]School of Medicine, University of California Davis, USA*
*[2]Center for Bioengineering in Medicine, University of California Davis, USA*
*[3]Department of Surgery, University of California Davis, USA*
*[4]Department of Neurology, University of California Davis, USA*

### Abstract

Intracerebral hemorrhage (ICH) causes high early mortality and long-term disability, yet objective molecular markers that predict recovery remain limited. Extracellular vesicles (EVs) circulate in blood and carry protein cargo that can report systemic responses and central nervous system injury biology. Here we performed a pilot, compartment-resolved EV proteomics study to identify outcome-associated EV signatures after ICH. EV protein cargo from plasma collected 3 days post-ICH in a pilot cohort was stratified by 90-day clinical outcome. Total plasma EVs were isolated using EXODUS, and neuron-derived EVs were enriched via immunoprecipitation, followed by data-independent acquisition coupled with liquid chromatography-tandem mass spectrometry. Differential abundance analysis in Spectronaut with downstream visualization and enrichment (Python; DAVID/STRING). Analyses revealed outcome-associated structure in both compartments, with enrichment for immune/complement and acute-phase programs showing compartment-specific patterns. These preliminary findings support EV "liquid

biopsy" stratification after ICH and motivate validation in larger cohorts with standardized timing and clinical covariates.

## Radio Frequency Exposure Modulates Neuronal Cell Growth Dynamics and Necrosis Under Inflammatory Conditions

**Shadi Rashed, Brian Budgell, Devin Coran, Dawson Nancekievill and Guy Sovak***

*Canadian Memorial Chiropractic College, Toronto, Canada*

### Abstract

The effect of radio frequency (RF) exposure on neuronal cell activity during the inflammatory process is still not well understood. This study examined the influence of RF exposure on cell proliferation and viability under both normal and lipopolysaccharide (LPS)-induced inflammatory conditions in a hybrid neuronal cell line developed from rat dorsal root ganglion (F-11) RF. F-11 cells were cultured in two groups (N = 8 each), one exposed to RF (Test) and the other not exposed (Control), with parallel groups either having LPS stimulation or not. Proliferation was determined by repeated cell counting at different time points. Data were analyzed using a linear mixed-effects model for growth curves, which included linear and quadratic time effects and random effects at the level of culture replicates. Cell viability, apoptosis and necrosis were evaluated using viability assays. Proliferation increased in a non-linear way over time for all the tested conditions. Although RF exposure did not bring about any major change in the absolute numbers of cells at separate time points, it did significantly ($p < 0.05$) influence the overall proliferation curve, thus there was a temporal modulation of growth dynamics. The rates of apoptosis and necrosis were similar in both groups during basal conditions. However, RF-exposed LPS-treated cells had higher rates of necrosis compared to non-exposed cells. These findings suggest that RF exposure alters the time-dependent control of neuronal cell growth under inflammatory stress. This points to a possible interaction between RF exposure and inflammatory signaling pathways.

## Docosahexaenoic Acid Deficiency in Early Stages of Oxygen-induced Retinopathy Worsens Retinal Vascular Pathology

**Michelle Lee*, Yan Zeng, Myriam Boeck, Victoria Hirst, Mengxu Ge, Hiroyuki Komatsu, Lois EH Smith and Zhongjie Fu**

*Boston Children's Hospital, Harvard Medical School, USA*

### Abstract

Retinopathy of prematurity (ROP) causes visual impairment in premature infants and is characterized by retinal vaso-obliteration (Phase 1) followed by uncontrolled retinal neovascularization (Phase 2). Omega-3 docosahexaenoic acid (DHA) and omega-6 arachidonic acid (ARA) are key factors in fetal and postnatal retinal development but are decreased in premature infants. In a previous study, we observed that supplementation of DHA and ARA at 1:2 ratio in mice modeling hyperglycemic phase 1 ROP improved retinal vascularization compared to DHA alone. However, the impacts of ARA and DHA supplementation on oxygen-induced retinal pathology remain unknown, considering hyperoxia and hypoxia are major risk factors for ROP pathogenesis. In the current study, we first analyzed retinal lipid changes using global lipidomics in mice with oxygen-induced retinopathy (OIR). We identified a loss of DHA and PUFA-containing phospholipids with an increase in compensatory enrichment of MUFA species. To further examine the impact of DHA supplementation, we fed the nursing dam of OIR mice from postnatal day (P)1. There was a significant decrease in retinal vascular pathology in DHA-enriched vs DHA-depleted diet-fed mice. Altogether, our findings suggest a switch in lipid compositions during OIR progression and DHA to be an important factor in protecting the retinas.

