# 11th Annual

# Lipids@Wayne

# Symposium

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## 1st May 2024

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**Student Center, Hilberry F**
**Wayne State University**
**5211 Gullen Mall**
**Detroit MI 48202**

## Scope

This symposium addresses the vital roles of lipids in cellular processes required for normal physiological function. Perturbation of lipid metabolism contributes to numerous pathologies, including cancer, and cardiovascular, metabolic, and neurological disorders. We invite you to participate in this exploration of the lipid frontier.

## Organizing Committee

**Xuequn Chen, Ph.D.** Department of Physiology
**Jagadananda Ghosh,** Cancer Research, Henry Ford Health
**Miriam L. Greenberg, Ph.D.** Department of Biological Sciences
**Hanna Hariri, Ph.D.** Department of Biological Sciences
**Christopher Kassotis, Ph.D.** Department of Pharmacology
**Christopher Kelly, Ph.D.** Department of Physics and Astronomy
**Seongho Kim, Ph.D.** Department of Oncology
**Wanqing Liu, Ph.D.** Department of Pharmaceutical Sciences
**Krishna Rao Maddipati, Ph.D.** Department of Pathology
**Emilio Mottillo, Ph.D.** Pathology, Henry Ford Health
**Izabella Podgorski, Ph.D.** Department of Pharmacology
**Stephanie Tucker, Ph.D.** Department of Pathology

## Program

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|---|---|
| 8:30 – 9:45 AM: | Registration and Poster setup |
| 9:45 AM: | Opening Remarks (Stephanie Hartwell, Dean, CLAS) |
| **10:00 - 11:00 AM:** | **Short Talks (**Session 1. Moderator: Michael Adu) |
| 10:00 AM: | ***Zhuqing Liang, Ph.D.,*** *Dual mechanisms contributing to pyruvate dehydrogenase activity deficiency in a Barth syndrome cell model* |
| 10:20 AM: | ***Alexis Wilson, Graduate student,*** *Stearoyl-CoA Desaturase: A key modulator of adipocyte-driven stress pathways supporting survival of metastatic prostate cancer in bone* |
| 10:40 AM: | ***Daniel Adebayo, Graduate student,*** *Mdm1 coordinates the crosstalk between fatty acid and sphingolipid biosynthesis: Implications for aging* |
| **11:00 – 11:30 AM** | **Flash Talks –** Introduction of posters (Moderator: Michael Adu) |
| 11:00 AM: | ***Abdo Najy, Ph.D.,*** *HACD2-mediated Fatty Acid Signaling for Bone-resident Prostate Cancer Dormancy* |
| 11:05 AM: | ***Yaroslav Balytskyi, Ph.D.,*** *Deep learning-guided reconstruction of the ABHD5 interactions and functions* |
| 11:10 AM: | ***Tyler Ralph-Epps, Graduate student,*** *CL-deficiency leads to acyl-carnitine accumulation in TAZ-KO cells* |
| 11:15 AM: | ***Jaspreet Singh, Ph.D.,*** *Integrative Analysis Unveils Correlation of MicroRNA and Metabolic Pathways Underlying Cerebral Disease in X-Linked Adrenoleukodystrophy Patient Postmortem Brain Tissue* |

11:20 AM: ***Laimar Garmo, Graduate student,*** *Pro-adipogenic activity of perfluorohexane sulfonate (PFHxS) in bone: exploring molecular targets and impact on skeletal metastases from prostate cancer*

11:30-11:40 AM: Coffee Break

**11:45 – 12:40 PM:** **Keynote Address I** (Moderator: Hanna Hariri)

Speaker: Dr. ***Jean Vance, Professor,*** University of Alberta, Edmonton, Canada. *Phospholipid Import into Mitochondria via Mitochondria-associated Membranes (MAM)*

## 12:45 PM: Lunch

**1:15 – 2:40 PM:** **Poster Viewing and Judging**

**2:45 – 3:35 PM:** **Short Talks** (Session 2. Moderator: Mohammad Chakkour)

2:45 PM: ***Eseiwi Folorunsho Obaseki, Graduate student,*** *ER-Lysosome contact sites in regulating autophagy and lipid metabolism.*

3:05 PM: ***Raja Narayanasamy, Ph.D.,*** *Molecular Insights on PS-PLA1 Lipase Activity of Human ABHD16B Protein*

3:25 PM: ***Chisom J Onu, Graduate student,*** *Valproic acid causes inositol depletion by increasing nuclear translocation of Opi1 repressor and decreasing INO1 transcription*

**3:45 – 4:40 PM:** **Keynote Address II (**Moderator: Mike Schmidtke)

**Speaker: Dr. *Tamas Balla, Senior Investigator,*** *NICHD/NIH, Bethesda, MD.*
*Phosphatidylinositol 4-phosphate: a minor lipid with major roles in the control of cellular lipid metabolism*

## 4:45 PM: Concluding Remarks and Award Presentation

# Keynote Talk I

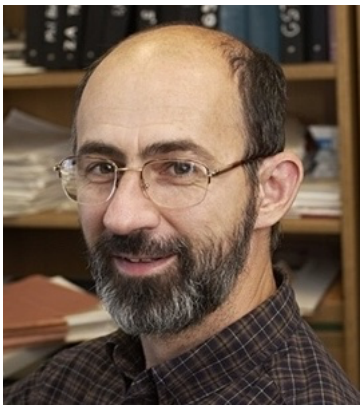
## *Phospholipid Import into Mitochondria via Mitochondria-associated Membranes (MAM)*

