Women in Autophagy

Beth Levine's Legacy Network

4th Annual Symposium Webinar

October 26-27, 2023

Organizers: **Mondira Kundu** (Faculty chair) ✦ **Ee Phie Tan** (Fellow chair)
Maho Hamasaki (Faculty cochair) ✦ **Rebecca Sereda** (Fellow cochair)

KEYNOTE SPEAKERS

Eric Baehrecke, PhD | UMass Chan
Anne Simonsen, PhD | Univ of Oslo

Trainee sessions |
Mentoring Workshop |
Poster sessions |

2-Days, 4-Sessions for global participation

October 26, 2023: New York 10:00 – 14:00 ✦ New York 18:00 – 21:50
October 27, 2023: New York 09:00 – 12:15 ✦ New York 18:00 – 21:15

Sponsored by:

The Company of Biologists

www.womeninautophagy.com

Dr. Beth Levine

1960 – 2020

Beth Levine deemed scientific conferences the most effective venues to facilitate isolated ideas to become the groundbreaking discoveries that have pushed forward the autophagy field for in the last twenty years.

Born in Newark, New Jersey, on April 7, 1960, Dr. Levine graduated magna cum laude from Brown University in 1981. She received her medical degree from Cornell University Medical College in 1986, followed by a residency in internal medicine at the Mount Sinai Hospital in New York. She established her laboratory at Columbia University in New York and was recruited to University of Texas Southwestern Medical Center in 2004 where she served as Director of the Center for Autophagy Research.

Dr. Beth Levine discovered the first mammalian autophagy gene, which she named *beclin 1*, demonstrating that autophagy played a critical role in cancer. An autophagy pioneer, her laboratory unraveled conserved mechanisms underlying the regulation of autophagy and provided the first evidence that autophagy is crucial in antiviral host defense, tumor suppression, neurodegenerative diseases, lifespan extension, metazoan development, diabetes, and the beneficial metabolic effects of exercise. Furthermore, she developed a potent autophagy-inducing cell-permeable peptide, Tat-beclin 1, with potential therapeutic applications in a range of human diseases.

Women in Autophagy (WIA) was established as Beth Levine's Legacy Network in 2020. This international network was founded to continue her dedication to scientific research, her commitment to mentoring and empowering women in science.

Beth Levine's "joy of scientific curiosity and discovery" was inspiring to anyone who met her - scientists, physicians, and non-scientists alike. Asked once about her secret to success she commented, "*I think what was most critical to my success was my willingness to follow my scientific intuition and curiosity and pursue questions that I thought were important*". She kept THE nitrocellulose membrane, on which she had identified Beclin1, for many years and loved to always show it in her talks to remind everybody how every big discovery starts with a simple experiment - embodying how passionate, Beth Levine the scientist, really was.

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Program Day 1

Zoom: https://us06web.zoom.us/j/86480617048

October 26, 2023 ✦ Times in EST USA

Session I

10:00 – 10:20 **INTRODUCTION** Report on WIA activities (Ana Maria Cuervo, MD PhD)

10:20 – 10:30 **Milton Packer, MD** | Baylor University (Mondira Kundu, MD PhD)

10:30 – 11:20 KEYNOTE SPEAKER

Anne Simonsen, PhD | University of Oslo, Norway
Regulation of autophagy and cellular bioenergetics by lipid-binding proteins

-- 10 minutes break ----------

11:30 – 12:45 TRAINEE SESSION I | Moderator: Ee Phie Tan, PhD

Kajal Kamble, National Institute of Immunology, India (PhD Student)
A novel ER stress regulator ARL6IP5 induces ER-phagy to reduce the prion burden

Nathalia Chica, PhD, Oslo University Hospital, Norway (Research Scientist)
Genome-wide profiling of the hierarchical control of autophagy dynamics using deep learning

Irene Sambri, PhD, Telethon Institute of Genetics and Medicine, Italy (Research Associate)
RagD auto-activating mutations impair MiT/ TFE activity in kidney tubulopathy and cardiomyopathy syndrome

Adriana Covarrubias-Pinto, PhD, Goethe University, Germany (Postdoc)
Ubiquitination regulates ER-phagy and remodelling of endoplasmic reticulum

Bishal Basak, PhD, University of Pennsylvania, USA (Postdoc)
Mitochondrial damage triggers degradation of negative regulators of neuronal autophagy

-- 10 minutes break ----------

12:55 – 13:55 POSTER SESSION I

13:55 – 14:00 Reminder for upcoming
SESSION II 18:00 – 21:50 EST USA

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Program Day 1

Zoom: https://us06web.zoom.us/j/86480617048

October 26, 2023 ✦ Times in EST USA

Session II

18:00 – 18:20 **INTRODUCTION** Report on WIA activities (Ana Maria Cuervo, MD PhD)

18:20 – 18:30 **Milton Packer, MD** |Baylor University (Mondira Kundu, MD PhD)

18:30 – 19:20 KEYNOTE SPEAKER

Eric Baehrecke, PhD |University of Massachusetts Chan Medical School, USA
Ouroboros, autophagy, mitochondria, ER and disease

-- 5 minutes break

19:25 – 20:40 TRAINEE SESSION II | Moderator: Rebecca Sereda, MS

Mariana Sofia Tadic, University of Buenos Aires, Argentina (PhD Student)
The autophagy-related transmembrane protein VMP1 is unconventionally secreted as a component of extracellular vesicles

Rabia Khawaja, PhD, Albert Einstein College of Medicine, USA (Postdoc)
Chaperone-mediated autophagy regulates neuronal excitability by remodeling the synaptic proteome

Camille Lacarriere-Keita, University of Sherbrooke, Canada (PhD Student)
Autophagy inhibition favors drosophila intestinal enteroendocrine cell differentiation, most likely by increasing JAK-STAT signaling

Annan Cook, University of California Berkeley, USA (PhD Student)
Structural pathway for autophagic class III PI 3 - kinase activation by the myristoylated GTP - binding pseudokinase VPS15

Josephine Thinwa, MD, PhD, University of Texas Southwestern, USA (Assistant Professor)
The neurodevelopmental gene CDKL5 regulates p62-mediated virophagy of neurotropic viruses

-- 5 minutes break

20:45 – 21:45 POSTER SESSION II

21:45 – 21:50 Reminder for upcoming
SESSION III 9:00 – 12:15 EST USA SESSION IV 18:00 – 21:15 EST USA

Women in Autophagy

Beth Levine's Legacy Network

4th Annual Symposium Webinar

October 26-27, 2023

Organizers: **Mondira Kundu** (Faculty chair) ✦ **Ee Phie Tan** (Fellow chair)
Maho Hamasaki (Faculty cochair) ✦ **Rebecca Sereda** (Fellow cochair)

Program Day 2

Zoom: https://us06web.zoom.us/j/86480617048

October 27, 2023 ✦ Times in EST USA

Session III

9:00 – 9:05 **INTRODUCTION** (Mondira Kundu, MD PhD)

9:05 – 9:55 **KEYNOTE SPEAKER**

Eric Baehrecke, PhD |University of Massachusetts Chan Medical School, USA
Ouroboros, autophagy, mitochondria, ER and disease

-- 5 minutes break ----------

10:00 – 11:00 **TRAINEE SESSION II** | Moderator: Rebecca Sereda, MS

Mariana Sofia Tadic, University of Buenos Aires, Argentina (PhD Student)
The autophagy-related transmembrane protein VMP1 is unconventionally secreted as a component of extracellular vesicles

Rabia Khawaja, PhD, Albert Einstein College of Medicine, USA (Postdoc)
Chaperone-mediated autophagy regulates neuronal excitability by remodeling the synaptic proteome

Camille Lacarriere-Keita, University of Sherbrooke, Canada (PhD Student)
Autophagy inhibition favors drosophila intestinal enteroendocrine cell differentiation, most likely by increasing JAK-STAT signaling

Annan Cook, University of California Berkeley, USA (PhD Student)
Structural pathway for autophagic class III PI 3 - kinase activation by the myristoylated GTP - binding pseudokinase VPS15

Josephine Thinwa, MD, PhD, University of Texas Southwestern, USA (Assistant Professor)
The neurodevelopmental gene CDKL5 regulates p62-mediated virophagy of neurotropic viruses

-- 5 minutes break ----------

11:05 – 12:05 **MENTORING WORKSHOP** | Stress in the Lab

12:05 – 12:15 **CLOSING REMARKS and AWARDS** (Mondira Kundu, MD PhD)
Introduction for WIA Annual Symposium 2024 (Maho Hamasaki, PhD)

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Program Day 2

Zoom: https://us06web.zoom.us/j/86480617048

October 27, 2023 ✦ Times in EST USA

Session IV

18:00 – 18:05 **INTRODUCTION** (Mondira Kundu, MD PhD)

18:05 – 19:05 **MENTORING WORKSHOP** | Stress in the Lab

-- 5 minutes break ----------

19:10 – 20:00 **KEYNOTE SPEAKER**

Anne Simonsen, PhD |University of Oslo, Norway
Regulation of autophagy and cellular bioenergetics by lipid-binding proteins

-- 5 minutes break ----------

20:05 – 21:05 **TRAINEE SESSION I** | Moderator: Ee Phie Tan, PhD

Kajal Kamble, National Institute of Immunology, India (PhD Student)
A novel ER stress regulator ARL6IP5 induces ER-phagy to reduce the prion burden

Nathalia Chica, PhD, Oslo University Hospital, Norway (Research Scientist)
Genome-wide profiling of the hierarchical control of autophagy dynamics using deep learning

Irene Sambri, PhD, Telethon Institute of Genetics and Medicine, Italy (Research Associate)
RagD auto-activating mutations impair MiT/ TFE activity in kidney tubulopathy and cardiomyopathy syndrome

Adriana Covarrubias-Pinto, PhD, Goethe University, Germany (Postdoc)
Ubiquitination regulates ER-phagy and remodelling of endoplasmic reticulum

Bishal Basak, PhD, University of Pennsylvania, USA (Postdoc)
Mitochondrial damage triggers degradation of negative regulators of neuronal autophagy

21:05 – 21:15 **CLOSING REMARKS and AWARDS** (Mondira Kundu, MD PhD)
Introduction for WIA Annual Symposium 2024 (Maho Hamasaki, PhD)

Beth Levine's Legacy Network

WIA was founded to carry on Beth Levine's dedication to mentoring and training. Our mission is to promote the careers of trainees and young scientists interested in autophagy through a dynamic and global network of mentors and trainees. WIA provides a platform for targeted mentoring activities and workshops and hosts the Beth Levine's Legacy Annual Symposia.

Founding Members

Patricia Boya ✦ Ruey-Hwa Chen ✦ Charleen Chu ✦ Marisa Colombo ✦ Ana Maria Cuervo ✦ Laura Delgui ✦ Eeva-Liisa Eskelinen ✦ Maho Hamasaki ✦ Malene Hansen ✦ Congcong He ✦ Marja Jäättelä ✦ Adi Kimchi ✦ Claudine Kraft ✦ Mondira Kundu ✦ Alicia Melendez ✦ Sophie Pattingre ✦ Tassula Proikas-Cezanne ✦ Salwa Sebti ✦ Katja Simon ✦ Anne Simonsen ✦ Sharon Tooze ✦ Maria Ines Vaccaro ✦ Xiaochen Wang ✦ Eileen White ✦ Yan Zhao

Annual Symposium Committee

To organize the WIA annual symposium

Mondira Kundu **Faculty chair**
Ee Phie Tan **Fellow chair**

Maho Hamasaki
Rebecca Sereda

Carl Ash
Dongfang Li
Eunice Dominguez Martin
Floralba Gjergjova
Gamze Guney Eskiler
Leonard Yoon
Lorelei Ayala-Guerrero
Lydia Ambaye
Malena Herrera Lopez
Mariana Tadic
Maryam Jafari Rasht
Mika Minami
Paloma Liton
Sandra Pelka
Saori Yoshii
Sarah Norman
Susmita Kaushik
Thuy Nguyen
Yuhong Ma
Zi Gao
Thabata Duque **(Comm. L.)**

Journal Club Committee

Acquire deeper understanding of autophagy
Learn technical skills
Discuss review process of the articles
Present and promote publications of WIA members

Karla Alvarez-Valadez **Chair**
Aurore Claude-Taupin **Co-chair**
Carine Joffre **Faculty Advisor**

Aishwarya Chhatre
Christin Naumann
Eugenia Almacenas
Marie Nollet
Myra Chavez
Pamela Mattar
Prasoon Jaya
Rabia Khawaja
Swathy Krishna **(Comm. L.)**

Beth Levine's Legacy Network

Annual Symposium 2023

Scientific Advice Committee

Coordinate the share-for-feedback platform of the WIA website member portal, where members ask questions about experimental setup, troubleshoot analysis and results

Organize workshops on specific aspects of autophagy

Raquel Gómez-Sintes **Chair**
Shahla Shojaei **Co-chair**
Marta Martinez-Vicente **Faculty Advisor**

Ghada Alsaleh
Kristina Ames
Laury Lescat
Madhulika Tripathi
Muriel Mari
Pooneh Mokarram
Rut Valdor
Shirin Porteymour
Tayyebeh Madrakian
Virginie Hubert
Idil Orhon **(Comm. L.)**

Fundraising Committee

To raise funds to support the activities of WIA program, including travel grants for fellows

Ghita Ghislat **Co-chair**
Ana Rosa Saez **Co-chair**

Clara Sokn
Daniela Stanga
Inmaculada Tasset
Jin Zhou
Katharina Bell
Natalia Reglero
Stephanie Kermorgant
Valerie Pierrefite-Carle
Mericka McCabe **(Comm. L.)**

Mentoring Committee

Promote careers of trainees and young scientists interested in autophagy

Generate a global network of e-mentors providing trainees with access to expert advice on autophagy-related topics, career development and work-family balance

Alysia Vrailas-Mortimer **Chair**
Caroline Mauvezin **Co-chair**

Alejandra Suares
Anne Simonsen
Carolina Alquezar
Eileen White
Evelina Gatti
Ghita Ghislat
Ivana Novak
Lisa Frankel
Luciana Rodrigues Gomes
Malene Hansen
Maria Soledad Alvarez
Meiyan Jin
Mondira Kundu
Muriel Mari
Naomi Okugbeni
Natalia Jimenez Moreno
Nathalia Chica Balaguera
Nesibe Peker
Patricia Boya
Salwa Sebti
Saska Ivanova
Sharon Tooze
Stephanie Kermorgant
Surbhi Verma
Waleska Kerllen Martins
Beatriz Dias **(Comm. L.)**

Communications Committee

To raise funds to support the activities of WIA program, including travel grants for fellows

Marina Garcia-Macia **Chair**
Elise Jacquin **Co-chair**
Anne Simonsen **Faculty Advisor**
Yan Zhao **Faculty Advisor**

Azin Amin
Beatriz Dias
Cristina Vanrell
Idil Orhon
Laura Cervera-Carles
Malena Herrera Lopez
Mariana Tadic
Mericka McCabe
Roxana Resnik
Satya Surabhi
Shruti Ghai
Swathy Krishna
Theresa Bub
Thabata Duque

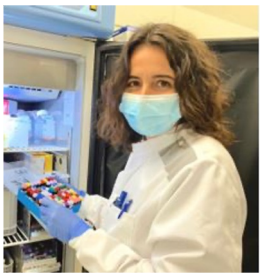
Annual Symposium 2023

WORKSHOP: Stress in the Lab

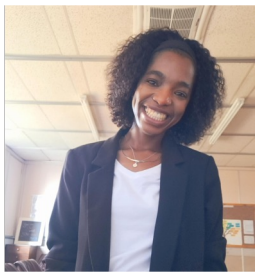
October 27, 2023

Session III: 11:05 EST USA

Carlos Velasco
Associate Professor
BI Norwegian Business School
Norway

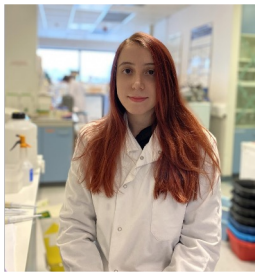


Meritxell Espino Guarch
Junior PI
Sidra Medical and Research Center
Qatar



Claudia Matlakala Ntsapi
Lecturer
University of Free State
South Africa

Jennifer Kunselman
Postdoc
National Institutes of Health
USA



Carla Salomo Coll
PhD Student
University of Edinburgh
UK

Join us for our workshop on dealing with Stress in the Lab!