## Mitochondrial Metabolic Reprogramming in Early Diabetic Retinopathy

**Hiroyuki Komatsu[1*], Yan Zeng[1], Hitomi Yagi[2], Deokho Lee[3], Taku Kasai[4-6], Victoria Hirst[1], Michelle Lee[1], Mengxu Ge[1], Sasha A. Singh[4-6], Masanori Aikawa[4-6], Zhongjie Fu[1] and Lois EH Smith[1]**

*[1]Department of Ophthalmology, Boston Children's Hospital, Harvard Medical University, Boston, USA*
*[2]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
*[3]Korean Institute of Nutrition, Hallym University, Republic of Korea*
*[4]Center for Interdisciplinary Cardiovascular Sciences, Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, USA*
*[5]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*
*[6]Center for Excellence in Vascular Biology, Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, USA*

## Abstract

**Background:** Diabetic retinopathy (DR) is a leading cause of blindness in working-age adults worldwide. Although vascular permeability and neural dysfunction have been reported in early-stage DR, the molecular alterations underlying early pathogenesis remain poorly characterized. This study aimed to evaluate early metabolic changes in DR leading to vascular and neural abnormalities in a mouse model.

**Methods:** Type 1 diabetes mellitus was induced in C57BL/6J mice by streptozotocin administration. At 6 months post-induction, electroretinography (ERG), proteomics, metabolomics, and electron microscopy (EM) were assessed. Mitochondrial oxygen consumption rate (OCR) was measured *ex vivo* using the BaroFuse.

**Results:** ERG revealed age-related signal attenuation that was further exacerbated under diabetic conditions, particularly in photoreceptor function. Proteomic analysis showed increased abundance of proteins associated with oxidative phosphorylation (OXPHOS) and mitochondrial ATP synthesis pathways in diabetic retinas. Consistently, *ex vivo* OCR measurements demonstrated elevated basal mitochondrial respiration in diabetic retinas supplemented with 5mM glucose. EM revealed increased mitochondrial number with reduced organelle size in both photoreceptors and retinal pigment epithelium, suggesting structural remodeling. Metabolomic profiling further identified decreased abundance of metabolites associated with mitochondrial fatty acid oxidation, suggesting that energy production through this pathway may be impaired in the early-stage diabetic retina.

**Conclusion:** This early DR mouse model demonstrated metabolic alterations characterized by photoreceptor dysfunction, adaptive upregulation of OXPHOS, and impaired fatty acid oxidation. These findings highlight mitochondrial metabolic reprogramming as a hallmark of early disease, and targeting this pathway may represent a promising therapeutic strategy to prevent progression to vision-threatening stages.

## Limiting Branched-chain Amino Acids Reduces Early Vessel Loss and Retinal Neovascularization in Retinopathy of Prematurity

**Victoria Hirst*, Yan Zeng, Mengxu Ge, Hiroyuki Komatsu, Michelle Lee and Zhongjie Fu**

*Department of Ophthalmology, Boston Children's Hospital, Harvard Medical School, USA*

## Abstract

Retinopathy of prematurity (ROP) is a leading cause of blindness in preterm infants, caused by early retinal vaso-obliteration (VO) followed by pathological neovascularization (NV). Essential nutrients like branched-chain amino acids (BCAAs) are reduced after premature birth. BCAAs defend against oxidative stress, so restoring them may protect against ROP. Yet, their role in ROP pathology remains unclear. We investigated how BCAA deficiency affects retinal VO and NV using the oxygen-induced retinopathy (OIR) mouse model. C57BL/6J neonatal mice and their nursing dams were exposed to 75% oxygen from postnatal day (P)7 to P12 to induce VO, then returned to room air to trigger NV through P17. Nursing dams were fed an isocaloric control diet or a low BCAA (two-third reduction) diet from P1 to P12, and retinas were collected at P12 to assess the effect on VO. To evaluate NV, a separate cohort of nursing dams received the isocaloric control diet from P1 to P17 or until P12 then switched to the low BCAA diet. Retinas were collected at P17 to assess the effects on NV. BCAA reduction significantly suppressed hyperoxia-induced VO at P12 (18% decrease, $p < 0.001$). At P17, reduced BCAAs suppressed retinal NV (20% decrease, $p < 0.01$) while VO increased (19% increase, $p < 0.01$). These results show that decreased BCAA availability has phase-dependent effects on OIR, mitigating early VO while worsening VO later despite decreasing pathological NV. This indicates a complex relationship between nutrient status and ROP pathology that could lead to future therapeutic strategies.