***Dr. Jean Vance, Ph.D. FRSC,***
*Professor, Department of Medicine,*
*University of Alberta, Edmonton, Canada*

**Abstract:** The functioning and survival of eukaryotic cells depend on compartmentalization of metabolic processes within specific subcellular organelles. Many hydrophobic membrane phospholipids are generated by organelle-specific enzymes and are transported through the aqueous cytosol for assembly into other organelle membranes. However, mechanisms of inter-organelle lipid transport remain largely unclear. Many organelles in eukaryotic cells form heterotypic membrane contact sites that mediate inter-organelle communication. For example, close juxtaposition (10-80 nm) between the endoplasmic reticulum (ER) and mitochondrial outer membranes occurs via transient tethering via a specific region of the ER (designated mitochondria-associated membranes/MAM). MAM-mitochondria contact sites are required for import of ER-derived phosphatidylserine (PS) into mitochondria for conversion to phosphatidylethanolamine (PE) via PS decarboxylase (PSD) that resides in mitochondrial inner membranes. These contact sites are also required for other key cellular events including calcium transport between ER and mitochondria, and maintenance of mitochondrial morphology and dynamics. The majority of PE in mitochondria is produced *in situ* from ER-derived PS via PSD, whereas very little PE derived from CDP-ethanolamine in the ER is imported into mitochondria. Our demonstration that genetic elimination of PSD in mice is embryonic lethal established that PS-derived PE is required for survival. We also found that PS is required for viability since mice lacking both PS synthases (PSS1 and PSS2) do not survive, whereas PSS1- or PSS2-deficient mice are viable. Recent clinical studies have implicated the PS/MAM/PSD pathway in human diseases. Inter-organelle membrane contacts between other membrane pairs (such as ER, plasma membrane, Golgi, lysosomes, peroxisomes, lipid droplets) have also been characterized. Several of these contact sites appear to be required for inter-organelle lipid transport.

# Keynote Talk II

## *Phosphatidylinositol 4-phosphate: a minor lipid with major roles in the control of cellular lipid metabolism*



***Dr. Tamas Balla*, MD, *Ph.D.*,**
*Senior Investigator, National Institute of Child Health and Human Development-NIH, Bethesda, Maryland*

**Abstract:** Cellular organization of eukaryotic cells relies upon compartmentalization of their signaling nodes on special membrane platforms forming the various organelles. Unique lipid composition of the different organelle membranes not only defines their identity but also is critical for the proper assembly and functioning of the protein signaling complexes associated with them. Inositol phospholipids (PPIns), a class of regulatory lipids, play a critical role in defining membrane identity and forming membrane microdomains with unique signaling properties. Recent developments in lipid membrane biology revealed that phosphatidylinositol (PI) 4-phosphate (PI4P) gradients and the PI 4-kinases that form them drive non-vesicular transport of several structural lipids against their concentration gradients at membrane contact sites (MCSs). These processes clearly depend on the delivery of PI from its site of synthesis in the ER to the membranes where PI4Ks convert them to PI4P. Therefore, our recent efforts have been focused on the question of how PI synthesis and PI transport systems provide the means of proper PI delivery to their other membrane destinations. In this presentation, I will share some historical aspects of PI4P research and show our recent data on the generation and use of molecular tools to visualize and manipulate PI metabolism and delivery and demonstrate, how they can help us better understand the central role of PI4P and other lipids in defining the overall lipid landscape of eukaryotic cells.

# Poster Presentations

| No. | Presenter | Title |
|---|---|---|
| **1** | Daniel Adebayo | Mdm1 coordinates the crosstalk between fatty acid and sphingolipid biosynthesis: Implications for aging |
| **2** | Yaroslav Balytskyi | Deep learning-guided reconstruction of the ABHD5 interactions and functions |
| **3** | Sumit Bandyopadhyay | Identification of Snx13 Interaction Partners at Endoplasmic Reticulum-Lysosomes Membrane Contact Sites |
| **4** | Tyler Ralph-Epps | CL-deficiency leads to acyl-carnitine accumulation in TAZ-KO cells |
| **5** | Laimar Garmo | Pro-adipogenic activity of perfluorohexane sulfonate (PFHxS) in bone: exploring molecular targets and impact on skeletal metastases from prostate cancer |
| **6** | Yogesh Joshi | Inhibition of innate immune activation improves Drosophila model of Vps13D-associated neurodegeneration |
| **7** | Dr. Patricia Kane | Organelle Disruption via Debris and Cell Recovery in Dementia, Alzheimer's, and Neurological Disease |
| **8** | Zhuqing Liang | Dual mechanisms contributing to pyruvate dehydrogenase activity deficiency in a Barth syndrome cell model |
| **9** | Camellia Mashal | Optimizing Gelatin Microspheres for Growth Factor Delivery in Peripheral Neuropathy Treatment |
| **10** | Abdo Najy | HACD2-mediated Fatty Acid Signaling for Bone-resident Prostate Cancer Dormancy |
| **11** | Raja Narayanasamy | Elucidating the Functional Role of Human ABHD16B Lipase in Regulating Triacylglycerol Mobilization and Membrane Lipid Synthesis in Saccharomyces cerevisiae |
| **12** | Eseiwi Folorunsho Obaseki | ER-Lysosome contact sites in regulating autophagy and lipid metabolism |
| **13** | Chisom J Onu | Valproic acid causes inositol depletion by increasing nuclear translocation of Opi1 repressor and decreasing INO1 transcription |
| **14** | Shahnaz Parveen | Membrane biophysics of lipid droplets: investigating ABHD5 behavior and its impact on metabolic regulation |
| **15** | Zachary Pomicter | Nvj3 is a Regulator of Lipid Homeostasis |
| **16** | Abu Ramim | Loss of tafazzin results in decreased levels of branched-chain amino acids (BCAA) and upregulation of BCAA degradation enzymes |
| **17** | Jaspreet Singh | Integrative Analysis Unveils Correlation of MicroRNA and Metabolic Pathways Underlying Cerebral Disease in X-Linked Adrenoleukodystrophy Patient Postmortem Brain Tissue |