Panelists from all career stages and institutions across the world will discuss their sources of stress in the lab and what tools they use to handle that stress.
We will also have a Q&A with the panelists.

Annual Symposium 2023

WORKSHOP: Stress in the Lab

October 27, 2023

Session IV: 18:05 EST USA

Maria Vaccaro
Full Professor
University of Buenos Aires
Argentina

Jung Kim
Assistant Professor
University of Hong Kong
China

Louise Uoselis
Postdoc
Monash University
Australia

Azin Amin
Postdoc
University of Melbourne
Australia

Sadia Sultana
PhD student
Illinois State University
USA

Join us for our workshop on dealing with Stress in the Lab!

Panelists from all career stages and institutions across the world will discuss their sources of stress in the lab and what tools they use to handle that stress.

We will also have a Q&A with the panelists.

Name: Kajal Kamble
Position: PhD student
Email: kajalkamble@nii.ac.in
Institution: National Institute of Immunology, New Delhi, India
Keywords: Prion disease, selective autophagy, ER-phagy, ER stress, ARL6IP5

Title: **A novel ER stress regulator ARL6IP5 induces ER-phagy to reduce the prion burden**

Abstract:
Prion disease is a fatal and infectious neurodegenerative disorder caused by the trans-conformation conversion of PrPC to PrPSc. Accumulated PrPSc-induced ER stress causes chronic UPR activation which is one of the fundamental steps in prion disease progression. Here, we demonstrated that ARL6IP5 is compensatory upregulated in response to chronically activated UPR in the cellular prion disease model (RML-ScN2a). ARL6IP5 overcomes ER stress by lowering the expression of chronically activated UPR pathway proteins. We found that ARL6IP5 induces ER-phagy to degrade the prion burden by releasing ER stress. Furthermore, the knockdown of ARL6IP5 leads to inefficient autophagic flux and elevated PrPSc burden. Our study discovered that ARL6IP5-induced ER-phagy is dependent on Ca2+ mediated AMPK activation and can also induce 3MA-inhibited autophagy flux. ARL6IP5-induced ER-phagy involves interaction with the soluble ER-phagy receptor CALCOCO1 and the lysosomal marker LAMP1. Here, we delineate the role of ARL6IP5 as a novel ER stress regulator and ER-phagy inducer that can effectively degrade the misfolded PrPSc burden.

Name: Nathalia Chica, PhD
Position: Research Scientist
Email: nathac@uio.no
Institution: Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway
Keywords: autophagy, dynamics, systems-wide mechanisms, high-throughput screening, deep learning

Title: Genome-wide profiling of the hierarchical control of autophagy dynamics using deep learning

Abstract:
Autophagy involves the breakdown and recycling of cellular material. It is initiated in response to cell stress or nutrient scarcity and is essential for maintaining cell function and homeostasis in health and disease. Despite a comprehensive understanding of the core autophagy regulating pathways, there is a limited grasp of systems-wide mechanisms influencing its dynamics and context-dependent regulation, which has limited the development of predictive models. This knowledge gap underscores the need to explore the temporal aspects of autophagy signaling, execution and feedback control.

To address this objective, we characterized the autophagy dynamic response to nitrogen starvation and replenishment in a genome-wide yeast gene deletion library, using high-content microscopy. We employed a deep learning-based approach to classify autophagic cell states and quantify the kinetic autophagy response of 6000 mutants to create the first genome-wide autophagy profiling repository. We subsequently integrated these profiles with genetic and protein-protein interaction networks to characterize the cellular hierarchical control of autophagy execution, along with multi-omics modeling strategies of dynamical changes in autophagy levels. This strategy allowed us to identify the RTG pathway as a previously unknown regulatory module that limits uncontrolled autophagy initiation by buffering the expression of Atg1 and Atg13 while ensuring the commitment to autophagy progression through transcriptional inhibition of TORC1 signaling.

Our findings constitute a valuable resource for comprehending genome-wide dynamical control of autophagy. Moreover, our study serves as a proof-of-principle for high-content data-driven biological discovery and the characterization of global regulation of cellular processes using machine learning.

Name: Irene Sambri, PhD
Position: Research Associate
Email: i.sambri@tigem.it
Institution: Scuola Superiore Meridionale and TIGEM- Telethon Institute of Genetics and Medicine, Italy
Keywords: RAGD mutations, MiT/ TFE transcription factors, autophagy, cardiomyopathy, kidney tubulopathy

Title: **RagD auto-activating mutations impair MiT/ TFE activity in kidney tubulopathy and cardiomyopathy syndrome**

Abstract:
Heterozygous mutations in the gene encoding RagD GTPase were shown to cause a novel autosomal dominant condition characterized by kidney tubulopathy and cardiomyopathy. We previously demonstrated that RagD, and its paralogue RagC, mediate a non-canonical mTORC1 signaling pathway that inhibits the activity of TFEB and TFE3, transcription factors of the MiT/TFE family and master regulators of lysosomal biogenesis and autophagy. Here we show that RagD mutations causing kidney tubulopathy and cardiomyopathy are "auto- activating", even in the absence of Folliculin, the GAP responsible for RagC/D activation, and cause constitutive phosphorylation of TFEB and TFE3 by mTORC1, without affecting the phosphorylation of "canonical"mTORC1 substrates, such as S6K. By using HeLa and HK-2 cell lines, human induced pluripotent stem cell-derived cardiomyocytes and patient-derived primary fibroblasts, we show that RRAGD autoactivating mutations lead to inhibition of TFEB and TFE3 nuclear translocation and transcriptional activity, which impairs the response to lysosomal and mitochondrial injury. These data suggest that inhibition of MiT/TFE factors plays a key role in kidney tubulopathy and cardiomyopathy syndrome.

Name: Adriana Covarrubias-Pinto, PhD
Position: Postdoc
Email: agross@age.mpg.de
Institution: Institute of Biochemistry, Goethe university, Germany
Keywords: ubiquitination, FAM134B, AMFR and ER-phagy

Title:
Ubiquitination regulates ER-phagy and remodelling of endoplasmic reticulum

Abstract:
The endoplasmic reticulum (ER) undergoes continuous remodelling via a selective autophagy pathway, known as ER-phagy1. ER-phagy receptors have a central role in this process2, but the regulatory mechanism remains largely unknown. Here we report that ubiquitination of the ER-phagy receptor FAM134B within its reticulon homology domain (RHD) promotes receptor clustering and binding to lipidated LC3B, thereby stimulating ER-phagy. Molecular dynamics (MD) simulations showed how ubiquitination perturbs the RHD structure in model bilayers and enhances membrane curvature induction. Ubiquitin molecules on RHDs mediate interactions between neighbouring RHDs to form dense receptor clusters that facilitate the large-scale remodelling of lipid bilayers. Membrane remodelling was reconstituted in vitro with liposomes and ubiquitinated FAM134B. Using super-resolution microscopy, we discovered FAM134B nanoclusters and microclusters in cells. Quantitative image analysis revealed a ubiquitin-mediated increase in FAM134B oligomerization and cluster size. We found that the E3 ligase AMFR, within multimeric ER-phagy receptor clusters, catalyses FAM134B ubiquitination and regulates the dynamic flux of ER-phagy. Our results show that ubiquitination enhances RHD functions via receptor clustering, facilitates ER-phagy and controls ER remodelling in response to cellular demands.

Name: Bishal Basak, PhD
Position: Postdoc
Email: bishal.basak@pennmedicine.upenn.edu
Institution: Department of Physiology, University of Pennsylvania, Philadelphia, USA
Keywords: mitophagy, Rubicon, neurodegeneration, proteasome, lysosomes

Title:
Mitochondrial damage triggers degradation of negative regulators of neuronal autophagy

Abstract:
Quality control of organelles such as mitochondria is mediated by autophagy and is key to maintaining normal neuronal physiology. Mutations in genes that regulate this process have been reported in patients suffering from Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS). Therapeutic advances to addressing the challenge of increasing mitochondrial turnover in neurons of PD and ALS patients require detailed molecular understanding of this selective autophagy pathway, termed as 'mitophagy'. In this study we show that increasing mitochondrial damage evokes a graded stress response involving the coordinated degradation of negative regulators of autophagy which include Myotubularin related protein (MTMR)-5, MTMR2 and Rubicon. We show that the targeted degradation of this class of regulators is specific to neurons and is mediated by the ubiquitin-proteasomal system (UPS). Our data indicates that the ubiquitination is brought about by the E3 ligase Cullin-5. We term this response to mitochondrial damage as the Mitophagic Stress Response (MSR) pathway. Under basal conditions, we find that MTMR5-MTMR2 represses mitochondrial turnover by trafficking autophagosomes inhibiting early steps of mitochondrial quality control. Rubicon which stably associates with lysosomes, prevents autophagosome-lysosome fusion, thereby inhibiting final steps of autophagy. Thus, during mitochondrial damage, the degradation of MTMR5 and MTMR2 promotes the uptake of damaged mitochondria by autophagosomes, and the degradation of Rubicon facilitates the fusion of autophagosomes carrying damaged mitochondria with lysosomes. Our work identifies three potential targets of the MSR pathway whose depletion can trigger clearance of damaged mitochondria in neurons from PD and ALS patients where mitophagy is compromised.

Name: Mariana Sofia Tadic
Position: PhD Student
Email: tadic.mariana@gmail.com
Institution: University of Buenos Aires, Faculty of Pharmacy and Biochemistry, Institute of Biochemistry and Molecular Medicine, Argentina
Keywords: autophagy, secretory pathway, extracellular vesicles, VMP1

Title:

The autophagy-related transmembrane protein VMP1 is unconventionally secreted as a component of extracellular vesicles

Abstract:
Cellular stress activates various mechanisms, including autophagy and vesicular trafficking, to maintain homeostasis and cope with pathological conditions. One of these mechanisms involves the unconventional secretion of extracellular vesicles (EVs). Our study focuses on Vacuole Membrane Protein 1 (VMP1), an autophagy-related protein implicated in pancreatitis and cellular stress management. We demonstrate that VMP1 is secreted into the extracellular medium as a part of EVs. Using cell lines expressing different VMP1-tag variants, we successfully identified and purified these VMP1-containing EVs (VMP1-EVs) from the extracellular medium using ultracentrifugation and immune isolation techniques. Our data confirm that the secretion of VMP1-EVs is affected by autophagy inducers like starvation and mTOR inhibitors, including PP242. Furthermore, VMP1-EV secretion mediates the associated release of autophagy markers LC3II and p62, and is altered under different conditions, including the use of autophagy inhibitors and exposure to cerulein in a pancreatitis cell model. These VMP1-EVs can be internalized by various cell lines, including pancreatic cells, and can induce LC3 recruitment in host cells. Our findings reveal for the first time that VMP1 is unconventionally secreted as a component of EVs and suggest that this secretion may have a functional role in cellular responses to stress and disease.

Name: Rabia Khawaja, PhD
Position: Postdoc
Email: rabia.khawaja@einsteinmed.edu
Institution: Albert Einstein College of Medicine, New York, USA
Keywords: chaperone-mediated autophagy, neurodegeneration, synaptic neurotransmission, hyperexcitability

Title:
Chaperone-mediated autophagy regulates neuronal excitability by remodeling the synaptic proteome

Abstract:
Chaperone-mediated autophagy (CMA) regulates multiple cellular processes by selective and timely degradation of key intracellular proteins to terminate their function. Loss of neuronal CMA causes neurodegeneration and behavior changes compatible with neuronal dysfunction; however, the role of CMA in normal neuronal physiology is largely unknown. We investigated whether CMA regulates neuronal activity. We found that loss of CMA caused neuronal hyperactivity in primary neurons cultures and that CMA-deficient mice displayed hyperactivity in open field, exhibited spontaneous seizures and had increased susceptibility and mortality in response to pharmacologically induced seizures. Similar hyperexcitability and increased susceptibility to seizures was also evident in an Alzheimer's disease (AD) mouse model and in old mice, in agreement with the reduced CMA activity in both models. Conversely, CMA preservation in excitatory neurons in old mice brains through genetic manipulations prevented these age-related excitatory electrophysiological changes. Furthermore, pharmacological activation of CMA reduced seizure susceptibility and enhanced learning and memory in old mice and in the AD mouse model. Field recordings in hippocampus of CMA-deficient mice revealed excitatory electrophysiological changes in presynaptic neurotransmitter release and postsynaptic response from AMPA receptors. Comparative quantitative proteomic analysis of control and CMA-deficient brains revealed that presynaptic proteins such as Neuronal Pentraxin 1 (NP-1), Syntenin and Synapsin-1, accumulate in aggregates in CMA-deficient neurons, but preservation of CMA prevented their accumulation in old mice brains. We propose that disruption of their regulated degradation contributes to the observed defective synaptic neurotransmission. These findings unveil a previously unknown role of CMA in the regulation of neuronal excitability through remodeling of a subset of the synaptic proteome. Our findings support that age-related failure in the regulation of synaptic proteome by CMA contributes to neuronal hyperexcitability and, consequently, defective synaptic neurotransmission in aging and neurodegeneration, and highlight the potential value of targeting CMA to prevent decline of brain function with age.

Name: Camille Lacarriere-Keita
Position: PhD student
Email: camille.lacarriere@usherbrooke.ca
Institution: University of Sherbrooke, QC, Canada
Keywords: Intestinal stem cell, Drosophila

Title:
Autophagy inhibition favors drosophila intestinal enteroendocrine cell differentiation, most likely by increasing JAK-STAT signaling

Abstract:
As the intestinal epithelium faces many stresses, dysregulation of essential mechanisms governing gut homeostasis, like autophagy, has been associated with inflammatory bowel pathologies. Using Drosophila model, recent work demonstrated that autophagy inhibition, specifically in adult intestinal stem cells (ISCs) or differentiated cells, caused intestinal stem cell hyperproliferation. Our laboratory has previously identified Rab21 as a critical regulator of autophagosome-lysosome fusion, an essential step to degrade and recycle intracellular components. Interestingly, a genome-wide interfering RNA screen identified Rab21 as a potential ISC regulator. Since the modulation of autophagy affects the switch between stemness and differentiation of dermal or hematopoietic cells, we hypothesized that Rab21 in intestinal stem cells may as well regulate cell differentiation to contribute to intestinal epithelium maintenance. As in mammals, Drosophila ISCs divide into specific progenitors that differentiate into nutrient-absorptive enterocytes or hormone-secreting enteroendocrine cells. We expressed RNAi against Rab21 or other Autophagy genes, specifically in adult ISCs and/or progenitor cells, for 10 days and quantified the different cell populations by immunofluorescence. Autophagy inhibition, specifically in ISCs, did not affect their number but increased the proportion of enteroendocrine cells. By screening various regulators of pathways involved in stem cell fate, we determined that Rab21 depletion in ISCs increased JAK-STAT signaling. Moreover, the co-depletion of the transcription factor STAT92E rescued the expansion of enteroendocrine cells caused by Rab21 knock-down in ISCs. Taken together, our data suggest that, in homeostatic conditions, autophagy regulates the differentiation of intestinal cells into enteroendocrine cells, possibly by modulating JAK-STAT signaling.

