TOR de France

4st edition

CONFERENCE PROGRAM

9-10 October 2025

Saint Paul Hotel, Nice, France

Welcome

Dear colleagues,

It is already the fourth time that we welcome you to St. Paul Hotel in Nice to participate in the "TOR de France" meeting, the only conference in the world entirely dedicated to the TOR signaling pathway.

This 2025 edition is truly special, as we celebrate two historic milestones: the 50th anniversary of the discovery of rapamycin, a remarkable drug that gave rise to an entire field of research many of us are so passionate about, and the 60th anniversary of the Medical Expedition to Easter Island (METEI), during which soil samples containing the bacterium that produced rapamycin were first collected.

Over the past half-century, TOR research has expanded tremendously, now encompassing virtually all major aspects of cellular function and development. For this edition, we are delighted to bring together the pioneers who made the first groundbreaking discoveries on TOR composition and mechanisms of action, alongside young researchers just beginning their scientific journey. This year, we are especially pleased to welcome PhD students and professors from the newly created European doctoral network MENTOR, established to train experts at the crossroads of metabolism, chemistry, and mTOR signaling. Together, we will explore the latest discoveries on molecular mechanisms of mTOR regulation, its roles in physiology and disease, and innovative strategies for developing mTOR inhibitors.

Fifty years after its discovery, rapamycin continues to inspire and now symbolically passes the torch to a new generation of scientists dedicated to advancing the mTOR field.

- Stéphanie Baulac, Svetlana Dokudovskaya, Mario Pende and Adrien Schramm

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Location
AND PRACTICAL INFORMATION

ADDRESS OF THE CONFERENCE VENUE

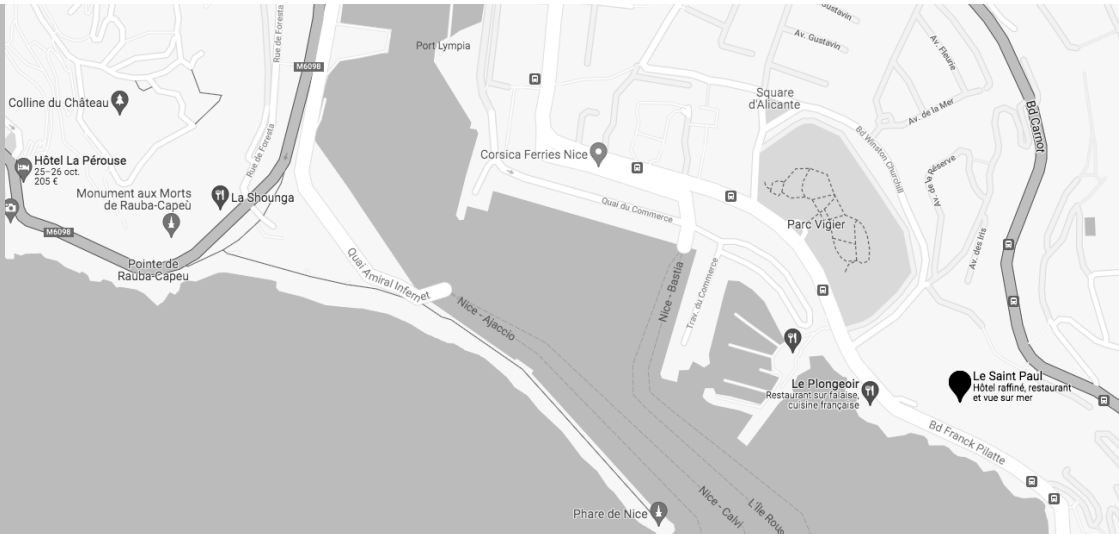
Le Saint Paul Hotel
29 boulevard Franck Pilatte
06300 Nice - France

HOW TO GET TO LE SAINT PAUL HOTEL

> By car:
A8. Exit 50 Promenade des Anglais – towards the port
A8. Exit 55 Nice Est – towards the port

> From the train station:
Bus n° 30 (Direction Riquier). Stop *La Réserve*.