**Daniel Adebayo**

(Mentor: Dr. Hanaa Hariri)

**1**

*Department of Biological Sciences, Wayne State University, Detroit, MI*

# Mdm1 coordinates the crosstalk between fatty acid and sphingolipid biosynthesis: Implications for aging

Lipid synthesis and homeostasis involves a complex network of pathways, including fatty acid, neutral lipid, phospholipid, and sphingolipid synthetic pathways. While several studies have focused extensively on synthesis or degradation in individual pathways, little is known about the crosstalk of these different lipid networks in the cell or how they are spatially organized and coordinated at the subcellular level to maintain an efficient homeostatic system. Over the last two decades, researchers have begun to appreciate inter-organelle membrane contact sites (MCS) as hubs for the subcellular compartmentalization and homeostasis of lipid metabolism. MCSs are defined as appositions between organelles maintained by tether proteins that allow for their physical interaction, affecting the function of either organelle or both organelles. Recently, studies in yeast revealed the role of Mdm1, a conserved protein tether that connects the endoplasmic reticulum (ER) and vacuole/lysosome (also called NVJ), in regulating neutral lipid metabolism.

My current work seeks to understand the role of Mdm1 in regulating the link between fatty acid and sphingolipid biosynthesis. Sphingolipids are a diverse class of lipids that have a sphingosine backbone comprising of long chain bases (LCBs), ceramides and complex ceramides. Furthermore, accumulating evidence in budding yeast indicates that reduction in sphingolipid biosynthesis promotes longevity. Interestingly, the sphingolipid synthetic pathway requires very long-chain fatty acids (VLCFAs) to make ceramides.

My current findings show that deleting MDM1 decreases VLCFAs and ceramides containing fatty acids with 24 and 26 chain lengths (C24 and C26). Furthermore, cells lacking Mdm1 have enhanced chronological lifespan. These findings reveal a novel role of the NVJ as a regulatory conduit that coordinates fatty acid and sphingolipid biosynthesis with aging.

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**Yaroslav Balytskyi**

(Mentor: Dr. Christopher Kelly)

*Department of Physics and Astronomy, Wayne State University, Detroit, MI*

## Deep learning-guided reconstruction of the ABHD5 interactions and functions

The metabolism of triacylglycerol (TAG) is vital for human health, as disruptions in this process can contribute to conditions such as obesity, diabetes, high blood pressure, and neurodegenerative disorders like Alzheimer's and Parkinson's diseases. These conditions incur significant treatment costs, amounting to billions of dollars annually in the U.S. alone. Central to TAG metabolism is patatin-like phospholipase domain-containing protein 2 (PNPLA2 or ATGL), which plays a crucial role in breaking down TAGs into fatty acids and glycerol. Another key player is α/β hydrolase domain-containing protein 5 (ABHD5 or CGI-58), which acts as a primary activator of ATGL and exhibits strong interactions with other members of the PNPLA family. However, to gain deeper insights into ABHD5 functions, elucidate the regulation of PNPLA family members, and achieve high-precision temporal resolution of ABHD5 dynamics—which occur on a timescale of seconds—there is a pressing need for the development of highly selective ligands.

We developed a novel ensemble of Neural Network architectures to meet this challenge, providing targeted and accurate predictions for experimentalists. Our approach starts by identifying the most favorable conformations of ABHD5 for ligand binding and identifying key residues in the binding pocket. Subsequently, we determine the binding pose of the ligand by performing molecular docking. Our findings show that this integrated approach significantly improves the accuracy of our predictions.

Furthermore, we identified the key residues responsible for binding ABHD5 both to ligands and other proteins and computationally confirmed that the mutation of these residues significantly weakens the binding strength. Our theoretical predictions were validated by experimental results obtained from the Integrative Biosciences Center. Our work is poised to have far-reaching and long-lasting impacts on a variety of lipid metabolic pathways, spanning both scientific research and translational applications in medicine and healthcare.

**Sumit Bandyopadhyay**

(Mentor: Dr. Hanaa Hariri)

*Department of Biological Sciences, Wayne State University, Detroit, MI*

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## Identification of Snx13 Interaction Partners at Endoplasmic

Snx13 is a triorganelle tether found at the membrane contact sites between lysosomes, the ER, and the lipid droplets. Previous studies have suggested that Snx13 is a regulator of intracellular cholesterol homeostasis and is a predicted lipid transfer protein. However, the molecular mechanism of Snx13-dependent cholesterol transfer between lysosomes and the ER remains unknown. To elucidate the mechanism of Snx13-dependent cholesterol transfer between lysosomes and the ER, I am using the proximity labeling technique to identify proteins that may coordinate with Snx13 at the ER-lysosomes contact sites and help it transfer cholesterol between these two organelles.