Name: Annan Cook
Position: PhD student
Email: zeke_cook@berkeley.edu
Institution: University of California Berkeley, USA
Keywords: PI3KC3-C1, RAB1A, Cryo-EM, mechanism, initiation

Title:
Structural pathway for autophagic class III PI 3 - kinase activation by the myristoylated GTP - binding pseudokinase VPS15

Abstract:
The class III phosphatidylinositol (PI) 3-kinase complex I (PI3KC3-C1) is a central player in the initiation of macroautophagy in mammals. Through three-dimensional classification of a large cryo-EM dataset of human PI3KC3-C1 bound to the small GTPase RAB1A, we were able to map the structural pathway of enzyme activation. The inactive conformation is stabilized by an N-myristoyl modification of the pseudokinase (PK) subunit VPS15. The N-myristate is sequestered in the N-lobe of the VPS15 PK domain, which stabilizes a series of interactions whereby VPS15 sequesters and blocks the catalytic and membrane-binding units of the VPS34 lipid kinase. In the activated conformation, the N-myristate and the VPS34 lipid kinase domain are liberated to interact with membranes and catalyze PI3P formation. The VPS15 PK contains a unique Arg at the gatekeeper position and binds tightly to GTP. GTP binding structurally stabilizes the N-myristate "in" conformation, which promotes the inactive conformation. This pathway provides a general mechanism for PI3KC3 activation in autophagy.

Name: Josephine Thinwa, MD, PhD
Position: Assistant Professor
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Institution: University of Texas Southwestern, USA
Keywords: p62/SQSTM1, virophagy, selective autophagy, neurotropic viruses

Title:
The neurodevelopmental gene CDKL5 regulates p62-mediated virophagy of neurotropic viruses

Abstract:
During virus infection, large quantities of viral proteins accumulate and can overwhelm cellular degradative capabilities causing cytotoxicity. Therefore virophagy, the selective autophagosomal engulfment and degradation of viral components, plays a crucial role in cell-intrinsic antiviral immunity particularly in non-renewable cells like neurons. However, the mechanisms leading to viral antigen recognition and autophagy induction remain poorly understood. In this work, we characterized the autophagy role of CDKL5, which was identified from a genome-wide siRNA screen designed to identify novel regulators of virophagy. We infected CDKL5 knock out (KO) HeLa cells and mouse primary cortical neurons with Sindbis virus (SINV), a proteotypic neurotropic RNA virus. Compared to wild type (WT) cells, we found CDKL5 KO cells robustly accumulate the SINV structural protein capsid, have increased cytotoxicity and minimal p62/SQSTM1 protein degradation suggesting that CDKL5-deficient cells have impaired virophagy. To determine how CDKL5 regulates virophagy, we generated a CDKL5 kinase-dead mutant, a pathogenic mutation associated with a human neurodevelopmental disorder, and performed co-immunoprecipitation studies. We found that CDKL5 directly associates with p62 and its kinase activity is required for p62 and capsid interaction. To elucidate the mechanism of CDKL5-mediated p62/capsid interaction, we performed in vitro kinase assays and discovered that CDKL5 phosphorylates p62 at T269/S272, which facilitates the formation of p62 condensates that bind and target capsid to autophagic degradation. Finally, CDKL5 KO mice infected with several neurotropic viruses have enhanced mortality compared to WT mice. Overall, our findings identify a cell-autonomous innate immune mechanism for autophagy activation to clear toxic viral proteins.

POSTER SESSION I

October 26, 2023

12:55 – 13:55 EST USA

Hosts: Sarah Norman and Dongfang Li

Room 1 : Group 1 posters 1-8
Moderator: Sarah Norman
Zoom: https://einsteinmed.zoom.us/j/98733119374

Room 2 : Group 2 posters 1-7
Moderator: Yuhong Ma
Zoom: https://einsteinmed.zoom.us/j/98733119374

Room 3 : Group 3 posters 1-7
Moderator: Dongfang Li
Zoom: https://einsteinmed.zoom.us/j/98480660402

Room 4 : Group 4 posters 1-7
Moderator: Paloma Liton
Zoom: https://einsteinmed.zoom.us/j/98480660402

POSTER SESSION I – Group 1

Moderator: Sarah Norman

#	Presenter and Title
1	**Rashmi Arora** Unveiling the intricate role of NR2 in mechanism of autophagy
2	**Letizia Brogi** 1.3, 1.6 B-Glucans restore impaired autophagy and mitochondrial respiration in the aging brain via a direct action
3	**Maria Colonna** Insights into lysosomal, autophagic and mitochondrial alterations in novel neuronal cell models for mucopolysaccharidosis IIIA
4	**Juliet Goldsmith** Proteomic profiling elucidates new aspects of neuronal autophagy in health and disease
5	**Raquel Gomez-Sintes** Chaperone-mediated autophagy as therapeutic target in retinal degeneration in Parkinson's disease
6	**Danijela Stevanovic** Trehalose differently regulates mitochondrial-oxidative stress damage in 6-OHDA and MPP+ -induced toxicity in SH-SY5Y neuroblastoma cells by modulating p38, JNK, AMPK/Akt/mTOR-signaling pathway
7	**Katerina Veverova** Mitophagy biomarkers in biofluids: Correlation with AD biomarkers and neuropathology
8	**Leonard Yoon** mTOR-independent autophagy activator ameliorates tauopathy and prionopathy

POSTER SESSION I – Group 2

Moderator: Yuhong Ma

#	Presenter and Title
1	**Priscila Campos** The E3 ubiquitin ligase Smurf2 regulates the immune response to Mycobacterium tuberculosis
2	**M. Esther Pérez-Pérez** ATG3 is activated by reduction to ensure ATG8 lipidation and autophagy progression in response to stress
3	**Kathryn Rahlwes** Regulation of host immunity to Mycobacterium tuberculosis infection by deubiquitinases
4	**Clémence Taisne** How Chlamydia trachomatis affects the powerhouse of the cell
5	**Nimna Wijewantha** The autophagic degradation of glycogen granules is a non-selective process in yeast
6	**MengYao Wu** Sestrin2 modulates BNIP3-mediated mitophagy and immune function in post-sepsis dendritic cells: insights into signaling mechanisms
7	**Wenxin Zhang** Autophagosome membrane expansion is mediated by the N-terminus and cis-membrane association of human ATG8s

POSTER SESSION I – Group 3

Moderator: Dongfang Li

#	Presenter and Title
1	**Lydie Barbeau** Elevating autophagy-dependence of hypoxic glioblastoma cells for increased therapeutic benefit
2	**Mehran Erfani** ARID1A acts as a regulator of autophagy in colorectal cancer: A promising candidate therapeutic target
3	**Xiaofen Li** TM9SF1 mediated lipophagy to promote the progression of HER2-positive breast cancer
4	**Arissa Mercer** Mechanism of liver fibrosis caused by autophagy-deficiency
5	**Khushbu Patel** Role of chaperone-mediated autophagy in initiation and progression to Hepatocellular carcinoma
6	**Nasim Rahmani-Kukia** ERMP1 accelerates the proliferation of CRC cells
7	**Katarina Wendy Schmidt** Selective autophagy impedes KSHV entry by recruiting to virus-containing endosomes via membrane damage sensor galectin-8

POSTER SESSION I – Group 4

Moderator: Paloma Liton

#	Presenter and Title
1	**Minal Ayachit** Atg1 modulates mitochondrial homeostasis during oogenesis in *Drosophila melanogaster*
2	**Rocío Barragán Arnal** Differential peripheral expression of selected autophagy-related genes in healthy subjects following short-term fasting versus frequent meal consumption: A randomized crossover trial
3	**Prasoon Jaya** Identification and functional analysis of substrates & regulators of starvation-induced endosomal microautophagy in *Drosophila*
4	**Gregory Krause** Molecular determinants of the crosstalk between endosomal microautophagy and chaperone-mediated autophagy
5	**Noelia Leopardo** Autophagy in the corpora lutea of the gestating South American plains vizcacha (Lagostomus maximus): a model of pseudo-ovulation during pregnancy
6	**Mija Marinkovic** Unveiling the BNIP3L/NIX-mediated mitophagy: novel insights into dimerization regulation and upstream mechanisms
7	**Tabassum Tasmi** Upf3 is a post-transcriptional negative regulator of autophagy

POSTER SESSION II

October 26, 2023

20:45 – 21:45 EST USA

Hosts: Zi Gao and Mericka McCabe

Room 1 : Group 1 posters 1-6
Moderator: Zi Gao
Zoom: https://einsteinmed.zoom.us/j/95900415773

Room 2 : Group 2 posters 1-6
Moderator: Malena Herrara Lopez
Zoom: https://einsteinmed.zoom.us/j/95900415773

Room 3 : Group 3 posters 1-6
Moderator: Mika Minami
Zoom: https://einsteinmed.zoom.us/j/96974926283

POSTER SESSION II – Group 1

Moderator: Zi Gao

#	Presenter and Title
1	**Taylor Benske** N-methyl-D-Aspartate Receptors' proteostasis is modulated by autophagic flux
2	**Ying He** Targeting PGRMC1-mediated autophagy by ultrasound-triggered microbubble destruction enhances the radiosensitivity of glioblastoma
3	**Juan Ignacio Jiménez-Loygorri** Urolithin A prevents acute retinal degeneration through selective autophagy
4	**Sierra Palumbos** Compensatory secretion in stressed neurons
5	**Xia Zhang** Mitophagy in Alzheimer's disease: a bibliometric analysis from 2002 to 2022
6	**Liyu Zheng** The effect of RETREG1-mediated ER-PHAGY on necroptosis of dendritic cells under septic exposure

POSTER SESSION II – Group 2

Moderator: Malena Herrara Lopez

#	Presenter and Title
1	**Yibo Guo** Association between *Porphyromonas gingivalis* infection and the progression of autophagic flux in esophageal squamous cell carcinoma
2	**Khushbu Patel** Role of Chaperone-mediated autophagy in initiation and progression to Hepatocellular carcinoma
3	**Isaiah Reeves** Inhibition of c-MYC in hepatocellular carcinoma via AAV8 mediated gene transfer
4	**Bing Sun** TRIM13 regulates autophagy through modulating both the transcription and the ubiquitination of SESN2 in sepsis
5	**Ruchi Umargamwala** Novel ubiquitin ligases in the regulation of autophagy
6	**Ariadne Vlahakis** Autophagy regulation of breast cancer metastasis: Unveiling the role of Cib1 as a key autophagy target for the regulation of invadopodia

POSTER SESSION II – Group 3

Moderator: Mika Minami

#	Presenter and Title
1	**Minghao Chen** Structure and activation of the human autophagy-initiating ULK1:PI3KC3-C1 supercomplex
2	**Carolina Franco** Vitamin B6 is governed by the local compartmentalization of metabolic enzymes during growth
3	**Esther Wong** TFEB activator screen identified anti-psychotic drug Sertindole as a novel TFEB activator for geroprotection
4	**Ana Rožić** Determination of key kinase governing BNIP3L/NIX mitophagy modulation
5	**Olaya Santiago-Fernández** Decline of chaperone-mediated autophagy with age contributes to loss of skeletal muscle integrity
6	**Kavitha Thirumurugan** Connecting senescence and autophagy in murine adipose cells

Abstract # 1

Name: Rashmi Arora
Position: PhD Student
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Institution: CSIR-Institute of Microbial TECHnology
Keywords: NR2, p62, autophagy, neurodegeneration, Parkinson's disease model

Title:
Unveiling the intricate role of NR2 in mechanism of autophagy

Abstract:
Introduction: NR2 (name encrypted), an orphan nuclear receptor, regulates the expression of dopaminergic neurons. Autophagy, a self-eating process, protects against neurodegenerative diseases.
Aim and objectives: To study the correlation between NR2 and autophagy.
Methodology: In vitro experiments examined protein expression of LC3II and p62 in SH-SY5Y cells treated with Bafilomycin with or without the ligand or overexpression of NR2 followed by western blotting and confocal imaging. The binding of the transcription factor NR2 to its response element in p62 gene was confirmed by EMSA and ChIP-qPCR experiments. In vitro Parkinson disease model was generated by transfecting SH-SY5Y cells with α-synuclein aggregates with or without the ligand or overexpression of NR2 and monitoring p62 levels.
Results: NR2 agonist or overexpression increases LC3II and p62 protein levels, while p62 levels increased whereas LC3 levels remain constant at transcriptional level. NR2 modulates autophagy initiation and autophagic flux. CHIP and EMSA experiments depicts the binding of NR2 to the novel response element identified on p62 promoter region. In vitro PD model was generated by ectopic α-synuclein aggregates showed decreased p62 levels which were restored upon ligand treatment or NR2 overexpression. Future studies will be needed for in vivo validation of novel p62 gene regulation in autophagy.
Discussion and conclusion: Increased p62 levels replenish the autophagy adaptor protein which are correlated with NR2 levels. The persevering of p62 protein may lead to enduring autophagy that can protect the neurons from chronic neuronal insults. This study reveals the mechanistic role of NR2 in regulating the autophagy.

Abstract # 2

Name: Letizia Brogi, MSc
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Institution: Scuola Normale Superiore, Pisa, Italy
Keywords: 1.3, 1.6 B-glucans, autophagy, aging, mitochondria, killifish

Title:
1.3, 1.6 B-Glucans restore impaired autophagy and mitochondrial respiration in the aging brain via a direct action

Abstract:
1.3-1.6 ß-glucans are natural food ingredients with immunomodulatory, antioxidant, anti-inflammatory and antineoplastic actions. We have used 1.3-1.6 ß-glucans as a chronic dietary supplement in the short-lived killifish Nothobranchius furzeri, a convenient model organism for investigating the effects of interventions on longevity and age-related pathologies in Vertebrates.
Administration of 1.3-1.6 ß-glucans reduces multiple aging hallmarks in several organs, including the brain. To investigate whether 1.3-1.6 ß-glucans could influence the brain directly, we used an ex vivo brain culture system for Nothobranchius furzeri. This system replicates age-dependent reduction of autophagy observed in vivo. Acute treatment with 1.3-1.6 ß-glucans restores impairment of autophagy and induces biogenesis of mitochondria and lysosomes in aged ex-vivo brains. Proteomic analyzes in ex vivo adult and old brain slices confirmed the positive activity of 1.3-1.6 ß-glucans on autophagy and mitochondrial respiration. We are currently studying the role of microglia in this effects.
These results indicate that 1.3-1.6 ß-glucans can slow progression some age-related markers. 1.3-1.6 ß-glucans act directly on the brain, normalizing cellular processes that are impaired during the aging even in acute treatments. As ß-glucans are part of our normal diet, our results advocate diets rich in ß-glucans to promote human longevity.