> From the airport:
Tramway to the terminus (Port), then 10min walk till the hotel



REGISTRATION

The registration desk will be located at the entrance to the conference room, in the hotel lobby. **It will open on October 9th at 10:00.**

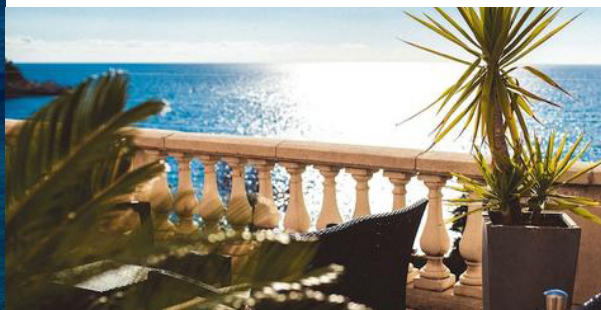
INTERNET ACCESS

> Wi-Fi network:
LE SAINT PAUL network
> Password:
riviera

CATERING

> Thursday, October 9th:
Lunch, a coffee break and a cocktail after the poster session will be provided.

> Friday, October 10th:
A lunch and coffee break will be provided at the hotel. The gala dinner will be held at the Le Boccaccio restaurant (7 Rue Massena, 06000 Nice).



Schedule

THURSDAY, 9 OCTOBER 2025

09:30 - 10:30	**Arrival and registration**
10:30 - 10:45	**Opening and Welcome** Members of organizing committee
50 YEARS OF RAPAMYCIN DISCOVERY	
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mTOR AND METABOLISM	
11:00 - 11:30	Michael Hall
11:30 - 12:00	Wilhelm Palm
12:00 - 12:15	Fried Zwartkruis
12:15 - 12:30	Nesli Ece Sen Arsovic
12:30 - 13:00	Nicolas Dupont
Lunch	
MOLECULAR MECHANISMS OF mTOR REGULATION	
14:15 - 15:00	Constantinos Demetriades • Keynote Lecture
15:00 - 15:15	Riko Hatakeyama
15:15 - 15:30	Wim Annaert
15:30 - 15:45	Stephanie Fernandes
15:45 - 16:15	Bernadette Carroll
Coffee break	
16:45 - 17:15	David Sabatini
17:15 - 17:45	Matthias Gehringer
17:45 - 18:20	**Flash Talks students** • Shiwani Kumari, Alessia Perciavalle, Vonda Koka, Jiyoung Pan, Samuel Laurent, Giorgia Piccoli, Ann-Sofie De Meulemeester
18:20 - 19:30	**Poster Session**
19:30 - 21:30	**Cocktail**

FRIDAY, 9 OCTOBER 2025

NEUROBIOLOGY AND BRAIN DISORDERS	
09:30 - 10:15	Clemence Blouet • Keynote Lecture
10:15 - 10:45	Gaia Novarino
10:45 - 11:00	Mike Fainzilber
Coffee break	
11:30 - 12:15	Mauro Costa-Mattioli • Keynote Lecture
12:15 - 12:30	Paul Dutchak
12:30 - 12:45	Joseph Bateman
12:45 - 13:00	**Flash talks PI** • Dorota Ciołczyk-Wierzbicka, Julian Martinez-Agosto, Jacek Jaworski, Andrea Oeckinghaus
Lunch	
TUBEROUS SCLEROSIS COMPLEX	
14:15 - 15:00	Lisa Henske • Keynote Lecture
15:00 - 15:30	Anna Jansen
15:30 - 15:45	Floor Jansen
15:45 - 16:00	Justyna Zmorzynska
16:00 - 16:15	Salvatore Gagliotta
Coffee break	
GROWTH & DISEASE	
16:45 - 17:00	Mojgan Djavaheri-Mergny
17:00 - 17:15	Viktor Korolchuk
17:15 - 17:30	Matthias Wymann
17:30 - 17:45	Alejo Efeyan
17:45 - 18:15	Andrei Chagin
18:15 - 18:30	**Awards and closing**
19:30	**Gala dinner in Nice center**