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**Tyler Ralph-Epps**

(Mentor: Dr. Miriam Greenberg)

*Department of Biological Sciences, Wayne State University, Detroit, MI*

## CL-deficiency leads to acyl-carnitine accumulation in TAZ-KO cells

Cardiolipin (CL) is a unique phospholipid found predominantly in the inner mitochondrial membrane where it is essential for optimal mitochondrial function and bioenergetics. The importance of CL is underscored by the life-threatening genetic disorder Barth syndrome (BTHS), whereby mutations in the TAFAZZIN (TAZ) gene lead to decreased CL levels. BTHS patients commonly suffer from cardiomyopathy, skeletal myopathy, neutropenia, and severe metabolic abnormalities such as increased levels of lactic acid and 3-methylglutaconic acid. While cardiomyopathy and skeletal myopathy point to defects in mitochondrial bioenergetics, increased lactic acid and 3-methylglutaconic acid specifically suggests alterations in metabolic pathways further upstream such as glycolysis, amino acid oxidation, and fatty acid oxidation (FAO), which supply the substrates necessary for optimal mitochondrial bioenergetics. To further explore how TAZ-deficiency influences energy metabolism, 242 metabolites were quantified via mass spectrometry in WT and TAZ-deficient C2C12 (TAZ-KO) cells. Interestingly, we found that 5 different species of acyl-carnitines, intermediates in mitochondrial fatty acid transport (the carnitine transport system), are significantly increased in TAZ-KO cells relative to WT cells. Furthermore, gene expression levels of all three carnitine transport enzymes, carnitine O-palmitoyl transferase 1 (CPT1), carnitine acyl-carnitine transporter (CACT), and carnitine O-palmitoyl transferase 2 (CPT2) are significantly upregulated in TAZ-KO cells compared with WT controls. We also found that TAZ-KO cells have a significantly decreased maximal oxygen consumption rate (OCR) when supplemented with palmitate (a long chain fatty acid). However, when TAZ-KO cells are supplemented with butyrate (a short chain fatty acid), which can bypass the mitochondrial carnitine transport system, maximal OCR is restored to WT levels in TAZ-KO cells. Taken together, these data suggest that CL-deficiency leads to defective acyl-carnitine transport in TAZ-deficient cells. This could help explain why BTHS patients have a decreased rate of palmitate oxidation during exercise, and commonly experience fatigue and exercise intolerance.

**Laimar Garmo**

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(Mentor: Dr. Izabela Podgorski)

*Department of Pharmacology, Wayne State University School of Medicine, Detroit, MI*

## Pro-adipogenic activity of perfluorohexane sulfonate (PFHxS) in bone: exploring molecular targets and impact on skeletal metastases from prostate cancer

Per- and polyfluoroalkyl substances (PFAS) are environmental contaminants that tend to accumulate in bone. Coincidentally, metastatic prostate cancer (mPCa) has a predilection for bone, an adipocyte-rich organ that serves as a source of lipids and energy for the tumor cells. Peroxisome proliferator-activated receptors (PPARs), notably PPARα and PPARγ, play essential roles in lipid metabolism and the differentiation of adipocytes (adipogenesis). PFAS have been reported to activate PPARs and play roles in lipid metabolism and adipocyte differentiation. However, little is known about their potential impact on bone marrow adipocytes and mPCa progression. We previously established that adipocytes contribute to the progression of mPCa by altering tumor metabolism through activation of oxidative and ER stress response and increase of lipid uptake and transport mechanisms. We hypothesized that PFAS accumulating in bone alter the marrow by promoting adipogenesis, creating a suitable environment for the growth and survival of mPCa. Tibiae and femurs from mice exposed to mixture of 5 environmentally relevant PFAS were extracted and used for histological and RNA analyses. Histological analysis of the tibiae from exposed mice revealed an increase in the number of bone marrow adipocytes, further supported by an augmented expression of adipogenesis-associated genes as determined by RT PCR. Subsequent RNAseq analyses conducted on tibia samples indicated alterations in bone metabolism and turnover due to PFAS exposure, hinting at a possible shift in adipogenesis and osteoblastogenesis pathways. Our in vitro assays utilizing isolated murine bone marrow derived mesenchymal stromal cells (BMSCs) confirmed that PFAS mixture induces adipogenesis and identified PFHxS as a key compound that promotes fat cell differentiation. RNAseq analyses of differentiated BMSCs with PFHxS compared to a vehicle control revealed significant enrichment of the PPAR pathway and, more specifically, augmented downstream target genes of PPARγ, suggesting PFHxS-induced adipogenesis works through activation of PPARγ signaling. Additionally, treatment with a PPARγ specific inhibitor diminished PFHxS-mediated adipogenesis, further confirming PPARγ is a target of PFHxS. Notably, we also observed that exposure of mPCa cells to adipocytes differentiated in the presence of PFHxS, leads to an increased expression of lipid transport, and oxidative stress genes, which we have previously established to be drivers of mPCa. Collectively, our results strongly suggest that exposure to PFHxS may enhance the tumor-supportive role of adipocytes in the bone marrow, contributing to mPCa progression.