Abstract # 3

Name: Maria Colonna
Position: Undergraduate student
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Institution: Laboratorio de disfunción celular en enfermedades neurodegenerativas y nanomedicina, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales/IQUIBICEN, UBA-CONICET.
Keywords: mucopolysaccharidosis, lysosomal storage disorder, autophagy, mitophagy, LC3-II

Title:
Insights into lysosomal, autophagic and mitochondrial alterations in novel neuronal cell models for mucopolysaccharidosis IIIA

Abstract:
Mucopolysaccharidosis type III (MPSIII), also known as Sanfilippo syndrome, is a rare lysosomal storage disorder (LSD) characterized by early childhood neurodegeneration. MPSIIIA arises from mutations in the gene coding N-sulfoglucosamine sulfohydrolase (SGSH), involved in heparan sulphate degradation in lysosomes. Currently, no nervous system-based MPSIIIA cellular models are available. Our lab developed SGSH-deficient HT22 neuronal cell lines, named 12, 124 and 15. In this study, we aim to characterize these models, elucidating the impact of SGSH deficiency on lysosomal/autophagic pathways and mitochondrial integrity. Previous results showed an increase in acidic vesicles (AV) number, size and AVs area/cell area, and a greater diffusion of the dye to the cytosol, suggesting an expansion of the lysosomal compartment together with the loss of the lysosomal membrane potential in MPSIIIA lines. Despite AVs alterations, the autophagic flux remained functional and possibly increased, as determined by LC3-II expression (WB). MPSIIIA cells displayed fragmented mitochondrial networks, likely related to cellular stress. Yet, MitoSpy FM staining showed total mitochondrial mass remained unchanged. Moreover, research on mitophagy suggests higher mitochondria-LC3 colocalization in lines 12 and 124, implying enhanced mitochondrial elimination by mitophagy. MPSIIIA cell lines´ viability is not affected under basal conditions, but it is lower than the control line under exposure to an oxidizing agent. Initial studies suggest increased glycosaminoglicans accumulation in MPSIIIA lines. These results provide new evidence on lysosomal, autophagic and mitochondrial alterations in our MPSIIIA cell lines. We expect our models and our findings, to be useful for future research on this disease.

Abstract # 4

Name: Juliet Goldsmith, PhD
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Institution: University of Pennsylvania, USA
Keywords: neurodegeneration, mitochondrial DNA, mitophagy, secretion

Title:
Proteomic profiling elucidates new aspects of neuronal autophagy in health and disease

Abstract:
Autophagy is important in all cell types, but neurons uniquely require autophagy for homeostasis, and inhibition of autophagy leads to rapid neuron death. My postdoctoral research initially asked a simple but long unanswered question: what is autophagy degrading that is critical for neuronal health? Using unbiased proteomics from mouse brain-derived autophagic vesicles and validating in primary and iPSC-derived neurons in culture, I found that mitochondria containing the mitochondrial genome are a major cargo of autophagosomes in neurons (Goldsmith et al., Neuron 2022). Defects in autophagy are a pathological hallmark of neurodegenerative disease. Thus, I predicted that neurodegenerative disease mutations will affect the clearance of mitochondrial DNA (mtDNA). In preliminary data from human iPSC-derived cortical-like and lower motor neurons that harbor an ALS/FTD – linked genetic mutation, I have found that a stressed autophagy pathway has impaired clearance of mtDNA. Using this model system, I can investigate the functional consequence of impaired mtDNA clearance in neurons in culture. In parallel postdoctoral work, I characterized how autophagy cargos change in two mouse models of Parkinson's disease, finding that compensatory mechanisms are upregulated. In PINK1-/- mice, levels of alternate mitophagy adaptors are increased, while in LRRK2G2019S mice, secretion of extracellular vesicles and autophagy cargo such as the pathological molecule alpha-synuclein is upregulated. Such adaptive mechanisms highlight the critical nature of autophagy in supporting neuron homeostasis. However, the compensatory pathways remain a stopgap fix. We predict that the resulting moderate phenotypes may lead to accumulation of damage with age and thus sensitize neurons to degeneration.

Abstract # 5

Name: Raquel Gomez-Sintes, PhD
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Institution: [1]Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain. [2]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY
Keywords: Parkinson's disease, retina, degeneration, chaperone mediated autophagy

Title:
Chaperone-mediated autophagy as therapeutic target in retinal degeneration in Parkinson's disease

Abstract:
Parkinson's disease (PD) is characterized by α-synuclein deposits in neurons, leading to dopaminergic neuron loss in central nervous system (CNS). Although much attention has focused on the degeneration of the substantia nigra, structural and functional changes in the retina are also a common feature in PD patients. Disturbances in the protein quality control systems contribute to neurodegeneration by interfering with the removal of PD-related pathogenic proteins. We are interested on a selective lysosomal degradation pathway known as chaperone-mediated autophagy (CMA) that has been previously shown to malfunction in experimental mouse models and brains of PD patients. Our ongoing studies support defective CMA activity and signs of retinal degeneration and deficits in visual behavioural tests in a PD mouse model. We are currently testing if genetically and pharmacologically restoration of normal CMA activity in the retina of this PD mouse model will improve α -synuclein degradation, neuronal homeostasis and slow down neuronal retinal loss related to PD. We believe that our suggested CMA activation approach would be able to reverse this phenotype, thus delaying the disease progression in the retina.

Abstract # 6

Name: Danijela Stevanovic
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Institution: Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
Keywords: trehalose, 6-OHDA, MPP+, mitochondria, p38

Title:
Trehalose differently regulates mitochondrial-oxidative stress damage in 6-OHDA and MPP+ -induced toxicity in SH-SY5Y neuroblastoma cells by modulating p38, JNK, AMPK/Akt/mTOR-signaling pathway

Abstract:
6-OHDA and MPP+ are the most common neurotoxins used to induce experimental models of PD. Neurotoxicity induced by 6-OHDA and MPP is mediated by mitochondrial dysfunction and oxidative stress leading to cell death. Trehalose has antioxidant properties and the ability to remove aggregates, damaged proteins, and organelles. In this study, we investigated the effects of trehalose on 6-OHDA- and MPP+-induced toxicity in neuroblastoma cells SH-SY5Y. As shown by immunoblot and flow cytometry analyses of mitosoxRed-stained cells, trehalose pretreatment decreases caspase 3 cleavage/activation and mitochondrial superoxide production in 6-OHDA-exposed cells. In MPP-exposed cells, trehalose increased the cleavage/activation of caspase 3 and intensity of mitosoxRed fluorescence. Accordingly, TEM analysis in 6-OHDA-exposed cells pretreated with trehalose showed mitochondria with typical ultrastructure in an electron-dense matrix without signs of damage. In combination of MPP+ with trehalose, TEM revealed large, swollen mitochondria with fragmentation and disorganization of cristae in a markedly electron-lucid matrix. In addition, immunoblot showed that trehalose decreased phosphorylation of p38 and phosphorylation of Akt in 6-OHDA treatment, whereas increasing these molecules in MPP+-treated cells. Moreover, in both 6-OHDA and MPP-treated cells, trehalose decreased phosphorylation of AMPK, and increased phosphorylation of S6 kinase, but only in 6-OHDA-exposed cells trehalose decreased JNK phosphorylation. While trehalose differentially regulated p38, the antioxidant N-acetyl cysteine (NAC) decreased phosphorylation of p38 in both 6-OHDA and MPP+ treatments. In conclusion, trehalose differentially regulates oxidative stress and mitochondrial damage induced by two different neurotoxins, suggesting further research on signaling pathways and localization of signaling molecules to preserve mitochondrial function.

Abstract # 7

Name: Katerina Veverova, PhD
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Institution: Memory Clinic, Department of Neurology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic
Keywords: Alzheimer's disease, biofluid biomarkers, autophagy, mild cognitive impairment, mitophagy

Title:
Mitophagy biomarkers in biofluids: Correlation with AD biomarkers and neuropathology

Abstract:
INTRODUCTION
Mitophagy impairment has been identified as an important pathophysiological hallmark of Alzheimer's disease (AD) in animal models, cell models and brain biopsies of AD. However, whether these changes are reflected in biofluids of individuals with AD is unknown.
METHODS
We evaluated levels of mitophagy markers (PINK1, BNIP3L, TFEB) in cerebrospinal fluid and serum samples from 242 biomarker-defined individuals with AD and cognitively unimpaired individuals (CU) from the Czech Brain Aging Study. Levels of mitophagy markers were correlated with biomarker, cognitive and brain atrophy profiles.
RESULTS
Our data identified mitophagy impairment as reflected by increased levels of mitophagy inducers (PINK1 and BNIP3L) and reduced levels of regulator of mitophagy flux (TFEB) in AD individuals compared to CU. Also, these changes were associated with more advanced AD pathology demonstrated by increased positivity of AD biomarkers, severity of AT(N) profile and cognitive impairment.
DISCUSSION
This study reveals mitophagy impairment reflected in biofluid markers of individuals with AD and associated with more advanced AD pathology.

Abstract # 8

Name: Leonard Yoon
Position: PhD Student
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Institution: The Scripps Research Institute
Keywords: High-throughput phenotypic screen, pharmacological autophagy activation, neurodegeneration, TFEB, mTORC1-independent

Title:
mTOR-independent autophagy activator ameliorates tauopathy and prionopathy

Abstract:
Autophagy activation holds the promise of alleviating the characteristic features of neurodegenerative diseases, such as protein aggregation, disruptions in lipid levels, and defects in axonal trafficking. To identify compounds that accelerate the removal of lipid droplets, we conducted a comprehensive screening using high-content imaging, evaluating a library of 940,000 small molecules. Out of the 77 validated hits, which exhibited diverse structural profiles, 24 were found to enhance the activity of an autophagy flux reporter. Notably, we highlight CCT020312 as an autophagy activator that operates independently of inhibiting the mammalian target of rapamycin and instead promotes TFEB nuclear localization and transcription activation.
CCT020312 exhibited a dose-dependent ability to reduce cytotoxic aggregates of mutant prion protein within endosomes in primary murine hippocampal neurons, thereby rectifying axonal trafficking deficits. Furthermore, this compound demonstrated efficacy in clearing phosphorylated insoluble tau and reducing tau-related vulnerability to neuronal stress in cellular models derived from patients.
Moreover, CCT020312 displayed the capability to lower intracellular A□ levels in neurons directly induced from fibroblasts of both familial and sporadic Alzheimer's disease patients. In summary, our findings present a promising avenue for uncovering novel pharmacological modulators of autophagy, which hold the potential to decelerate the progression of neurodegenerative diseases.

Abstract # 1

Name: Priscila Campos, PhD
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Institution: UT Southwestern Medical Center
Keywords: Mycobacterium tuberculosis, ubiquitination, type I IFN

Title:
The E3 ubiquitin ligase Smurf2 regulates the immune response to Mycobacterium tuberculosis

Abstract:
Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB), resulting in 1-2 million deaths annually. Long treatment courses pose significant challenges and lead to the emergence of multidrug resistance. Thus, there is an urgent need for innovative approaches against TB, including host-directed therapies that enhance innate immunity. We previously identified Smurf1, an E3 ubiquitin ligase, as an essential component of host defense against Mtb via K48-linked ubiquitination of Mtb containing organelles for subsequent autophagosomal degradation. Regulating Smurf1 activity during infection could enhance Mtb ubiquitination and its targeting for selective autophagy. Smurf1 is negatively regulated by Smurf2 via ubiquitination. We hypothesize that preventing Smurf1 degradation could lead to increased ubiquitination of Mtb and its targeting for selective autophagy. Smurf2 knockout in immortalized murine microglial BV2 cells resulted in decreased Mtb replication in BV2 macrophages, coinciding with increased LC3B lipidation and K48 ubiquitination of Mtb. We also found that the absence of Smurf2 significantly reduced type I interferon (IFN) production, possibly by affecting the stability of components of the cGAS/STING pathway. We propose that Smurf2 modulates Smurf1-dependent autophagic responses while concurrently decreasing type I IFN levels by affecting one or more components of the cGAS/STING pathway, and these coordinated responses work together to restrict intracellular growth of Mtb in macrophages. This work will not only lead to a better understanding of innate immunity to Mtb infection but also provide a framework for developing compounds that could enhance antimicrobial activity in macrophages.

Abstract # 2

Name: M. Esther Pérez-Pérez, PhD
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Institution: Plant Biochemistry and Photosynthesis Institute (IBVF)-CSIC
Keywords: ATG3, ATG8 lipidation, ROS, stress

Title:
ATG3 is activated by reduction to ensure ATG8 lipidation and autophagy progression in response to stress

Abstract:
Autophagy is a major degradative pathway by which eukaryotic cells eliminate and recycle damaged or superfluous cellular components such as proteins or even organelles to maintain cellular homeostasis and cope with unfavorable conditions. Mounting evidence indicates a strong interplay between the generation of ROS and the activation of autophagy. Although a tight redox regulation of autophagy has been shown in several organisms including microalgae, the molecular mechanisms underlying this control remain poorly understood. We have performed an in-depth in vitro and in vivo redox characterization of ATG3, an E2-activating enzyme involved in ATG8 lipidation and autophagosome formation, from two evolutionary distant unicellular model organisms: the green microalga Chlamydomonas reinhardtii and the budding yeast Saccharomyces cerevisiae. Our results indicated that ATG3 activity from both organisms is subjected to redox regulation since these proteins require reducing equivalents to transfer ATG8 to the phospholipid phosphatidylethanolamine. We established the catalytic Cys of ATG3 as redox target in algal and yeast proteins, and showed that the oxidoreductase thioredoxin efficiently reduces ATG3. Moreover, in vivo studies revealed that the redox state of ATG3 from Chlamydomonas undergoes profound changes under different autophagy-activating stress conditions, such as the absence of photoprotective carotenoids or high light stress. Finally, we performed a detailed in silico analysis of representative genomes from the entire microalgal lineage to analyze whether redox signals might also modulate autophagy in these organisms.

Abstract # 3

Name: Kathryn Rahlwes, PhD
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Institution: UT Southwestern ME
Keywords: Mycobacterium tuberculosis, Deubiquitinases, K63 ubiquitin, xenophagy

Title:
Regulation of host immunity to Mycobacterium tuberculosis infection by deubiquitinases

Abstract:
Emerging drug resistance in Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, threatens global health. Degradative autophagy targets intracellular pathogens such as Mtb for lysosomal destruction. The E3 ubiquitin ligases Smurf1 and Parkin play key roles in restricting Mtb infection via the recruitment of autophagy receptors to Mtb-containing organelles, and mice lacking Smurf1 or Parkin are more susceptible to Mtb infection. E3 ubiquitin ligase activity is countered by deubiqutinase (DUB) enzymes that remove ubiquitin chains from ubiquitinated targets. We tested the hypothesis that DUBs play important roles in the host response to Mtb infection in macrophages. To identify specific DUBs that modulate Mtb survival and/or the stability of ubiquitin on Mtb-containing organelles, we screened a DUB shRNA knockdown library in the BV2 macrophage cell line for the impact of individual DUBs on intracellular Mtb replication. We identified 10 and 12 DUBS, respectively, whose depletion either enhanced or restricted Mtb replication. We focused on one DUB whose knockdown led to reduced Mtb replication. BV2 macrophages lacking this DUB demonstrated enhanced K63 ubiquitination of Mtb and recruitment of autophagy machinery. BV2 DUB knockout cells, primary mouse macrophages, and primary human macrophages transfected with a DUB-targeting shRNA restricted Mtb replication more than wild-type cells. Using an in vitro DUB activity assay and a stereoselective probe library, we identified unique and stereoselective DUB inhibitors that also restrict Mtb replication in wild-type macrophages. Taken together, we have identified regulators of Mtb intracellular replication that could serve as targets for “host-directed therapy” by enhancing selective autophagy.