Keynote SPEAKERS

CONSTANTINOS (COSTAS) DEMETRIADES

is a molecular cell biologist, currently an independent research Group Leader at the MPI-AGE, and associated PI at the CECAD Research Institute in Cologne. The work from his group investigates the molecular mechanisms of nutrient sensing, metabolic regulation, and cell growth control, focusing primarily on the cell biological processes and signaling networks that regulate—and are regulated by—mTOR.human disease.

Previously, following the completion of his PhD studies with George Mosialos in Greece, he joined the group of Aurelio Teleman at the German Cancer Research Center (DKFZ Heidelberg) as a postdoctoral researcher in 2010. He has received grants from the ERC (StG & PoC), the DFG, the Minna-James-Heineman Foundation, FEBS, the Alexander Onassis Foundation, and the Alexander Fleming Institute, won the 2019 Walther Flemming Award from the German Society for Cell Biology, and served as a speaker at the World Economic Forum Annual Meeting 2019 in Davos and the 2019 Berlin Science Week.

ELIZABETH (LISA) PETRI HENSKE

is the Director of the Center for LAM Research and Clinical Care at Brigham and Women's Hospital, Professor of Medicine at Harvard Medical School, an Associate Member of the Broad Institute of MIT and Harvard, and a practicing medical oncologist at the Dana-Farber Cancer Institute.

LAM (lymphangioleiomyomatosis) is a destructive lung disease of women leading to lung collapse and respiratory failure, often with rapid progression during pregnancy. Dr. Henske's laboratory made the pivotal discovery that LAM is caused by mutations in the tuberous sclerosis complex 2 (TSC2) gene, leading to clinical trials demonstrating efficacy of mTORC1 inhibitors. Her research laboratory is focused on the cellular, metabolic, and immunologic mechanisms underlying the pathogenesis of tuberous sclerosis complex (TSC) and LAM, supported by multiple grants from the National Institutes of Health. She is a member of the American Society for Clinical Investigation and the Association of American Physicians and has received awards for her research from the Tuberous Sclerosis Alliance, The LAM Foundation, the American Thoracic Society, and the Society for Women's Health Research (the Medtronic Prize). EHenske@BWH.Harvard.edu.

MAURO COSTA-MATTIOLI

is a Professor at Baylor College of Medicine and a Principal Investigator at the Altos Labs Bay Area Institute of Science. He was Full Professor and Cullen Foundation Endowed Chair in Neuroscience & the Director of the Memory & Brain Research Center at BCM.

He did his bachelor's degree in the University of the Republic (Uruguay), his PhD at the University of Nantes (France) and his post-doctoral fellowship at McGill University (Canada).

Mauro has elucidated central mechanisms underlying neurological dysfunction. Specifically, he discovered that the protein homeostasis network dubbed the integrated stress response (ISR) is a universal regulator of long- term memory formation, and its activation the main causative mechanism underlying cognitive dysfunction in a wide range of memory disorders. More recently, Mauro discovered how specific microbes in the gut modulate brain function and complex behaviors in both animal models and humans. He has received multiple awards, including the International Eppendorf & Science Prize in Neurobiology, the Searle Scholar Award, the International Society for Neurochemistry Young Investigator Award, the Michael E. DeBakey Excellence in Research Award, and the UCSF Presidential Award. Mauro also serves in several editorial boards, including the editorial board of the journal Neuron and the board of Life Science of the National Academies of Science, Engineering, and Medicine.

CLEMENCE BLOUET

is a Programme Leader at the MRC Metabolic Disease Unit, embedded in the Institute of Metabolic Science at the University of Cambridge.