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**Yogesh Joshi**

(Mentor: Dr. Ryan Insolera)

*Department of Ophthalmology, Visual and Anatomical Sciences, Wayne State University School of Medicine, Detroit, MI*

## Inhibition of innate immune activation improves Drosophila model of Vps13D-associated neurodegeneration

A complex combination of factors contributes to neurodegenerative disease. Two increasingly common themes in the pathogenesis of neurodegenerative disease are mitochondrial dysfunction and activation of the immune system in the brain. We seek to examine the relationship between these two common pathologies using the powerful genetic tools available in Drosophila melanogaster in a model of neurodegenerative disease. Mutations in the Vps13D gene in humans cause the neurodegenerative disease ataxia, and our previous work has shown that loss of Vps13D in Drosophila neurons results in severe mitochondrial dysfunction, compromised mitophagy, neurodegeneration, and lethality. We hypothesize that activation of the innate immune system in the brain contributes to the neurodegeneration and lethality caused by Vps13D loss. We find that genetically blunting innate immune response in neuronal Vps13D knocked down flies, manipulating the transcription factor Relish, enhances survival. Our results show that loss of Vps13D causes the accumulation of the innate immune protein Kenny (homolog of mammalian optineurin), associated with mitochondria stalled in mitophagy. We are currently testing whether the accumulation of Kenny on stalled mitophagy intermediates is sufficient to sensitize neurons to trigger a cellular immune response. To mimic conditions of Kenny accumulation caused by mitophagy defects, we find that direct overexpression of Kenny in neurons is sufficient to activate cellular innate immunity and results in the upregulation of neurotoxic anti-microbial peptides (AMPs) in the brain. Through future work, we seek to causally associate mitophagy defects in neurons in this model with immune activation to provide a molecular mechanism for these common neurodegenerative disease-associated pathologies.

**Dr. Patricia C. Kane**
*Chief Research Officer*

*Cellular Health Foundation, Richmond, Virginia*

## Organelle Disruption via Debris and Cell Recovery in Dementia, Alzheimer's, and Neurological Disease

Cellular debris is a significant obstacle in overcoming states of disease. Debris often originates from epigenetic insult creating a disturbance of organelle interplay reflected in both aberrant lipids (ceramides, lipid rafts, VLCFAs, sphingomyelin, oxidized lipids & cholesterol) and aberrant proteins (misfolded, aggregated, unfolded) throughout the cellular components (ER, mitochondrion, peroxisome, cytosol, nuclear envelope, membranes). Altered gene expression following epigenetic insult results in impaired peroxisomal and mitochondrial respiration, derangement of neural, cellular and organelle membranes, neuroinflammation, aberrant phospholipid architecture, ceramide formation and endoplasmic reticulum / ER stress (both smooth and rough ER are involved) with the unfolded protein response via mitochondria-associated membranes. Abnormal proteins, however, occur in unison with abnormal lipids and both must be addressed concomitantly. Derangement of neural, cellular and organelle membranes, including deficits of the phospholipids, serve as primary therapeutic targets towards clearing debris caused by epigenetic insult involving both nuclear and mitochondrial DNA adducts. Clearance of debris may be approached with chaperones glycerol butyrate, phenylbutyrate, and TUDCA to collapse the lipid rafts, abnormal lipids, and proteins characteristic in the composition of cellular debris. However, it is crucial to optimize membrane architecture with oral / intravenous phospholipids and bioactive lipids, including all the eicosanoids EPA, DHA and DGLA, to clinically address appropriate balance, fluidity, and phospholipid content of cellular organelles toward optimizing neurometabolic function. Our lipid therapeutic approach has yielded marked clinical improvement in subjects following 3 to 6 months of a targeted regimen corresponding with normalization in red cell lipids, clearance of cellular debris and DNA adducts in Alzheimer's, dementia, and neurological disease.

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**Zhuqing Liang**

(Mentor: Dr. Miriam Greenberg)

*Department of Biological Sciences, Wayne State University, Detroit, MI*

## Dual mechanisms contributing to pyruvate dehydrogenase activity deficiency in a Barth syndrome cell model

Barth syndrome (BTHS) is a rare genetic disease that results from mutations in the TAFAZZIN gene, which encodes the cardiolipin (CL) remodeling enzyme tafazzin (Taz). The mechanisms linking perturbation of CL remodeling and the pathological features of BTHS are not understood. We have recently reported that intermediary metabolism is perturbed in BTHS, and activity of the metabolic gatekeeper enzyme pyruvate dehydrogenase (PDH) is deficient in BTHS models. The mechanism whereby PDH is regulated by Taz is unknown, and this knowledge gap represents an obstacle to the development of therapeutics to treat BTHS.

Using an established C2C12 myoblast model of BTHS, TAZ-KO, I identified important mechanisms by which CL regulates PDH function. CL regulates PDH activity through activation of PDK4, and this is mediated by hyperactivation of AMPK activity, which facilitates FOXO1 nuclear translocation and subsequent increase in PDK4 expression in TAZ-KO cells. PDK4 upregulation leads to alterations in substrate utilization, consistent with observed metabolic changes in BTHS patients, including disrupted glucose and fatty acid oxidation and increased fatty acid accumulation in the heart and plasma.

In addition, CL regulates PDH activity directly by facilitating PDP1-mediated dephosphorylation of PDH. Of different CL species tested in isolated TAZ-KO mitochondria, only tetralinoleoyl-CL (TLCL) rescues PDH activity. Interestingly, when PDP1 activity is inhibited, the PDH activity was not rescued by CL. Additionally, PDP1 activity is decreased in TAZ-KO cells and TLCL interacted with PDP1 in vitro. These findings suggest that PDP1 is required for CL-activation of PDH activity. It is likely that TLCL serves as an anchor for concurrent binding of PDP1 and PDH, thereby facilitating their interaction. This is supported by the observed dose-dependency of TLCL rescue.

PDP1 activity is also likely reduced by the finding that mitochondrial calcium levels are decreased in TAZ-KO cells, as PDP1 activity is dependent on calcium. TAZ-KO cells exhibit decreased mitochondrial calcium levels and supplementation with calcium lactate (CaLac) rescues PDH activity and oxygen consumption rate (OCR). Overall, I proposed two mechanisms whereby CL regulates PDH, including regulation of PDK4 via FOXO1-AMPK and interaction with PDP1 or/and modifying mitochondrial calcium levels. These findings provide insight into the regulatory mechanisms of PDH activity by CL, linking CL function to substrate metabolism and mitochondrial calcium regulation.