## Therapeutic Efficacy of a New Age Antimicrobial, Synthetic Peptide DP1 Against Staphylococcus Aureus Systemic Infection

**Hemant Goyal[1,2*] and Avneet Saini[1]**

[1]*Department of Biophysics, Panjab University, Chandigarh, India*
[2]*Department of Medical Microbiology, Post Graduate Institute of Medical Education and Research, Chandigarh, India*

## Abstract

Microbial infections remain a significant cause of mortality projected to be responsible for 10 million deaths per year by 2050. Included in WHO priority pathogen list 2024, *Staphylococcus* aureus is one such nosocomial pathogenic responsible for a large number of bacterial infections in humans due to its ability to proliferate in the blood stream. It is a leading cause of systemic infections accompanied by organ dysfunction, apoptosis, and immunosuppression. Alternative novel drug candidates, such as antimicrobial peptides (AMPs) have evolved as promising antimicrobial agents, as antimicrobial resistance has put advances of modern medicine at risk. AMPs hinder initial bacterial attachment and adhesion to the target tissue, thus effectively intervening

in its potential proliferation. In this work, the therapeutic potential of a synthetic AMP, DP1, has been explored for its ability to inhibit the growth and proliferation of *S. aureus*. DP1 was validated for its antimicrobial activity, exhibiting a minimum inhibitory concentration (MIC) of 8 μM with an MIC/minimum bactericidal concentration ratio of 1, ceasing bacterial growth in time-killing kinetics. The results indicated its strong bactericidal effect due to disruption of cell membrane as validated by field emission scanning electron microscopy and dielectric spectroscopy analysis. DP1 also remained effective in combatting S. aureus infection *in vivo* mice with more than 55% reduction in bacterial bioburden in the liver, kidney, spleen and peritoneal fluid. Treatment with DP1 results in increased activity of certain enzymes that might have decreased oxidative and nitrosative stress while controlling elevated levels of kidney and liver biomarkers. These results suggested the potential role of the designed peptide as an effective antimicrobial agent to target staphylococcal infections.

## Neuroanatomical and Ocular Specializations in African Tree Squirrels: Structural Adaptations Supporting Arboreal Life

**Olanrewaju Fatola*, Kolade Aribisala, Taidinda Gilbert, Oluwasanmi Aina and James Olopade**

*Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Nigeria*

### Abstract

African tree-dwelling squirrels (*Heliosciurus gambianus* and *Funisciurus anerythrus*) are diurnal rodents with remarkable visual acuity, enabling effective navigation and foraging in complex arboreal habitats. This study presents a comprehensive histological and immunohistochemical examination of the ocular and visual brain structures that underpin these adaptive traits. Paraffin-embedded eye and brain samples from five squirrels captured at the University of Ibadan, Nigeria, were analyzed using routine histological techniques, periodic acid Schiff (PAS) special stain, and glial fibrillary acidic protein (GFAP) immunohistochemistry. The cornea exhibited densely packed stromal fibers with undulating basal epithelial layers, and a strongly PAS-positive basement membrane. Rich melanin pigmentation was observed in the choroid, iris, and ciliary epithelia. The retina displayed a well-stratified architecture with a densely packed ganglion cell layer, while GFAP+ astrocytic profiles with extensive fibrous processes were evident in the retinal nerve fiber layer and optic nerve. Neuroanatomical assessments revealed distinct structural adaptations in the pineal gland, dorsal lateral geniculate nucleus (DLG), and rostral colliculus (RC). The pineal gland featured pinealocytes clustered among astrocyte-like neuroglia and prominent capillary networks. The DLG and RC were enlarged and organized into layered neuronal zones, with heterogeneous neuronal populations suggesting a primate-like specialization in visual processing. These findings provide the first integrated histological and neuroanatomical insights into the visual and neuroendocrine adaptations of African tree squirrels. By highlighting their specialized features for sensorimotor integration and environmental responsiveness, this work positions *H. gambianus* and *F. anerythrus* as promising models for comparative neurobiology and the study of arboreal visual systems.

## *In Vitro* Absorption, Distribution, Metabolism, Excretion Evaluation and Physiologically Based Pharmacokinetic Modeling of C-19 tert-butyldiphenylsilyl Andrographolide Analog

**Nikhil Nunna, Tara Arun, Tia S. Ghoshal and Vasudha Salgotra***

*Department of Biological, Human and Life Sciences, Aspiring Scholars Directed Research Program, Fremont, USA*