She joined the Institute of Metabolic Science in 2013 after completing a postdoctoral training in the laboratory of Professor Gary Schwartz at the Albert Einstein College of Medicine, where she developed a specific interest in brain amino acid sensing. During her postdoc, Clemence was supported by consecutive postdoctoral fellowships from the France NIH, the American Heart Association, and a NIH-NIDDK K99 award. She received several postdoctoral prizes and published 7 first-author papers in world-leading journals including Cell Metabolism. Clemence's postdoctoral work produced some of the seminal findings still defining her research today, by demonstrating that amino acid sensing in brain appetite-regulating centres contributes to energy and glucose homeostasis via mTOR signalling. Additional interests in Clemence's lab include the mechanisms of action of anti-obesity drugs, and the role of oligodendrocyte plasticity in neuroendocrine functions.

Talks ABSTRACTS

mTOR AND METABOLISM

mTOR SIGNALING IN GROWTH, METABOLISM AND DISEASE

• **Michael N. Hall** | *Biozentrum, University of Basel, Switzerland*

TOR (target of rapamycin) is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, and cellular energy. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. TOR is found in two structurally and functionally distinct multiprotein complexes termed TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth. As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.

While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in disease and in coordinating and integrating overall body growth and metabolism in multicellular organisms. This will require elucidating the role of TOR signaling in individual tissues. Data on the role of mammalian TORC1 (mTORC1) and mTORC2 in controlling cellular processes and in specific tissues will be presented.

mTORC1 CONTROLS ENDO-LYSOSOMAL NUTRIENT ACQUISITION

• **Wilhelm Palm** | *German Cancer Research Center (DKFZ)*

Mammalian cells can acquire exogenous amino acids either in their free form or through macropinocytosis and lysosomal catabolism of extracellular proteins. We discovered that lysosomal degradation of extracellular proteins can generate sufficient amino acids to induce activation of mTORC1. In turn, mTORC1 suppresses lysosomal degradation of ingested proteins. Mechanistically, mTORC1 blocks lysosomal protein catabolism by suppressing V-ATPase-mediated acidification of lysosomes. Upon nutrient starvation, the ensuing inactivation of mTORC1 triggers rapid V-ATPase assembly into active proton pumps. The resulting drop in luminal pH increases protease activity, initiating degradation of lysosomal protein contents. As a surprising corollary, by promoting endo-lysosomal nutrient generation, mTORC1 inhibition enhances cell proliferation under nutrient-poor conditions in vitro and within vascularly compromised tumors in vivo. Conversely, constitutively active mTOR mutations restrict access to the nutrient stores of extracellular proteins and are thus not tolerated in most cancers. Together, these results define a dynamic crosstalk between mTORC1 and the endo-lysosomal system, enabling cells to adapt metabolism and growth to changing nutrient environments.

mTORC1 CONTROLS AMINO ACID HOMEOSTASIS VIA GLUTAMINE METABOLISM.

• **Fried Zwartkruis** | *Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, The Netherlands*

Uncontrolled mTORC1 activity is causative to devastating disorders like tuberous sclerosis (TSC) and focal cortical dysplasia (FCD). Enhanced glutaminolysis, driven by hyperactive mTORC1, may contribute to clinical manifestations of these diseases. Here we demonstrate that unrestricted mTORC1 activity lowers the concentration of a wide spectrum of intra-cellular amino acids in A549 cells, U373 cells and non-transformed neural progenitor cells mutant for TSC2. In A549 cells, expression of a hyper-active GLSS482C variant recapitulates the changes in intra-cellular amino acids. Conversely, lowering glutamine consumption with the GLS-inhibitor CB-839 or promoting pyruvate entry into the tricarboxylic (TCA) cycle with dichloroacetic acid (DCA), increases intra-cellular amino acids. These findings are in good agreement with predictions from a recently developed computational model of amino acid homeostasis in A549 cells that explains how amino acid transporters collectively control amino acid levels. Interestingly, cells with constitutively increased mTORC1 activity compensate for lower cytosolic amino acids by elevating compatible osmolytes in a cell-type specific manner. Our data demonstrate how hyper-active mTORC1 as present in TSC, shifts the homeostatic outcome of the amino acid transporter network to lower the amount of many amino acids via elevated glutamine consumption.