**Camellia Mashal**

(Mentor: Dr. Harini Sundararaghavan)

*Department of Biomedical Engineering, Wayne State University, Detroit, MI*

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# Optimizing Gelatin Microspheres for Growth Factor Delivery in Peripheral Neuropathy Treatment

Peripheral neuropathy is a devastating condition characterized by damage to peripheral nerves, resulting in numbness, weakness, and loss of reflexes. Treatment is particularly challenging when nerves undergo damage due to axonal degradation, requiring axon regeneration and reinnervation of the affected organ or muscle. Although neurotrophic factors show promise in promoting nerve regrowth, effective delivery remains a challenge. Gelatin microspheres offer a solution for targeted factor delivery due to their biocompatibility and controlled release.

This study aims to optimize gelatin microspheres for peripheral nerve regeneration by investigating the effects of gelatin and oil types on microsphere characteristics. Using a water-in-oil emulsion, gelatin microspheres were synthesized with type A and type B gelatin, alongside variations in oil types (extra virgin olive oil and pure olive oil). Results demonstrated that type B gelatin microspheres were significantly smaller (average 18µm) compared to type A (average 80-150µm). Further tests revealed that using a uniformly shaped stir bar yielded spherical microspheres, while pure olive oil led to clustering without size reduction.

Analysis suggests that gelatin type B and extra virgin olive oil are optimal for producing small, spherical microspheres suitable for growth factor delivery. Challenges such as gelatin denaturation and filtration inconsistency were identified as potential sources of error. Overall, this study provides insights into optimizing gelatin microspheres as carriers for growth factor delivery in peripheral neuropathy treatment, offering the potential for improved therapeutic strategies.

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**Dr. Abdo Najy**

*Assistant Professor - Research*

*Department of Pathology, Wayne State University School of Medicine, Detroit, MI*

## Identification of a novel regulator for the exit of bone-resident prostate cancer cells from dormancy

Bone metastases occur in the majority of prostate cancer (PCa) that progresses to metastatic castrate-resistant prostate cancer (mCRPC), resulting in incurable disease. In PCa, it has long been suspected that clinical disease recurrence is due to proliferation of disseminated tumor cells (DTCs) already present in the target organ at the time of initial therapy. We investigated the molecular and cellular mechanisms governing the dormancy of bone resident PCa cells. We present evidence that the Pten knockout mouse represents a model for "dormancy" of DTCs in the bone microenvironment. Through genome-wide knockdown of potential tumor suppressors in Pten-/- PCa cells and subsequent in vivo functional screening in an intratibial injection model, we identified HACD2, a 3-hydroxyacyl-CoA dehydratase which plays a critical role in the synthesis of very long chain fatty acid (VLCFA), as a potential regulator of bone-resident PCa cell dormancy. Our findings demonstrate that HACD2 functions as a tumor suppressor through the production of VLCFAs and secreted VLCFAs-mediated autocrine signaling induces dormancy signaling via their receptors, free fatty acid receptor (FFAR) 1 and 4. Therefore, downregulation (or loss) of HACD2 promotes a conversion of dormant PCa cells to a proliferative state. Moreover, the lack of VLCFA results in the recruitment of osteoclasts and/or inflammatory immune cells, contributing to bone remodeling and awakening bone-resident dormant cells. Our study has uncovered the pivotal role of an enzyme involved in very long chain fatty acid synthesis as a previously unrecognized tumor suppressor in regulating tumor dormancy and inhibiting intraosseous tumor growth.

**Raja Narayanasamy**

(Mentor: Dr. James G. Granneman)

*Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI*

**11**

# Elucidating the Functional Role of Human ABHD16B Lipase in Regulating Triacylglycerol Mobilization and Membrane Lipid Synthesis in Saccharomyces cerevisiae

Lipids are essential biological macromolecules that play a pivotal role in various physiological processes and cellular homeostasis. ABHD16B, a member of the α/β-hydrolase domain (ABHD) superfamily protein, has emerged as a potential key regulator in lipid metabolism. However, the precise role of human ABHD16B in lipid metabolism remains unclear. In this study, we reported the overexpression of ABHD16B in Saccharomyces cerevisiae to determine its physiological relevance in lipid metabolism. Through in vivo [14C]acetate labeling experiments, we observed that overexpression of ABHD16B causes a decrease in cellular triacylglycerol (TAG) levels and a concurrent increase in phospholipid synthesis in wild-type cells. Mass spectrophotometry LC-MS/MS) analysis further corroborated these findings, showing a significant decrease in TAGs with a carbon chain length of 48 and an increase in major phospholipid species, specifically 34:2, upon overexpression of ABHD16B. Confocal microscopy analysis revealed a reduction in the number of lipid droplets in strains overexpressing ABHD16B, consistent with the observed decrease in neutral lipids. Additionally, qRT-PCR analysis indicated a high phospholipid synthetic activity of ABHD16B and a potential decrease in TAG levels in wild-type yeast, possibly due to upregulation of endogenous TAG hydrolytic enzymes, as confirmed using 3tglsΔ mutant strain. Furthermore, GC-MS analysis revealed significant modifications in fatty acid composition upon ABHD16B overexpression. Collectively, our results underscore the influence of ABHD16B overexpression on TAG levels, phospholipid synthesis, lipid droplet dynamics, and fatty acid composition. These findings reveal a complex interplay between TAG hydrolysis and phospholipid synthesis, highlighting the critical involvement of ABHD16B in lipid homeostasis and providing further insights into its regulatory function in cellular lipid metabolism.