Abstract # 4

Name: Clémence Taisne, PhD
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Institution: Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, 2629 HZ Delft, The Netherlands
Keywords: mitochondria, infection, Chlamydia trachomatis.

Title:
How Chlamydia trachomatis affects the powerhouse of the cell

Abstract:
Chlamydia trachomatis (Ctr) is an obligate intracellular human pathogen causing blinding eye disease (trachoma) and is the most frequent sexually transmitted bacterial infection causing pelvic inflammatory disease linked to ectopic pregnancy and infertility. Once it enters the cell, the pathogen drives the conversion of the endocytic compartment into a replicative niche called "inclusion". To meet the energy requirements of its exclusive intracellular lifestyle, Ctr modulate host metabolism. In preliminary results of this project, we found that Chlamydia infection triggers reversible morphological changes of mitochondria, ranging from mitochondrial fragmentation to recovery of tubular morphology along progression through the infectious cycle. Mitochondria are highly dynamic organelles and undergo membrane remodelling through coordinated cycles of fission and fusion. Recent studies have shown that treatment with the protonophore carbonyl cyanide 3-chlorophenylhydrazone (CCCP) leads to irreversibly shortened and collapsed ring-shaped mitochondria referred to as mitochondrial spheroids, which is linked to chronic depolarization of the mitochondrial membrane. The phenotypic similarity of this process to our observations in Chlamydia-infected epithelial cells suggests that mitochondria may undergo similar, but reversible, morphological transitions in infected cells and that reactive oxygen stress early in the infection may trigger mitochondrial membrane depolarization. The surprising observation that infected cells can recover from this process and re-establish an elongated mitochondrial network raises interesting questions about the mechanism Ctr may use to revert mitochondrial dysfunction and avoid mitophagy and apoptotic cell death.

Abstract # 5

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Institution: Department of Biology, Georgia State University, Atlanta, GA
Keywords: autophagy. glycogen granules, yeast, vacuole, non-selective

Title:
The autophagic degradation of glycogen granules is a non-selective process in yeast

Abstract:
The intracellular recycling pathway of autophagy was initially characterized as a bulk degradation process that randomly sequesters and degrades cytoplasmic materials. In recent years, various types of selective autophagy have been discovered. Glycophagy, the autophagic degradation of glycogen granules, is one of them and delivers cytosolic glycogen to lysosomes. Overaccumulation of glycogen granules in lysosomes leads to glycogen storage diseases. The study of glycophagy is in its early stages aiming to understand the precise molecular mechanism, in several tissues under different conditions. We developed the Komagataella phaffii yeast as a model to study autophagic degradation of glycogen granules by western blotting and fluorescence microscopy under nitrogen starvation conditions. We converted the self-glucosylating initiator of glycogen synthesis, Glg1 (which has a covalent linkage with glycogen), into the Glg1-GFP glycophagy reporter. Our results revealed that vacuolar degradation of Glg1-GFP to free GFP strictly depended on autophagic machinery (exemplified by autophagic kinase, Atg1) and vacuolar proteolysis (represented by vacuolar proteases A and B). Importantly, this process was independent of Atg11, the scaffold protein common for many (but not all) selective autophagy pathways. The above requirements were also the same for delivery of Glg1-GFP from the cytoplasm to the vacuole, as indicated by fluorescence microscopy. Interestingly, the wildtype Glg1-GFP (which can synthesize glycogen) and mutated Glg1Mut-GFP (unable to synthesize glycogen, resides in the cytosol and is degraded by a non-selective autophagy) had similar levels of free GFP released suggesting the same trafficking mechanism. Also, they were equally well delivered to the vacuole, as judged by fluorescence microscopy. These results suggest that autophagy of glycogen is a non-selective process in nitrogen-starved yeast. Also, they raise an interesting possibility that autophagy of glycogen might be a non-selective process in the non-hepatic mammalian tissues.

Abstract # 6

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Keywords: Sestrin2, mitophagy, BNIP3, sepsis, immunity

Title:
Sestrin2 modulates BNIP3-mediated mitophagy and immune function in post-sepsis dendritic cells: insights into signaling mechanisms

Abstract:
Sepsis, a life-threatening condition, results from an aberrant host response to infection, causing organ dysfunction. Dysregulated cellular immunity is pivotal in sepsis pathogenesis. Dendritic cells (DCs), specialized antigen-presenting cells, crucially bridge innate and adaptive immunity. DCs' functional status significantly impacts T-lymphocyte activation or suppression, crucial for the inflammatory response in sepsis. Sestrin2 (SESN2), a stress-responsive protein, regulates DCs, ameliorating sepsis-induced immune dysfunction. SESN2 also plays a role in mitophagy. BNIP3, a mitophagy-associated receptor, exhibits promise in its interplay with SESN2. However, the precise mechanism by which SESN2 orchestrates DC homeostasis and immune function through BNIP3-mediated mitophagy in sepsis remains enigmatic.
Methods: Sepsis was induced in murine models via cecum ligation and puncture. Western blot quantified protein expression, while microscopy visualized co-localization of bnip3 and related proteins and mitochondrial morphology. Flow cytometry assessed surface molecule expression on DCs. ELISA quantified cytokine release. Co-cultivation with T cells evaluated proliferation and cytokine levels.
Results: In vivo and in vitro experiments revealed heightened SESN2 expression during sepsis, mitigating DC immune dysfunction via upregulated BNIP3-mediated mitophagy. NR4A1 repressed BNIP3 transcription, while SESN2 phosphorylated and suppressed NR4A1 function. Gain- and loss-of-function experiments validated SESN2's role in amplifying BNIP3-mediated mitochondrial autophagy, finely tuning DC immune function, and alleviating sepsis-induced immunosuppression.
Conclusions: Our study unveils a mechanism where SESN2, through phosphorylation-mediated NR4A1 inhibition, upregulates BNIP3 and mitophagy during sepsis, orchestrating DC immune function and mitigating sepsis-induced immunosuppression. Our findings promise novel insights and a theoretical foundation for precise regulatory strategies addressing sepsis-induced immune dysfunction.

Abstract # 7

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Institution: The Francis Crick Institute
Keywords: Autophagy, real-time ATG8 lipidation assay, cis-membrane association, atomistic MD simulation, membrane expansion

Title:
Autophagosome membrane expansion is mediated by the N-terminus and cis-membrane association of human ATG8s

Abstract:
Autophagy is an essential catabolic pathway to sequester and engulf cytosolic substrates via a unique double-membraned structure, termed autophagosome. ATG8 proteins play an important role in mediating autophagosome membrane expansion. They are the only ubiquitin-like proteins known to be conjugated on autophagosomes via lipidation at the C-termini. However, the function of lipidated ATG8 in membrane expansion remains obscure. Using a real-time in vitro lipidation assay, we revealed that the N-termini of human ATG8s (LC3B/GABARAP) are highly dynamic and associate with membrane post-lipidation. Moreover, the atomistic MD simulation and FRET assays indicate that N-termini of LC3B/GABARAP associate in cis on the membrane. We found that membrane expansion and size of autophagosomes in cells is mediated by this cis-membrane association of N-termini in LC3B/GABARAP, interestingly, independent on ATG8-cargo interaction. Our study provides new molecular insights into autophagosome membrane expansion, revealing the critical and unique functions of lipidated ATG8.

Abstract # 1

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Keywords: autophagy-dependence, hypoxia, glioblastoma, kinome drop-out screen

Title:
Elevating autophagy-dependence of hypoxic glioblastoma cells for increased therapeutic benefit

Abstract:
Most solid human tumors, including glioblastoma, contain regions that are poorly oxygenated. Tumor hypoxia is a very heterogeneous and dynamic feature and is associated with a more malignant phenotype due to higher resistance to chemo- and radiotherapy. Glioblastoma treatment response and survival is associated with the level of hypoxia and expression of hypoxia-associated genes. From a clinical point of view, reducing tumor hypoxia is highly desired. Essential for the survival of hypoxic cells, is the activation of autophagy. Previously, we showed that inhibition of autophagy (chloroquine, CQ) sensitizes cells to hypoxia, reduces the viable hypoxic fraction in tumors and subsequently sensitizes glioblastoma to irradiation. Nevertheless, large differences in CQ-mediated killing efficacy are observed between cancer types and within patient populations. Alterations in signaling pathways, such as expression of the mutated kinase EGFRvIII and/or BRAFv600E, increases the autophagy dependency and sensitizes cells to autophagy inhibition during hypoxia. This led to the hypothesis that targeting kinase signaling may result in sensitization of hypoxic cells to chloroquine-treatment through inhibition of autophagy redundancy pathways. Two independent CRISPR-Cas9 drop-out screens (kinome and whole-genome) on a library of CRISPR-Cas9-expressing primary and established GBM cell lines were performed. We identified two targetable hits that can be inhibited with clinically approved drugs. We showed that both drugs alter autophagy activity through EGFR and mTOR pathways. Finally, cell proliferation decrease during hypoxia when drugs were combined with CQ. These data show that survival rates of hypoxic GBM cells can be reduced by combining clinically applicable kinase- and autophagy inhibitors.

Abstract # 2

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Keywords: ARID1A, autophagy, colorectal cancer

Title:
ARID1A acts as a regulator of autophagy in colorectal cancer: A promising candidate therapeutic target

Abstract:
Background: ARID1A, a bona fide tumor suppressor, is frequently downregulated in multiple human cancers including colorectal cancer (CRC).
Aim: We planned to grab the latent impacts of ARID1A in regulating cell autophagy, a crucial cellular homeostatic mechanism that plays a pro-survival or pro-death role in cancer. Thus, to clarify ARID1A possible regulatory principle in CRC.
Methods: The expression levels of ARID1A and autophagic markers, Beclin1, P62 and LC3-II in CRC cells were detected via realtime quantitative PCR (qPCR) and western blot. ARID1A overexpression and shRNA-mediated knockdown were performed to indicate the role of ARID1A on autophagy process in CRC cells. Moreover, the impacts of ARID1A overexpression on CRC cells viability was assessed using MTT proliferation assay.
Results: Here we reported that ARID1A overexpression in SW48 cells that has relatively low ARID1A expression, is sufficient to suppress cell proliferation. ARID1A downregulation in HCT116 cells that has relatively low ARID1A expression, decreased the initiation of autophagy due to downregulation of beclin 1 and inhibited the autophagic flux, as evidenced by the accumulation of LC3-II and p62. Whereas ARID1A overexpression in SW48 cells exhibited opposite effects. We found that ARID1A downregulation inhibited the fusion between autophagosome and lysosome, resulting in the accumulation of autophagosomes accompanied with lysosomal alkalinization.
Conclusion: Based on our knowledge, the present study is the first to evaluate the effect of ARID1A in autophagy process. According to our cell line-based study, it is likely that ARID1A downregulation may promote CRC by inhibiting autophagy.

Abstract # 3

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Keywords: TM9SF1, lipophagy, HER2-positive breast cancer

Title:
TM9SF1 mediated lipophagy to promote the progression of HER2-positive breast cancer

Abstract:
Compared to normal tissue, the up-regulated genes in HER2-positive breast cancer are more clustered in the "phagocytosis" pathway. Autophagy plays an important role in tumors as a form of cellular phagocytosis. So, we searched for potential autophagy-related prognostic molecules in HER2-positive breast cancer, among which, TM9SF1 has not been studied and attracted our great attention. Herein, we found that under starvation induction, TM9SF1 can be elevated with increasing levels of autophagy. Validation of clinical samples showed that TM9SF1 was highly expressed in tumor tissues compared to normal tissues and was associated with a poor prognosis. After the intervention of TM9SF1 expression levels, we found that the level of autophagy was then consistently altered. Further studies had shown that TM9SF1 levels were negatively correlated with lipid droplet content. Interestingly, we observed that TM9SF1 affected the level of lipophagy, which could be reverted by autophagy inducer or inhibitor. We also observed that knockdown of TM9SF1 disrupted cellular energetic homeostasis and induced apoptosis and necrosis, whereas overexpression of TM9SF1 promoted the proliferation ability of cancer cells. In vivo animal experiments likewise yielded consistent phenomena. In conclusion, we identified that TM9SF1 mediated lipophagy to maintain homeostasis in cancer cells and promote their progression. Our findings may facilitate the development of new strategies for targeted therapy of HER2-positive breast cancer.

Abstract # 4

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Institution: Tulane University School of Medicine
Keywords: fibrosis, hepatic stellate cells, pathology

Title:
Mechanism of liver fibrosis caused by autophagy-deficiency

Abstract:
Autophagy-deficiency in hepatocytes presents liver pathologies such as liver injury, inflammation, and fibrosis similar to common liver diseases affected by autophagy impairment. It is unknown how fibrosis develops in livers that are autophagy deficient. This study aims to examine the kinetics and mechanism of liver fibrosis development in autophagy-deficient livers.
Conditional and inducible models of liver specific Atg7 knockout was characterized for liver fibrosis. Immunofluorescence and immunohistochemistry were used to assess the presence of fibrosis and the upregulation of fibrosis-related cells. Microarray data was used to analyze fibrotic markers and possible mechanistic pathways involved in the development of fibrosis. Quantitative polymerase reaction and immunoblotting were used to examine fibrotic genes and proteins.
We determined the kinetics of disease development in the liver and the upregulation of cells promoting fibrosis, such as Hepatic Stellate Cells (HSC). We found that the occurrence of fibrosis happens in the same timeframe as the upregulation of HSCs in our model. We also analyzed potential pathways involved in the activation of HSCs and found that the transforming growth factor beta (TGFβ) pathway is upregulated in our model.
Autophagy-deficiency in livers leads to the activation of HSCs which can then lead to the development of liver fibrosis. The underlying signaling mechanism of HSC activation and fibrosis development in the autophagy-deficient liver is currently being investigated. This study will be clinically relevant in understanding molecular events that are important in the early stages of liver diseases and potentially provide a basis for pharmacological intervention of liver fibrosis.