TORC2 IS ACUTELY REGULATED BY GLUCOSE AVAILABILITY

• **Nesli Ece Sen Arsovic** | *Lewith Lab, University of Geneva, Switzerland*

Target Of Rapamycin (TOR) protein complexes, TORC1 and TORC2, promote cellular growth under permissive conditions integrating multiple intra- and extra-cellular cues such as nutrient availability, cell volume/surface ratio and temperature. While the glucose-dependent stimulation of (m) TORC1 is well-established in yeast and mammalian cells, TORC2 is thought to be regulated only by plasma membrane tension. Our work here reveals for the first time that TORC2 is also subject to acute stimulation by glucose. Using classical yeast genetics and chemical biology tools, we identified mannose-6-phosphate as the sentinel signaling metabolite, and demonstrated the crosstalk between metabolic and PM-related input to TORC2. We are currently employing a multi-tiered proteomics approach that includes proximity biotinylation, phospho-proteomics and limited proteolysis mass spectroscopy to pinpoint the sensor protein. Following the dataset integration and functional validations, we will have identified a new pathway in TOR signaling, laying the groundwork for deeper investigations into the metabolic regulation of TORC2.

ROLE OF mTOR AND AUTOPHAGY IN MECHANOTRANSDUCTION TO CONTROL RENAL CELL HOMEOSTASIS

• **Nicolas Dupont** | *Université Paris Cité, INSERM UMR-S1151, CNRS UMR-S8253, Institut Necker Enfants Malades, F-75015 Paris, France*

Physical constraints, such as compression, shear stress, stretching and tension, play major roles during development, tissue homeostasis and pathologies. Cells and organelles face mechanical forces during many cellular processes, and investigations into how mechanical forces are translated into a wide panel of biological responses, including changes in cell morphology, membrane transport, metabolism, energy production and gene expression, is a flourishing field.

In this talk, I will describe the role of mTOR and autophagy in mechanotransduction to control renal cell homeostasis. Considering the modulation of mechanical forces during the progression of many diseases (including chronic renal disease), I will also discuss to what extent the mTOR/autophagy interplay controlled by physical forces could be instrumental in the development of many pathologies.

MOLECULAR MECHANISMS

SPATIAL SEPARATION OF NUTRIENT SIGNALING TO mTORC1

• **Constantinos Demetriades** | *Max Planck Institute for Biology of Ageing, Cologne, Germany*

Nutrients are the building blocks for cells to grow and proliferate, hence nutrient sensing mechanisms ensure that cells are metabolically active only when all necessary elements are available and all conditions are optimal. Importantly, how information about intra- and extracellular nutrient levels, stresses, and the overall metabolic state of a cell is integrated to form a coordinated response remains enigmatic. Our work aims to elucidate: i) how cells sense the availability of nutrients and the presence of stresses in their environment to adjust their growth, metabolism, and other functions accordingly, ii) how the dysregulation of these cellular mechanisms contributes to the development of human diseases and to ageing, and iii) how we can intervene pharmacologically to efficiently and specifically target such life-threatening conditions. Due to its role as a primary hub in metabolic and nutrient signaling, and a key coordinator of virtually all cellular functions, most of our projects center around the master cellular nutrient sensor, the mTOR kinase. In this talk, I will present highlights of our published and unpublished work on the intricate molecular and cellular mechanisms that reciprocally connect cell growth, metabolism, protein recycling and secretion; and will discuss how this machinery responds to starvation and stress in health and disease.

SPATIALLY AND FUNCTIONALLY DISTINCT TORC1 POOLS IN YEAST AND BEYOND: CONCEPT, APPROACH, AND IMPLICATIONS.