Keywords ABHD16B: Triacylglycerol: Phospholipid Synthesis: Mass Spectrometry: Lipid Droplets: Lipid Homeostasis.

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**Eseiwi Folorunsho Obaseki**

(Mentor: Dr. Hanaa Hariri)

*Department of Biological Sciences, Wayne State University, Detroit, MI*

## ER-Lysosome contact sites in regulating autophagy and lipid metabolism

Compartmentalization of eukaryotic cells, in the form of membrane-delimited organelles, is critical for the regulation and segregation of metabolic reactions. However, to maintain cellular homeostasis in a constantly varying environment, efficient inter-organelle exchange of signals and metabolites is crucial. Apart from vesicular trafficking and cytoplasmic diffusion, exchange occurs at inter-organelle junctions maintained by tether proteins, known as membrane contact sites (MCSs). Several studies on MCSs and associated proteomes have led to appreciation of their diverse role in biological functions. Expectedly, mutations in MCSs and associated proteome have been implicated in metabolic disorders, neurodegenerative diseases, and aging. However, a mechanistic understanding of how dysregulating MCSs contributes to metabolic imbalance and diseases is not clear. Here, we used budding yeast as a model system to investigate the role of ER-Lysosome contact sites in modulating autophagy. Our findings show that the conserved ER-lysosome/vacuole tether, Mdm1, is required for efficient specific and non-specific autophagy. Investigation into the regulatory mechanism of Mdm1 on autophagy indicates a role in facilitating autophagosome membrane formation. Together this data suggests a role for Mdm1 in coordinating autophagy through membrane lipid biosynthesis.

**Chisom J Onu**

(Mentor: Dr. Miriam Greenberg)

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*Department of Biological Sciences, Wayne State University, Detroit, MI*

## Valproic acid causes inositol depletion by increasing nuclear translocation of Opi1 repressor and decreasing INO1 transcription

Valproic acid (VPA) is a mood stabilizer widely prescribed and used for the treatment of bipolar disorder (BD). BD is a psychiatric disorder that affects 2% of the world's population. Although VPA has been in use for more than four decades, the therapeutic mechanism of action remains unknown. We have determined that VPA decreases inositol levels. Two other mood stabilizers, lithium, and carbamazepine have been shown to decrease inositol. The finding that three structurally distinct mood stabilizers decrease inositol suggests that inositol depletion may be a key therapeutic mechanism of action of the drugs. Using the yeast model in which the inositol biosynthetic pathway has been well studied, we aim to determine the mechanism by which VPA decreases inositol. Inositol is synthesized de novo from glucose-6-phosphate (G6P), a product of glucose phosphorylation by the rate-limiting enzyme Myo-inositol phosphate synthase (MIPS) encoded by the INO1 gene in yeast. Transcription factors Ino2 and Ino4 initiate INO1 transcription by forming a tetramer on the INO1 promoter. INO1 expression is robustly repressed by Opi1 protein, which interacts with Ino2 on the INO1 promoter. The repressor protein Opi1 is tethered to the endoplasmic reticulum (ER) by the Scs2 protein and phosphatidic acid (PA) when inositol is limiting. In the presence of inositol, Opi1 translocates to the nucleus, and INO1 expression is repressed. Our research has shown that VPA decreases INO1 mRNA and MIPS protein levels, increases Opi1 nuclear translocation, and that VPA-mediated inositol depletion is Opi1-dependent. In addition, VPA decreases total PA levels and, specifically, PA species 34:1, 34:2, and 32:1. Further, Scs2 protein is not altered with VPA treatment. These findings suggest that VPA depletes inositol by increased Opi1-mediated repression of INO1 expression and that depletion of individual PA species, but not total PA, is responsible for increased Opi1-nuclear translocation. These studies have implications for BD, as PA has been shown to modulate inositol hexakisphosphate kinase 1 (IP6K1) nuclear translocation to decrease MIPS protein levels in mammalian cells.

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## Membrane biophysics of lipid droplets: investigating ABHD5 behavior and its impact on metabolic regulation

The surface of lipid droplets (LDs) undergoes intricate remodeling upon the initiation of lipolysis, orchestrated by a multifaceted interplay involving proteins, phospholipids (PLs), and neutral lipids (NLs). Understanding the molecular mechanisms governing LD surface remodeling during lipolysis is essential for preventing metabolic disorders like obesity and diabetes. Our research involves the development of model LDs (mLDs) ranging from 0.1 to 40 microns in diameter with known compositions, and using high-resolution microscopy methods to study binding kinetics, diffusion dynamics, membrane sorting phenomena, and tension alterations contingent upon LD composition. We have developed coverslip adhered mLD caps that enable time dependent measurements of the monolayer. Our results demonstrates that phospholipid vesicles bound and formed a monolayer at the oil-water interface within 5 mins, but the monolayer tension was very slow to equilibrate. We also developed droplet embedded vesicle (DEVs) which enables monitoring of the spontaneous sorting of proteins between the phospholipid monolayer and bilayer as a function of phospholipid composition and tension. We observed a preferential partitioning of ABHD5 to the LD monolayer compared to the phospholipid (PL) bilayer. Ultimately, our studies aim to uncover the fundamental biophysics governing LD biology, providing insights into biophysical properties and molecular mechanisms crucial for metabolic health.