Abstract # 5

Name: Khushbu Patel, MS
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Keywords: Chaperone mediated autophagy, cancer, oncogenes

Title:
Role of Chaperone-mediated autophagy in initiation and progression to Hepatocellular carcinoma

Abstract:
Autophagy is an essential physiological process to degrade cellular components at the lysosomal compartment; it contributes to the maintenance of cellular homeostasis and energy balance -among other specific regulatory functions. The dysregulation of autophagy has been described to contribute to different human diseases, including cancer.
My project focus in Chaperone-mediated autophagy (CMA), a selective form of autophagy that has been related to oncogenesis -although detailed information is still limited. Physiological CMA activity in healthy conditions have an anti-oncogenic role in cells, whereas malfunction of this process contributes to cellular malignant transformation and tumor growth. The goal of this project is to determine the impact of CMA dysfunction in liver disease progression to malignant transformation and development of hepatocellular carcinoma (HCC).
We have developed cell type specific CMA-deficient mice: in hepatocytes (AlbL2AKO) and CD4 cells (CD4L2AKO); and use those with a genetically engineered experimental approach -that overexpress cMyc and mutate p53 in the liver -two of the genetic alterations most commonly found in HCC patients. We are using this to generate the disease and evaluate the impact of the selective lack of CMA activity in hepatocytes and T cells.
Our data (including histopathological characterization, survival and tumor biology studies -among others) show relevant differences supporting that deficiency of CMA activity has detrimental effects in liver tumorigenesis and HCC progression. Different omics analysis are helping us to identify targets and better understand the molecular mechanism behind the process. This end will hopefully contribute to open new potential therapeutical windows for this disease.

Abstract # 6

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Keywords: ERMP1, proliferation, colony formation, PI3K/AKT pathway, free β-catenin, cell surface GRP78, tumor microenvironment, environment stress

Title:
ERMP1 accelerates the proliferation of colorectal cancer cells

Abstract:
Background: Endoplasmic reticulum metallopeptidase 1 (ERMP1) is highly expressed in various cancers such as colorectal cancer (CRC). It indicates the potential role of this oncogenic protein in crucial signaling pathways for cancer development. We aimed to investigate the molecular function of ERMP1 in colorectal cancer cells proliferation and progression.
Methods: ERMP1 knock-down was performed using specific small hairpin RNA (shRNA) targeting ERMP1 in CRC cell line (HCT116). The ERMP1 gene overexpression vector was used to upregulate this gene gene in SW48 cells. MTT assay was used to measure cell proliferation. The expression of AKT, p-AKT, p-mTOR, β-catenin, p-β-catenin and GRP78 proteins was assessed by western blotting. The expression of ERMP1, cyclin D and c-Myc was evaluated by RT-qPCR. Flowcytometry assay was performed to determine the localization of GRP78, cell cycle distribution and apoptosis.
Results: The ERMP1 gene knock-down reduced cell proliferation, inactivated PI3K/AKT pathway, prompted G1 arrest, and attenuated free β-catenin and cyclin D expression. Opposite results were obtained in ERMP1 up-regulated cells. Knock down of ERMP1 also reduced GRP78 localization at cell surface. In addition, colony reproduction and cell survival were decreased in ERMP1 down-regulated HCT116 cells. Conclusion: ERMP1 functioned as an oncogene in CRC cells by mediating cell proliferation, promoting G1 to S progression and activating the PI3K/AKT/β-catenin pathway . The localization of GRP78 was closely related to the effects of ERMP1. Consequently, ERMP1 can be regarded as a novel target in therapeutic strategies related to CRC.

Abstract # 7

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Keywords: autophagy, KSHV, galectin-8, NDP52, viral entry, virophagy, cell-autonomous immunity

Title:
Selective autophagy impedes KSHV entry by recruiting to virus-containing endosomes via membrane damage sensor galectin-8

Abstract:
When microorganisms invade the host cell cytosol, host defense mechanisms are deployed to direct pathogens to degradation by selective autophagy. One deadly human pathogen is Kaposi sarcoma-associated herpesvirus (KSHV) which is an oncogenic ɣ-herpesvirus associated with several opportunistic malignancies, including the 4th most common cancer in Sub-Saharan Africa, Kaposi sarcoma. Numerous studies demonstrate the role autophagy plays upon lytic reactivation of KSHV, however the details of the host cell autophagy response during KSHV's initial entry have remained enigmatic. Using an in vitro infection model with human epithelial cell lines, we show the lipidation of autophagy hallmark LC3 is induced shortly after KSHV entry. When components of the autophagy conjugation complex are depleted, infection is facilitated, suggesting that autophagy hinders KSHV infection. Virus particles were observed co-localizing with both NDP52 and the endolysosome damage sensor galectin-8 upon KSHV entry and depletion of galectin-8 promoted KSHV infection, suggesting that it may be involved in recruiting autophagy to incoming virus. We followed KSHV entry by live cell imaging and found that in contrast to what was previously thought about enveloped viruses, KSHV could cause endolysosomal membrane damage, comparable to non-enveloped viruses and bacteria. Our study identifies an important antiviral role for galectin-8 in the host cell-autonomous immune response to KSHV infection by recruitment to virus-damaged endosomes, and places both galectin-8 and NDP52 early in the pathway leading to viral restriction by selective autophagy.

Abstract # 1

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Keywords: mitophagy, mitochondrial dynamics, Atg1, Marf, oogenesis

Title:
Atg1 modulates mitochondrial homeostasis during oogenesis in *Drosophila melanogaster*

Abstract:
During *Drosophila* oogenesis, germline stem cells (GSCs) divide and differentiate to produce the egg. During oogenesis mitochondria undergo morphological shift from fused in GSCs to fragmented in differentiated cyst cells (developing eggs). These dynamic changes in mitochondrial shape are regulated by GTPases Marf and Opa1 which promote mitochondrial fusion and Drp1 which catalyzes mitochondrial fission. Mitophagy (autophagy of mitochondria) ensures that only healthy mitochondria are inherited in the egg by selectively degrading damaged mitochondria. Atg1 is a serine threonine kinase required in mitophagy. Depleting Atg1 (Atg1KD) blocks autophagy as observed by accumulation of Ref(2)P puncta and decrease in mCherryAtg8a puncta. Surprisingly, we observed fused/clumped mitochondria in Atg1KD germ cells and this was mediated by upregulation of Marf protein. Our data suggest that Atg1 in addition to its crucial role in autophagy, modulates mitochondrial fragmentation which facilitates mitophagy. The egg formation and fecundity in Atg1KD females were not affected but we observed a significant decrease in the percentage of hatched eggs in Atg1KD. The fused-like mitochondrial phenotype in Atg1KD cysts was restored back to fragmented by either overexpressing Drp1 or by depleting Marf suggesting they interact genetically. Combined Marf and Atg1 KD led to the failure of oogenesis, as observed by reduced ovary size and absence of eggs. Upon further examination, we observed reduced number of Vasa positive germ cells in Marf and Atg1 double KD. Further experiments are being carried out to understand how absence of Atg1 affects mitochondrial dynamics during oogenesis.

Abstract # 2

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Keywords: fasting, autophagy, gene expression, transcriptomics, nutrigenomics

Title:
Differential peripheral expression of selected autophagy-related genes in healthy subjects following short-term fasting versus frequent meal consumption: A randomized crossover trial

Abstract:
Objectives: While the relevance of autophagy in animal models during fasting is well-established, humans studies are scarce. Our aim was to investigate the expression of selected autophagy-related genes (macro, micro, CMA, etc.) in humans undergoing fasting periods of 12 and 24 hours, and compare it with the expression after feeding.
Methods: We conducted a cross-over randomized trial (ISRCTN12651780) with 29 healthy volunteers. After a standardized breakfast, participants were randomly assigned to either the fasting group (24h-fasting) or the frequent meal group (we provided six standardized meals every three hours). After a washout-period, participants were switched to the alternative treatment arm. For this exploratory analysis a random sample of 13 participants who completed both treatments was selected. Gene expression was meaured (by Clariom-S-Human-Array) at 12h and 24h in leukocytes. After quality control and array normalization, differential gene-expression (fold change) of selected genes (153 from the literature) by time and intervention group was examined. Statistical analyses were undertaken, adjusting for sex, age, body-mass index and leukocytes.
Results and conclusions: After 12h fasting (compared to baseline), we detected several differentially expressed autophagy-related genes. The top-ranked were: BCL2 (p=0.001); LIPE (0.001); PARK2 (P=0.005); LAMP2 (P=0.006); PINK1 (P=0.009) TECPR2 (P=0.011); and ATG16L1 (P=0.015). At 24h-fasting we observed changes in the hits. After frequent meal intake the differentially expressed-genes varied (NBR1, P=0.008; STX7, P=0.007; SQSTM1, P=0.012). Similarly, a dynamic pattern was observed at 24h. When comparing the expression of autophagy-related genes between treatments, additional differences were found, supporting further research.

Abstract # 3

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Keywords: autophagy, endosomal microautophagy, ESCRT, proteomics, transcriptomics

Title:
Identification and functional analysis of substrates & regulators of starvation-induced endosomal microautophagy in Drosophila

Abstract:
Autophagy is an evolutionarily conserved cellular stress response that degrades a wide variety of substrates in lysosomes, including oxidized and aggregated proteins, organelles, and intracellular pathogens, and is thus required for cellular homeostasis and function. Autophagy dysfunction has been linked to a number of diseases, including HIV, cancer, metabolic disorders, liver diseases, and neurodegenerative diseases. To date, three autophagic pathways have been identified: macroautophagy (MA), chaperone-mediated autophagy (CMA), and endosomal microautophagy (eMI). The latter was discovered relatively recently, and its regulation and physiological role are still poorly understood. During eMI, substrates are engulfed into late endosomes either in bulk or selectively for proteins with a targeting KFERQ motif in a process requiring the Endosomal Sorting Complex Required for Transport (ESCRT) machinery. Our laboratory recently discovered that specific types of cellular stress, such as prolonged starvation and oxidative stress, but not ER stress, induce eMI in Drosophila, implying that eMI is required for proper cellular homeostasis. The in vivo eMI substrates and cellular processes regulated by eMI, however, are still unknown. To better understand the molecular basis for regulation and the physiological relevance of this pathway in Drosophila, I am using proteomics and transcriptomics to identify endogenous eMI substrates and biological pathways regulated by eMI. To accomplish this, I have used label-free quantitative proteomics to compare changes in the fat body proteome of starved larvae over different time periods (0h, 4h, and 25h), under different (genetically altered) conditions with fed larvae as a control. Additionally, I have used RNA-seq to examine how changes in gene expression correspond to variations in the protein content of the larval fat bodies over time. Our analysis has identified several ribosomal proteins as potential eMI substrates. Further research will provide a new perspective regarding eMI regulation on the pathogenesis of various diseases.

Abstract # 4

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Keywords: autophagy crosstalk, chaperone-mediated autophagy, chaperones, endosomal microautophagy, late endosome

Title:
Molecular determinants of the crosstalk between endosomal microautophagy and chaperone-mediated autophagy

Abstract:
Chaperone-mediated autophagy (CMA) and endosomal microautophagy (eMI) are pathways for selective degradation of cytosolic proteins in lysosomes and late endosomes in mammals, respectively. These autophagic processes share as a first step the recognition of the same five amino acid motif in substrate proteins by the Hsc70 chaperone, raising the possibility of coordinated activity of these pathways. In this work, we demonstrate the existence of a compensatory relationship between CMA and eMI and identify a role for the chaperone protein Bag6 in triage and internalization of eMI substrates into the late endosome. Using proteomics of isolated organelles, we have also identified the subproteome undergoing eMI in a Bag6-deependent manner. Association and dynamics of Bag6 at the late endosome membrane, visualized by super-resolution microscopy of isolated organelles, change during starvation which we found that, contrary to other autophagic pathways, causes a decline in eMI activity in mammals. Collectively, we demonstrate a coordinated function of eMI with CMA, identify the interchangeable subproteome degraded by these pathways and start to elucidate the molecular mechanisms that facilitate the switch between them.

Abstract # 5

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Keywords: autophagy, corpora lutea, vizcacha, ovary

Title:
Autophagy in the corpora lutea of the gestating South American plains vizcacha (*Lagostomus maximus*): a model of pseudo-ovulation during pregnancy

Abstract:
The corpus luteum (CL) is a transient endocrine gland that synthesizes peptide and steroid hormones, serving as the primary source of progesterone during estrous/menstrual cycles and playing a crucial role in pregnancy establishment and maintenance. While autophagy and apoptosis have been suggested as cooperative mechanisms, their interaction within the CL of pregnant mammals has not been extensively investigated. In order to deepen our understanding of the potential collaborative role of autophagy and apoptosis in the development, maintenance, and regression of the CL, we conducted an analysis of both mechanisms during pregnancy, in the South American plains vizcacha, *Lagostomus maximus*, a rodent that undergoes a decline in progesterone levels during mid-gestation, reactivation of the hypothalamus-hypophysis-gonadal axis, and the incorporation of new functional secondary CL, with retention of the oocyte. Our analysis of autophagy markers BECLIN1 (BECN1), SEQUESTOSOME1 (SQSTM1), LC3B-I/II, and LAMP1 and anti- and pro-apoptotic markers BCL2 and ACTIVE CASPASE 3 (A-C3) revealed interactive behaviors between both processes, with autophagy promoting either cell survival or cell death depending on the ovarian structure. Healthy primary and secondary CL exhibited positive expression of BECN1, SQSTM1, LC3B, and LAMP1, while regressed CL displayed enhanced expression of these autophagic markers along with nuclear A-C3. Transmission electron microscopy revealed a significant formation of autophagosomes, autolysosomes, and lysosomes in regressed CL during full-term pregnancy, whereas healthy CL exhibited a low number of autophagic vesicles. The co-expression of LC3B-SQSTM1 and LC3B-LAMP1 was observed in both healthy and regressed CL during pregnancy, while co-expression of BECN1-BCL2 was only detected in healthy primary and secondary CL. The presence of LC3B-A-C3 co-expression was detected in a subset of luteal cells within the regressing CL. We propose that autophagy could act as a survival mechanism in the primary and secondary CL, allowing the pregnancy to progress until full-term, while also serving as a mechanism to eliminate remnants of regressed CL, thereby providing the necessary space for subsequent follicular maturation.

Abstract # 6

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Keywords: BNIP3L/NIX, selective autophagy, mitophagy, regulation

Title:
Unveiling the BNIP3L/NIX-mediated mitophagy: novel insights into dimerization regulation and upstream mechanisms

Abstract:
To preserve both mitochondrial quality and quantity, cells selectively remove damaged or superfluous mitochondria through a specialized form of autophagy-mitophagy. Nevertheless, the mechanisms that control the selective elimination of dysfunctional mitochondria under steady-state or hypoxic conditions as well as upon erythrocyte differentiation are not entirely understood. Selective autophagy receptor BNIP3L/NIX was shown to be crucial for the programmed removal of healthy mitochondria during terminal erythropoiesis, its role in mitophagy regulation has been verified in many different cell types.
Recently, we have suggested a new mechanism underlying BNIP3L/NIX-mediated mitophagy. This mechanism involves the fine interplay between C-terminal BNIP3L/NIX dephosphorylation of Ser212 and BNIP3L/NIX dimerization as a consequence of BNIP3L/NIX intermembrane phosphorylation loss. BNIP3L/NIX dimerization is essential for the recruitment of autophagy machinery and also has a positive effect on mitophagy progression. This relationship between BNIP3L/NIX phosphorylation and dimerization is essential for proper BNIP3L/NIX-dependent mitophagy. Consequently, we are investigating the phosphatase and kinase that regulate BNIP3L/NIX dimerization. Detailed analysis of the potential BNIP3L/NIX Ser212 C-terminal dimerization regulators has revealed phosphatases/kinase candidates are the main focus of our current research.
Thus, understanding this novel upstream mechanism of BNIP3L/NIX dimerization provides a key missing piece in the molecular puzzle controlling receptor-mediated mitophagy important for cellular development and differentiation as well as understanding diseases where such mitophagy is abrogated.