### Abstract

Andrographolide, the primary bioactive compound derived from *Andrographis paniculata*, has long been used in traditional Chinese medicine to treat conditions such as fever and sore throat. It exhibits a broad spectrum of pharmacological activities, including anti-inflammatory, anticancer, antibacterial, antiviral, anti-obesity, immunomodulatory, and hypoglycemic effects. However, its clinical use is limited by poor oral bioavailability (~2.67%) due to rapid metabolism in the gastrointestinal tract and liver. To address this, a novel analog was synthesized by chemically modifying the C-19 hydroxyl group with a tert-butyldiphenylsilyl (TBDPS, $C_{16}H_{19}Si$) group to protect against enzymatic degradation and improve pharmacokinetic properties. This study evaluates the analog's pharmacokinetic enhancements by measuring lipophilicity, plasma protein binding, plasma stability, and hepatic microsomal stability using liquid chromatography–mass spectrometry and spectrophotometer analysis. Our results indicate that the analog is 50.8% more lipophilic, exhibits increased plasma protein binding (Analog PPB%: 85.99%, original PPB%: 54.79%), and demonstrate enhanced stability in plasma over time compared to unmodified andrographolide (Analog: 94.97% remaining, original: 80.43% remaining). Furthermore, the analog showed enhanced stability in hepatic microsomes (CLint = 4.69μL/min/mg protein, t1/2 = 1478 min) when compared to andrographolide (CLint = 17.3μL/min/mg protein, t1/2 = 400 min), suggesting reduced phase I metabolism. These findings suggest that the TBDPS structural modification can effectively enhance andrographolide's pharmacokinetic profile, potentially increasing oral bioavailability and therapeutic efficacy. Ongoing work includes physiologically based pharmacokinetic modeling to predict *in vivo* pharmacokinetics.

## Classifying Canonical and Vacancy-bearing G-quadruplexes in Human 5 UTRs and Testing Their Impact on Translation Initiation

**Merat Semma***

*Department of Biochemistry and Molecular Biology, Yale University, New Haven, USA*

### Abstract

G-quadruplexes (G4s) are guanine-rich RNA secondary structures that can inhibit translation initiation by impeding 43S ribosomal scanning along the 5 untranslated region (5 UTR). Recent studies have proposed a related class of noncanonical structures, guanine-vacancy-bearing quadruplexes (GVBQs), in which one guanine tract contains only two guanines, resulting in reduced intrinsic stability. Although predicted to form under cellular conditions, the role of GVBQs in translational regulation has not been well defined. The objective of this study was to determine whether GVBQ motifs within human 5 UTRs regulate translation initiation in a structure-dependent manner and to compare their effects with those of canonical G4s. We developed a computational pipeline using regular-expression–based pattern matching to identify canonical G4s and non-overlapping GVBQs across annotated human 5 UTRs. Representative candidate sequences were cloned in their wild-type form upstream of a dual-luciferase reporter to measure translation efficiency. To assess whether translational effects depended on RNA secondary structure rather than guanine content alone, corresponding G A mutations were introduced to disrupt predicted quadruplex formation in both canonical G4 and GVBQ-containing 5 UTRs. Translation efficiency measurements showed that 5 UTRs containing either canonical G4s or predicted GVBQ motifs reduced translation initiation relative to controls. Comparison of wild-type constructs with their G A mutants revealed relief of translational repression, indicating that reduced translation efficiency depends on quadruplex formation rather than guanine content alone. This work provides a framework for directly comparing canonical and noncanonical G4 variants and supports future high-throughput studies of RNA secondary structure–mediated translational regulation.

## Effects of SIRT1 and SIRT2 Activity Modulation on Limb Regeneration in *Ambystoma mexicanum*

**Ayla Aysar Kyler Núñez[1*], Belfran Alcides Carbonell Medina[1] and Claudia Marcela Arenas Gómez[2]**

*[1]Genetics, Regeneration, and Cancer Group, University of Antioquia, Colombia*
*[2]Biodiversity for Society Group, National University of Colombia, Colombia*

### Abstract

Epimorphic regeneration in urodeles like *Ambystoma mexicanum* (axolotl) involves tightly regulated cellular and molecular processes, including epigenetic mechanisms such as histone deacetylation. Sirtuins, a class of nicotinamide adenine dinucleotide dependent histone deacetylases, have been implicated in multiple biological processes, yet their role in limb regeneration remains unknown. In this study, we investigated the functions of SIRT1 and SIRT2 during axolotl limb regeneration. Through bioinformatic analysis, we confirmed the presence of seven sirtuin orthologs in axolotl. Using a SIRT1 activity assay, we confirmed enzymatic activity throughout the early stages of regeneration (n = 3/day). Pharmacological inhibition of sirtuins with sirtinol delayed regeneration, as evidenced by stage progression and limb growth retardation (n = 5/group). Sirtinol significantly reduced cell proliferation and apoptosis levels during early regeneration (n = 6/group). However, sirtinol-treated animals ultimately regenerated limbs with no significant defects in skeletal morphology, mineralization, or histological architecture. Transcriptomic analyses revealed altered expression of genes involved in vesicle transport, and muscle development processes upon sirtuin inhibition during blastema formation (n = 3/group). These findings suggest that SIRT1 and SIRT2 modulate early regenerative events without being essential for successful limb regeneration in axolotls.

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