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## Nvj3 is a Regulator of Lipid Homeostasis

Organelles were once believed to be isolated, intracellular compartments which functioned independently from the other components within the cytoplasm. However, emerging studies have shown that organelles are not isolated, but rather interact with each other. One of these modes of interaction is membrane contact sites (MCS). MCSs are regions where organelles are held in close proximity ranging from 10-35nm apart. This contact region is maintained by tether proteins which facilitate communication between the organelles. The nuclear ER-vacuole junction (NVJ) represents the contact site formed between the nuclear ER and vacuoles in yeast. This junction is also found in multicellular organisms between the ER and lysosomes. Studies have shown that the size of the NVJ is responsive to cellular nutrient levels. Lipid storage organelles known as lipid droplets accumulate at this junction during nutrient stress such as acute glucose restriction or acute nitrogen restriction. These suggest that NVJ has a role in cellular response to nutrient starvation and lipid droplet metabolism. Aside from the main tether proteins, several metabolic proteins reside at the NVJ. One of these proteins is the newly discovered Nvj3 which has been shown to localize to lipid droplets at the NVJ. The mechanism behind Nvj3's localization to lipid droplets remains unknown. Therefore, my project in the lab is to uncover this mechanism and determine why it occurs.

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## Loss of tafazzin results in decreased levels of branched-chain amino acids (BCAA) and upregulation of BCAA degradation enzymes

Barth syndrome (BTHS) is a severe genetic disorder resulting from mutations in the TAFAZZIN (TAZ) gene, resulting in decreased total cardiolipin (CL), elevated monolysocardiolipin, and deficient tetralinoleoyl-CL. Clinical symptoms include myopathy, fatigue, and sarcopenia among others; the precise mechanisms underlying these phenotypes are not understood. In TAZ knockout (TAZ-KO) mouse myoblasts, we have observed perturbations in aerobic energy metabolism, including decreased activity of pyruvate dehydrogenase, decreased flux of glucose to acetyl-CoA and the TCA cycle, and reduced ATP levels. Mass spectrometry analysis indicated significantly decreased levels of branched-chain amino acids (BCAAs) in TAZ-KO cells. RNA-seq data analysis revealed increased expression levels of enzymes involved in the degradation of leucine and isoleucine. Mitochondrial oxygen consumption rate (OCR) was increased with the supplementation of increased concentration of BCAAs. These observations suggest that the impaired energy production in TAZ-KO cells results in increased utilization of BCAAs, potentially limiting their availability for protein synthesis. Elucidating the relationship between TAZ deficiency, BCAA metabolism, and protein synthesis could provide insights for developing precise interventions to enhance muscle function and improve the quality of life for individuals affected by BTHS.

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# Integrative Analysis Unveils Correlation of MicroRNA and Metabolic Pathways Underlying Cerebral Disease in X-Linked Adrenoleukodystrophy Patient Postmortem Brain Tissue

Introduction: X-linked adrenoleukodystrophy (X-ALD) is a progressive neurodegenerative disease caused by mutations in peroxisomal ABCD1 gene. X-ALD males develop fatal cerebral demyelinating disease (cALD) as young as 3 years of age with death typically occurring within 2-5 years of onset of symptoms. The mechanism by which ABCD1 mutation initiates neuroinflammation and demyelination remains unknown.

Objective: Our objective was to identify potential novel pathways underlying the severity of disease progression. For this we employed multi-omics approach of untargeted metabolomics and next generation sequencing (HiSeq) to identify the regulatory (microRNA) and active (metabolite) pathways underlying the fatal neuroinflammation and demyelination in X-ALD.

Methods: Postmortem brain tissue (n=5) from healthy controls and ALD patients were processed for microRNA (miRNA) and metabolite extraction and analysis (HiSeq [Illumina] and Gas Chromatography Mass Spectrometry (GC-MS) [Agilent Technologies], respectively). Data analysis was performed by ""MetaboAnalyst 2.5"" (www.metaboanalyst.ca/) for GC-MS and Bioconductor (www.bioconductor.org) for miRNA.

Results: Each measured miRNA and metabolite was screened using appropriate ANOVA models to account for the study design. Thresholds for significance were set to control the estimated false discovery rate, per platform, at 5%.

We compared postmortem brain white matter of healthy controls (CTL) with normal looking area (NLA) and periphery of plaque/lesion (PLS) regions within the cALD brain white matter. Analysis of variance (P< 0.05) and Post-hoc t-tests identified nineteen miRNA and eleven metabolites that significantly differed (P< 0.05) across the three groups (control, NLA and PLS). Of the nineteen miRNA seventeen were increased with disease severity (PLS>NLA>CTL) and two were decreased (CTL>NLA>PLS). Seven metabolites were upregulated with disease severity (PLS>NLA>CTL) and four were downregulated (CTL>NLA>PLS). We calculated the Pearson's correlation coefficient between the expression of these 19 miRNAs and the metabolite intensities of significantly differential metabolites for putative links between the global gene expression modulators (miRNAs), and metabolites. Ingenuity pathway analysis of differentially altered metabolites and miRNA comparing CTL with NLA and NLA with PLS, identified several hubs of metabolite and signaling molecules and their upstream regulation by miRNA. Of special interest were the novel interactions predicted between miR-2114, miR378a-3p and miR-23a-3p with norrin protein, aspartic acid metabolism and CDP-choline metabolism.

Conclusion: Our novel ""transomic"" modeling identifies, for the first time, integrated miRNA and metabolite pathways underlying cerebral disease severity in fatal X-ALD.

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