Abstract # 7

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Keywords: Atg16, NMD, Nonsense-mediated mRNA decay, Upf3

Title:
Upf3 is a post-transcriptional negative regulator of autophagy

Abstract:
The goal of this study is to investigate the role of a novel post-transcriptional regulator of autophagy in the yeast Saccharomyces cerevisiae. Macroautophagy/autophagy is a cellular process that degrades and recycles cellular components to maintain homeostasis and survival under stressful conditions. Aberrant autophagy contributes to cancer, cardiovascular, neurodegenerative, pulmonary, and infectious diseases. To date, >40 AuTophaGy-related (ATG) genes have been identified in fungi. Autophagy is regulated at multiple checkpoints, including at the transcriptional, post-transcriptional, translational, and post-translational levels. Upf3 (UP- Frameshift suppressor 3) is a component of the nonsense-mediated mRNA decay (NMD) pathway. NMD mediates quality control in eukaryotes by degrading mRNAs containing premature termination codons (PTCs) or other structural abnormalities, although NMD also regulates normal cellular transcripts. Our work demonstrates that Upf3 functions as a negative regulator of autophagy through multiple assays. Upf3 expression decreases during autophagy, further supporting its role as a negative regulator. Loss of UPF3 enhances Atg16 expression, which is a key protein involved in autophagosome formation. These findings provide insight into the post-transcriptional regulation of autophagy through the NMD factor Upf3.

Abstract # 1

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Keywords: Proteostasis, NMDA Receptor, disease-associated variants, ER-phagy

Title:
N-methyl-D-Aspartate Receptors' proteostasis is modulated by autophagic flux

Abstract:
The proteostasis of receptor proteins is essential for their proper folding, trafficking, localization, functionality, and degradation. Membrane proteins have complex structures, which result in inefficient folding and assembly within the endoplasmic reticulum (ER). N-methyl-D-aspartate receptors (NMDARs) mediate excitatory neurotransmission and play a critical role in the formation and maturation of excitatory synapses and are implicated in learning, memory, and synaptic plasticity. The GRIN genes that encode NMDAR subunits are highly intolerant to genetic variation, indicating, mutation are likely to result in various neurological disorders including epilepsies and intellectual disabilities. We have established numerous disease-associated variants (DAVs) that have been identified within the GluN2B subunits result in misfolding, improper assembly, aggregation, defective trafficking, and impaired functionality on the cell surface. These nonfunctional GluN2B subunits accumulate within the ER and must be cleared to ensure physiological function of ER continues. These misfolded subunits can be degraded through the proteasome or the lysosome. Our work demonstrates that the proteostasis of GluN2B subunits is mediated via autophagy by two distinct pathways: ER-phagy and macroautophagy. Our results support that a highly conserved LC3-interacting region (LIR) motif within the GluN2B subunit is essential for targeting of NMDARs for autophagic clearance.

Abstract # 2

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Institution: Institute of Pathology and Southwest Cancer Centre, First Affiliated Hospital,Army Medical University
Keywords: UTMD, radiosensitivity, PGRMC1, autophagy, glioblastoma

Title:
Targeting PGRMC1-mediated autophagy by ultrasound-triggered microbubble destruction enhances the radiosensitivity of glioblastoma

Abstract:
Objectives: To explore the role of PGRMC1-mediated autophagy in ultrasound targeted microbubble destruction (UTMD)-mediated radiosensitivity of glioblastoma (GBM).
Methods: Murine GL261 GBM cells, human U251 cells and orthotopic GBM mice models were divided into different groups. PGRMC1 inhibitor(AG-205), PGRMC1 overexpression vector and PGRMC1 siRNA were added separately. Autophagic markers (LC3、p62) and PGRMC1 expression were detected by Western blot, the co-localization of PGRMC1 and LC3B2 was detected by immunofluorescence, cell viability was detect by a CCK-8 kit, cell death was detect by flow cytometry, and cell clonality was detect by colony formation assay. IVIS live animal imaging and high-resolution animal ultrasound were used to detect tumor growth, immunofluorescence was used to detect tumor tissue proliferation, and Kaplan-Meier survival analysis was used to evaluate the survival time.
Results: Compared with the radiotherapy group, UTMD combined with radiotherapy notably improved the sensitivity of GBM cells to radiotherapy. UTMD significantly increased the expression of LC3B2 and inhibited the degradation of p62 in IR-treated GBM cells and tumor tissues. Meanwhile, it was found that UTMD markedly inhibited PGRMC1 expression, decreased the colocalization of PGRMC1 and LC3B2 in GBM cells, inhibited tumor growth and prolong the survival time of GBM-bearing mice. Inhibition or targeted silencing of PGRMC1 can further enhance the inhibitory effect of UTMD on autophagy. In contrast, PGRMC1 overexpression abolished UTMD-induced blockade of autophagic degradation, and then reverse the radiosensitization effect of UTMD.
Conclusion: UTMD enhanced radiosensitivity of GBM through disrupting PGRMC1-mediated autophagy in vitro and in vivo.

Abstract # 3

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Keywords: AMD, RPE, UA, lysophagy, LMP

Title:
Urolithin A prevents acute retinal degeneration through selective autophagy

Abstract:
Purpose. Age-related macular degeneration (AMD) is the leading cause of blindness in elderly people in the developed world, and the number of people affected is expected to almost double by 2040. The retina presents one of the highest metabolic demands that is partially or fully fulfilled by mitochondria in the neuroretina and retinal pigment epithelium (RPE), respectively. Together with its post-mitotic status and constant photooxidative damage from incoming light, this context requires a tightly-regulated housekeeping system that involves autophagy. We want to assess the effects of selective autophagy modulation in the neuroretina and RPE given an AMD-like paradigm.
Methods. Sodium iodate (SI) was used as a model of late-stage AMD and Urolithin A (UA) as an autophagy inducer. C57BL6/J mice and ARPE-19 human cells were used as in vivo model and in vitro models, respectively. Tandem fluorescence reporters (MAP, mito-QC and tfGal3) and siRNA-mediated gene knockdown were used to further dissect the effects of SI and UA on selective autophagy pathways. Bioinformatic analysis of public human databases was also performed.
Results. UA induced autophagy in vivo and prevented degeneration both in the neuroretina and RPE. This amelioration was also associated with decreased lipid peroxidation, gliosis, increased photoreceptor survival and, most importantly, lead to preserved visual function in SI-treated mice. In vitro approaches showed that SI impaired autophagy due to lysosomal membrane permeabilization. UA was able to restore autophagic flux by promoting lysophagy-mediated lysosomal quality control.

Abstract # 4

Name: Sierra Palumbos, PhD
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Institution: University of Pennsylvania
Keywords: secretory autophagy, neuron, extracellular vesicle

Title:
Compensatory secretion in stressed neurons

Abstract:
Neurons rely on autophagy to constitutively degrade damaged proteins and organelles to maintain neuronal homeostasis. When neuronal autophagy is perturbed or degradation capacity is overwhelmed, aggregated proteins and damaged organelles accumulate, contributing to neuronal dysfunction or death. Indeed, autophagy dysfunction is consistently associated with neurodegenerative diseases, such as Parkinson's Disease. How do neurons respond when faced with the accumulation of dysfunctional proteins and organelles that overwhelm autophagy? My findings suggest that neurons can employ autophagy-dependent secretion as an alternative quality control mechanism to dispel their cellular waste when degradation is chronically or acutely strained. For example, I observe that cultured neurons treated with Bafilomycin A1, which effectively blocks lysosomal fusion, prompts the upregulation of secretion. Additionally, neurons expressing the Parkinson's Disease-causing mutation, LRRK2 G2019S, which exhibit strained degradation, shunt autophagy cargo toward secretion. I used live-cell imaging and immunoblots to confirm that the upregulation of secretion is dependent on LRRK2 kinase hyperactivity. Finally, analysis of plasma from LRRK2 G2019S animals suggest that the upregulation of secretion is likely occurring in vivo. These observations highlight an underappreciated quality control mechanism in mammalian neurons by describing the interplay between autophagy dependent degradation and secretion.

Abstract # 5

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Institution: Department of Neurology, Peking University Aerospace School of Clinical Medicine
Keywords: Alzheimer's disease, drug, mitochondrion, mitophagy, neurodegenerative diseases

Title:
Mitophagy in Alzheimer's disease: a bibliometric analysis from 2002 to 2022

Abstract:
Background: The study of mitophagy in Alzheimer's disease remains relatively understudied in the realm of bibliometric analysis, which is a widely adopted method for analyzing the influential publications of specific fields. This study aims to investigate the research progress in AD and mitophagy, providing researchers with comprehensive research trends and frontiers.
Methods: We analyzed the basic bibliometric information, targets, target-drug-clinical trial-disease of publications in the Web of Science Core Collection from 2007 to 2022 using bibliometric software.
Results: A total of 5146 publications were included in the study with a 16-year steadily increasing trend. The USA occupied the leading position in publications. PINK1 and Parkin, as key targets of mitophagy, have shown a rising trend in the recent six years. Keywords, such as insulin, aging, epilepsy, tauopathy, and mitochondrial quality control, have been emerging hotspots in the past three years. Despite donepezil already being approved by Food and Drug Administration, nine new drugs have been approved for clinical trials, such as curcumin, insulin and melatonin. Moreover, clinical trials and targets related to these drugs are included in the study.
Conclusions: Our study comprehensively presents the research trend of mitophagy in AD over the past 16 years, revealing that mitophagy is an emerging molecular mechanism of AD research and will be a penetrating point for AD candidate drug research. Moreover, this study is the first to introduce the targets and target-drug-clinical trial-disease into the field of bibliometric analysis, which will provide inspiration for bibliometric workers in different fields.

Abstract # 6

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Keywords: sepsis, dendritic cells, RETREG1, ER-phagy, necroptosis

Title:
The effect of RETREG1-mediated ER-PHAGY on necroptosis of dendritic cells under septic exposure

Abstract:
Introduction: Diminished dendritic cells (DCs) count is a key player in sepsis-induced immunosuppression, which could be largely attributed to necroptosis. ER-phagy plays an essential role in the maintenance of functional homeostasis and cellular viability. However, the role and regulatory mechanisms of ER-phagy in the necroptosis of DCs remain largely unclarified.
Methods: The splenic DCs were extracted by CD11c+ microbeads, followed by treatment of LPS+ z-VAD. Murine models of sepsis were reproduced by CLP in wild-type mice and mice with Retreg1 knockout. Flow cytometry and SYTOX Green were used to detect necroptotic cells. LSCM was used to observe necroptotic activity, ER-phagy and ER morphology. WB was applied to determine the expressions of ERS, ER-phagy and necroptosis-related proteins.
Results:
1. RETREG1-mediated ER-phagy was mobilized from 6 to 24 hours while decreased at 48 and 72 hours post-CLP surgery and LPS+z-VAD treatment.
2. Upon septic challenge, the proportion of DCs undergoing necroptosis elevated and the expression levels of necroptosis-related proteins p-RIPK1, p-RIPK3 and p-MLKL markedly upregulated. Nec-1 could rescue the necroptosis of DCs in sepsis.
3. Compared with WT mice, the necroptosis in Retreg1-/- mice was significantly increased. The mortality of Retreg1-/- septic mice were much higher than that of WT littermates.
4. The expression of UPR proteins, including HSPA5, p-EIF2AK3, ATF4, p-EIF2A and DDIT3 were significantly up-regulated, which were augmented by Retreg1 deficiency. Salubrinal could rescue the necroptosis of DCs in Retreg1-/- septic mice.
Conclusions: FAM134B-mediated ER-phagy is critically involved in regulating necroptosis of DCs via restraining overactivated ERS under septic exposure.

Abstract # 1

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Keywords: porphyromonas gingivalis, autophagy, ESCC

Title:
Association between *Porphyromonas gingivalis* infection and the progression of autophagic flux in esophageal squamous cell carcinoma

Abstract:
Objective:
Porphyromonas gingivalis (P.gingivalis) infection is closely related with the occurrence and development of esophageal squamous cell carcinoma(ESCC).The main purpose of the current research was to explore the association between P.gingivalis infection and the progression of autophagic flux in esophageal squamous cell carcinoma and provides a new scientific evidence for the etiological study and the formulation of clinical prevention and treatment strategies in ESCC.
Methods:
The P.gingivalis infection and autophagic protein-SQSTM1/p62 were detected by immunohistochemistry of ESCC patients tissue,and it's relation with the clinicopathological parameters and 5-years survival rate was analysed by x2 test and kaplan-meier survival analysis.The autophagosome of P.gingivalis infected ESCC cells was detected by transmission electron microscopy.Moreover,the effects of P.gingivalis infection on the autophagic flux of ESCC cells was monitored dynamically by confocal microscope.Furthermore the effect of autophagy activity on chemotherapy sensitivity of P.gingivalis infected ESCC patients was analysed by Patient-Derived Xenograft (PDX) model with 3-Methyladenine and Paclitaxel intervene.
Results:
ESCC patients with P.gingivalis infection and high expression of p62 were often accompanied by adverse indicators(late clinical stage and low 5-year survival rate).Moreover,large number of autophagosomes were emerged in cell cytoplasm after P.gingivalis infection according to the results of transmission electron microscopy.Additionally, with the continuous infection of P.gingivalis,the fusion amount of autophagosome and lysosome also continued to decrease,resulting in the accumulation of a large number of autophagosomes in the cytoplasm and the autophagy flux was then obstructed.PDX animal model was established by collecting tumors of P.gingivalis infected ESCC patients and then treated with Paclitaxel and 3-Methyladenine to illustrated that effective regulation of autophagy activity can be an important approach to improve the chemotherapy sensitivity of P.gingivalis infected ESCC patients.

Abstract # 2

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Keywords: Chaperone mediated autophagy, cancer, oncogenes

Title:
Role of Chaperone-mediated autophagy in initiation and progression to Hepatocellular carcinoma

Abstract:
Autophagy is an essential physiological process to degrade cellular components at the lysosomal compartment; it contributes to the maintenance of cellular homeostasis and energy balance -among other specific regulatory functions. The dysregulation of autophagy has been described to contribute to different human diseases, including cancer.
My project focus in Chaperone-mediated autophagy (CMA), a selective form of autophagy that has been related to oncogenesis -although detailed information is still limited. Physiological CMA activity in healthy conditions have an anti-oncogenic role in cells, whereas malfunction of this process contributes to cellular malignant transformation and tumor growth. The goal of this project is to determine the impact of CMA dysfunction in liver disease progression to malignant transformation and development of hepatocellular carcinoma (HCC).
We have developed cell type specific CMA-deficient mice: in hepatocytes (AlbL2AKO) and CD4 cells (CD4L2AKO); and use those with a genetically engineered experimental approach -that overexpress cMyc and mutate p53 in the liver -two of the genetic alterations most commonly found in HCC patients. We are using this to generate the disease and evaluate the impact of the selective lack of CMA activity in hepatocytes and T cells.
Our data (including histopathological characterization, survival and tumor biology studies -among others) show relevant differences supporting that deficiency of CMA activity has detrimental effects in liver tumorigenesis and HCC progression. Different omics analysis are helping us to identify targets and better understand the molecular mechanism behind the process. This end will hopefully contribute to open new potential therapeutical windows for this disease.

Abstract # 3

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Institution: St. Jude Children's Research Hospital
Keywords: MYC, HCC, Omomyc, AAV, gene therapy

Title:
Inhibition of c-MYC in hepatocellular carcinoma via AAV8 mediated gene transfer

Abstract:
Inhibiting the key transcription factor MYC has long been a goal due to its central role in human cancers; although, it has yet to be accomplished clinically. Hepatocellular carcinoma (HCC) is the most common liver malignancy and is often associated with MYC deregulation. The mini-protein Omomyc has been shown to induce tumor regression in a variety of cancer types by preventing the heterodimerization of c-MYC and it obligate binding partner MAX, thus preventing its transcriptional activation. We have shown that Omomyc expression in HCC cell lines in vitro induces a 8-20 fold inhibition of proliferation depending on cell type, including G2 cell cycle arrest (about 50%) in the high c-MYC expressing cell line Hep3B. We have demonstrated a 2-fold induction of autophagy, measured via mCherry-GFP-LC3 levels in HCC cell lines with a direct correlation to the amount of c-MYC expressed in these cell lines. When combined with conventional therapeutics like the multi-kinase inhibitor sorafenib, Omomyc induces autophagy another 20%. Mitophagy is also observed via electron microscopy. Additionally, Omomyc results in significant levels of cell death (20-60%), in direct correlation with the level of c-MYC protein expression. Our preliminary data suggests that this could be due to induction of autophagic cell death, with mitigation of the effect seen when treated with Chloroquine. Taken together, our data suggest that Omomyc can inhibit cellular proliferation, induce autophagy, and induce cell death in HCC which may prove to be a novel therapeutic approach for the treatment of HCC, especially when combined with conventional therapeutics.

Abstract # 4

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Institution: Fujian Medical University Union Hospital
Keywords: SEPSIS, TRIM13, SESN2, Sestrin2, ubiquitination

Title:
TRIM13 regulates autophagy through modulating both the transcription and the ubiquitination of SESN2 in sepsis

Abstract:
Sepsis refers to a life-threatening organ dysfunction caused by a dysregulated host immune response to infection. Dendritic cells (DCs), as crucial professional antigen-presenting cells, play pivotal roles in initiating adaptive inflammatory immune response. Sestrin2 (SESN2), a highly evolutionarily conserved protein, is critically involved in cellular response to various stresses and maintains homeostasis of the internal environment. Here, we demonstrate that TRIM13, a RING-type E3 ubiquitin ligase, regulates the stability of SESN2 protein directly or indirectly. The SESN2-Trim13 interaction was confirmed by co-immunoprecipitation. Additionally, TRIM13 ubiquitinates and activates SESN2. SESN2 ubiquitination activates mitochondrial autophagy, promotes aggregation of isolated bodies 1(SQSTM1, p62) on the surface of mitochondria, and clears damaged mitochondria. Importantly, TRIM13 deletion increase the expression of DRP1, which is a mitochondrial mitotic protein, and affects mitochondrial autophagy, leading to DC dysfunction and necrosis. In summary, TRIM13 plays a key role in preventing inflammatory reaction in sepsis by activating SESN2 mitochondrial phagocytosis through ubiquitination, providing new insights into sepsis immunotherapy mechanisms.

Abstract # 5

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Institution: Centre for Cancer Biology
Keywords: ubiquitination, *Drosophila*, autophagy regulation

Title:
Novel ubiquitin ligases in the regulation of autophagy

Abstract:
Autophagy is an evolutionarily-conserved, prosurvival pathway responsible for maintaining cell and tissue homeostasis. Cellular stressors upregulate autophagy, causing sequestration of bulk cytoplasmic substrates within double-membraned autophagosomes and their subsequent degradation via lysosomal-mediated pathways. Dysregulation of autophagy has been identified in numerous diseases such as breast cancer, lung cancer and neurodegenerative disorders. Given the context-dependent nature of these disorders, we propose that additional regulators function within autophagy which, when perturbed, contribute to a spectrum of autophagy-related disorders.
Recent findings have demonstrated roles for ubiquitination as an important posttranslational modification mechanism that regulates autophagic machinery. While studies have highlighted the significance of certain E3 ligases such as TRAF6 in autophagy induction and NEDD4 in autophagy inhibition, there are likely multiple other ubiquitin modification enzymes (UME) that have specific targets in autophagic machinery.
In order to screen for UME specific for autophagy regulation, we have utilised a unique developmental process within Drosophila melanogaster as our pipeline model. During metamorphosis, increase in the steroid hormone, ecdysone, initiates autophagic degradation of the obsolete larval midgut. Importantly, inhibition of autophagy impedes midgut degradation, allowing us to determine how knockdown of different UME can affect autophagy-dependent midgut remodelling.
Through a comprehensive genetic screen of over 250 UME in the Drosophila midgut, we have uncovered 21 E3 ligases and 1 E2-conjugating enzyme which, upon knockdown, alter autophagy dynamics during midgut histolysis. Further characterisation of these candidates using Drosophila and mammalian cell models will enhance our understanding of autophagy in the context of human disease.

Abstract # 6

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Keywords: invadopodia, Cib1, breast cancer, autophagy, metastasis

Title:
Autophagy regulation of breast cancer metastasis: Unveiling the role of Cib1 as a key autophagy target for the regulation of invadopodia

Abstract:
Autophagy is a critical stress response that promotes tumor adaptation to cancer-related stressors, though its involvement in metastasis remains inadequately understood. Emerging evidence in breast cancer suggests that diminished autophagic activity plays a pivotal role in driving metastasis. This study aims to uncover how autophagy suppresses pro-metastatic invasive behaviors in breast cancer and identify specific autophagy-dependent targets responsible for regulating metastasis. We identified a novel autophagy target protein, calcium and integrin binding protein 1 (CIB1), which exhibits a strong correlation with cancer cell invasion. Cib1 is responsible for the maturation of invadopodia—pro-metastatic cellular protrusions implicated in extracellular matrix (ECM) degradation and the secretion of metastasis-promoting factors like matrix metalloproteases (MMPs) among many others. Our findings suggest that autophagy subdues the formation of pro-metastatic invadopodia structures by targeting Cib1 to the lysosome and limiting the degradative capacity of invadopodia. Likewise, inhibiting autophagy in metastatic breast cancer cells leads to an accumulation of Cib1-dependent invadopodia structures and increased ECM degradation—two behaviors strongly associated with aggressively metastatic cells.
This discovery challenges the prevailing notion that cancer cell autophagy invariably promotes late-stage cancer progression. Instead, our research demonstrates an anti-metastatic function for autophagy and raises the possibility that pharmacological autophagy inhibitors, including anti-malarial drugs, may carry long-term risks by enhancing pro-metastatic cellular behaviors. Increasing autophagy turnover in metastatic cells may represent a promising therapeutic strategy to reduce metastasis rates. However, a comprehensive understanding of autophagy and late-stage cancer is imperative for developing therapies that modulate autophagy effectively to attenuate breast cancer metastasis.

Abstract # 1

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Keywords: autophagy initiation, ULK1 complex, PI3KC3-C1 complex, autophosphorylation, cryo-EM

Title:
Structure and activation of the human autophagy-initiating ULK1:PI3KC3-C1 supercomplex

Abstract:
Autophagy is essentially orchestrated by six core complexes. Among them, the unc-51-like kinase protein kinase complex (ULK1C) and the class III phosphatidylinositol (PI) 3- kinase complex I (PI3KC3-C1) are the most upstream and central players in the initiation of autophagy. On one hand, the ULK1C provides an assembly site for the autophagy adaptors and phospho-regulates the downstream mitophagy core complex, on the other hand, The C1 complex produces phosphatidylinositol-3-phosphate (PI(3)P), which is the main membrane-resident second messenger in the mitophagy signaling cascade. Here, we determined the high-resolution 3D structures of both the ULK1C core region and the full-length PI3KC3-C1 individually, along with a moderate resolution structure of their supercomplex using cryo-electron microscopy. These structural insights shed light on the recognition mechanisms employed by these two complexes. Intriguingly, the presence of PI3KC3-C1 induced an alternation of the stoichiometry of ULK1C, prompting us to further investigate this by combining mutagenesis and biochemical analysis. Our findings unveiled a novel mechanism governing the initiation of autophagy, involving PI3KC3-C1-induced dimerization and transphosphorylation of ULK1 kinase. This study uncovered a positive feedback loop between ULK1C and PI3KC3-C1, providing new concepts for their therapeutic targeting in the future.

Abstract # 2

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Keywords: methylation, lysosomes, degradation, metabolism, pyridoxine

Title:
Vitamin B6 is governed by the local compartmentalization of metabolic enzymes during growth

Abstract:
Vitamin B6 is a vital micronutrient across cell-types and tissues and dysregulated B6 levels contribute to human disease. Despite its importance, how B6 vitamer levels are regulated is not well understood. Here, we provide evidence that B6 dynamics are rapidly tuned by precise compartmentation of pyridoxal kinase (PDXK), the rate-limiting B6 enzyme. We show that canonical Wnt rapidly led to the accumulation of inactive B6 by shunting cytosolic PDXK into lysosomes. PDXK was modified with methyl-arginine Degron (MrDegron), a protein tag for lysosomes, which enabled delivery via microautophagy. Hyperactive lysosomes resulted in the continuous degradation of PDXK and B6 deficiency that promoted proliferation in Wnt-driven colorectal carcinoma cells (CRCs). Importantly, pharmacological or genetic disruption of the coordinated MrDegron proteolytic pathway was sufficient to reduce CRC survival in cells and organoid models. In sum, this work contributes to the repertoire of micronutrient-regulated processes that enable cancer cell growth and provides insight into the functional impact of B6 deficiencies for survival.

Abstract # 3

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Keywords: TFEB, geroprotectors, aging, sertindole, longevity

Title:
TFEB activator screen identified anti-psychotic drug Sertindole as a novel TFEB activator for geroprotection

Abstract:
Repurposing drugs and natural products are considered a leading approach to slow aging. This project aimed to identify geroprotectors that sustain health and slow aging. Given that loss of macroautophagy is a major hallmark of aging and augmenting macroautophagy underlies many longevity regimes, we proposed screening of FDA approved and pharmacopeial drug libraries for macroautophagy activators to increase the chances of identifying effective geroprotectors.
Transcription Factor EB (TFEB), the master regulator of lysosomal biogenesis and autophagy, is of great interest in our screen as a molecular target for macroautophagy activators as TFEB expression and activity have been shown to modulate rate of aging.
We established and validated a high content screening platform to identify novel TFEB activators through the detection and analysis of TFEB-GFP nuclear translocation and LC3-GFP puncta formation via fluorescence microscopy in transgenic HeLa cells. Out of 2,964 compounds from the FDA approved and pharmacopeial drug library, our screening platform identified 56 hits. One of which was Sertindole, an anti-psychotic drug. Compared to control, Sertindole induced ~3-fold increase in TFEB nuclear translocation and significantly upregulated autophagic flux with low toxicity in HeLa cells. Further validation in HEK293 cells confirmed Sertindole induced TFEB nuclear translocation, which was accompanied by an ATG7-dependent macroautophagy upregulation and increased lysosomal biogenesis. Sertindole was found to inhibit mTOR activity and promote TFEB dephosphorylation. Pilot lifespan experiment in flies suggests that Sertindole may promote longevity. Hence, repurposing drugs by applying at a lower dosage that enhances macroautophagy represents potential therapeutics for aging and age-related diseases.

Abstract # 4

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Keywords: autophagy, mitophagy, BNIP3L/NIX, phosphorylation, erythrocyte differentiation

Title:
Determination of key kinase governing BNIP3L/NIX mitophagy modulation

Abstract:
Mitophagy, a specialized pathway of selective autophagy of mitochondria, involves the programmed removal of unnecessary or damaged mitochondria. Alongside mitochondrial fusion and fission, this process is essential to control mitochondrial quality and quantity. Dysfunctions in mitophagy have been linked to various conditions, including neurodegenerative and hematological disorders, cancer, and metabolic abnormalities. Notably, programmed mitophagy of healthy mitochondria, primarily orchestrated by mitophagy receptors, plays a significant role in the development of different cell types, such as erythrocytes, retinal ganglion cells, and oligodendrocytes. Over a decade ago, the BNIP3L/NIX mitophagy receptor was identified as indispensable for the programmed removal of mitochondria during the terminal stages of erythropoiesis. Recent studies reveal that regulation of BNIP3L/NIX-mediated mitophagy depends on receptor dimerization, coupled with phosphorylation of the LC3-interacting region (LIR), a prerequisite for the recruitment of autophagic machinery to mitochondria. The primary focus of this study is to extensively investigate the kinases responsible for receptor phosphorylation. Phosphorylation of the BNIP3L/NIX LIR domain provides the formation of stronger interactions between BNIP3L/NIX and autophagosomal LC3 proteins, thereby enhancing the recruitment of mitochondria toward autophagosomes. Our main focus centers on elucidating signaling pathways that initiate and govern mitophagy within the erythroid lineage.

Abstract # 5

Name: Olaya Santiago-Fernández, PhD
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Keywords: chaperone-mediated autophagy, aging, skeletal muscle

Title:
Decline of chaperone-mediated autophagy with age contributes to loss of skeletal muscle integrity

Abstract:
Chaperone-mediated autophagy (CMA) plays a key role in proteostasis maintenance as it selectively degrades a subset of cytosolic proteins in lysosomes. Levels of CMA's limiting component, the lysosomal receptor LAMP-2A, decrease during aging in multiple tissues. Concurrent with this reduction in LAMP-2A, in skeletal muscle we have also observed a decrease in CMA activity in aged mice. However, the role of CMA in this tissue and the consequences of decline in skeletal muscle CMA with age are poorly understood. Here, we have generated a muscle-specific LAMP-2A knockout mouse model (HSA-Cre:L2Af/f), which we found shows progressive development of myopathic features including reduced muscle force, degenerative changes in myofibers and dystrophin loss. At the cellular level, CMA-defective skeletal muscle displays pronounced alterations in mitochondria and sarcoplasmic reticulum morphology and Z-line derangement. Proteomic analyses of isolated lysosomes to identify the CMA substrates responsible for the observed phenotype revealed the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) as a potential CMA substrate, and its faulty degradation through CMA as responsible for dysregulation in calcium storage in myofibers. Together, our work highlights the importance of CMA in the control of skeletal muscle homeostasis and myofiber integrity.

Abstract # 6

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Institution: Vellore Institute of Technology, India
Keywords: adipose, senescence, autophagy, SASP

Title:
Connecting senescence and autophagy in murine adipose cells

Abstract:
There are changes in gene expression during the differentiation of pre-adipocytes into mature adipocytes in murine adipose cells. RT-PCR results show altered expression of senescence-associated secretory phenotype markers (p16, p21), and autophagy-like genes (ATG) in early- and late-passage murine adipose cells (3T3-L1). Staining the pre- and mature adipose cells with beta-galactosidase displayed variation in the number of senescent cells. Treating the cells with hydrogen peroxide, phytochemicals, and DNA damaging agents showed differential expression of ATG genes and SASP markers. In the H2-DCFDA ROS assay, the intensity of free radicals produced upon oxidative stress show elevated ROS levels, and cells treated with phytochemicals (naringenin, embelin) display reduced fluorescence intensity. Further research will focus on protein expression and mechanism of action.

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