• **Riko Hatakeyama** | *University of Aberdeen, UK*

An emerging paradigm in TORC1 signaling is its spatial compartmentalization. Distinct subcellular pools of TORC1 localize to distinct organelles, where they regulate specific biological processes by locally phosphorylating nearby proteins. This "spatially and functionally distinct TORC1 pools" model explains why TORC1 can orchestrate diverse cellular processes occurring at remote subcellular locations. Dissecting local TORC1 signaling branches would enable us to design novel, more targeted therapeutic approaches against human diseases by selectively manipulating a desired subset of TORC1 functions.

In this presentation, I will introduce our experimental approach to separately measure and manipulate lysosomal and endosomal TORC1 activities in the budding yeast model. I will share our recent advances regarding the formation, function, and regulation of the yeast endosomal TORC1 pool, as well as preliminary evidence of its conservation in mammalian cells. I hope our research inspires wider efforts to dissect TORC1 pools in diverse organelles and organisms, leading to a deeper understanding of cell growth regulation.

Key references: Hatakeyama et al. (2019) Mol. Cell. 73:325-338; Muneshige and Hatakeyama (2025) J. Cell Biol. 224 (5): e202407021.

VPS13C INTERACTION WITH GATOR2 IS REQUIRED FOR mTORC1 ACTIVATION IN PLASMA MEMBRANE-ENDOPLASMIC RETICULUM CONTACT SITES

• **Wim Annaert** | *Lab for Membrane Trafficking, VIB-Center for Brain and Disease Research & KU Leuven, Leuven, Belgium*

Mutations in VPS13C have been linked to Lewy Body diseases, including Parkinson's disease and Dementia with Lewy Bodies. VPS13C functions as a lipid transfer protein in contacts between late endosomes/lysosomes and endoplasmic reticulum as well as lipid droplets. In this study, we identify the endogenous VPS13C interactome and reveal an association with GATOR2, a key complex regulating mTORC1 activation. This interaction occurs not on lysosomes but at the cell surface, where VPS13C is recruited to de novo formed plasma membrane-ER contact sites, particularly in response to nutrient availability. In the absence of VPS13C, mTORC1 is still recruited to membrane ruffles but fails to become activated due to an impaired GATOR2 complex recruitment. Further, VPS13C functionally cross-talks with the small GTPase, ARF5, required for the upstream recruitment of mTORC1 to the plasma membrane. Mutations in VPS13C recapitulate these defects underscoring a loss-of-function in Dementia with Lewy Bodies. Pharmacological activation of mTORC1 signaling rescued mitochondrial dysfunction in VPS13C deficient and mutant cells, highlighting a role for non-lysosomal mTORC1 in downstream mitochondrial fitness.

Defects in lysosome positioning, mTORC1 activation and mitochondrial function could be recapitulated in VPS13C deficient iPSC-derived human induced dopaminergic neurons, placing VPS13C central in pathways shown to be vulnerable in Lewy Body diseases.

THE TUMOR SUPPRESSOR CYLD ACTS AS A DEUBIQUITINASE FOR mTOR TO CONSTRAIN ITS ACTIVITY

• **Stephanie Fernandes** | *Max Planck Institute for Biology of Ageing, Cologne, Germany*

Proper control of mTOR (mechanistic Target of Rapamycin) signaling is relevant for health, disease and ageing. Information from intra- and extra-cellular cues is transmitted to mTOR through an intricate signaling network that regulates its localization and activity. Interestingly, although mTOR is a heavily ubiquitinated protein, the role of this post-translational modification (PTM) in regulating its activation status remains poorly understood. Here, through an unbiased RNAi screen, we identified the tumor suppressor CYLD deubiquitinase (DUB) as a direct negative regulator of both mTORC1 and mTORC2 activities. Mechanistically, CYLD interacts with mTOR and removes non-degradative, K63-linked ubiquitin (Ub) chains from multiple of its residues. Consequently, CYLD loss-of-function cells are characterized by mTORC1/2 hyperactivation, elevated rates of protein synthesis, increased cell size, and resistance to serum-starvation-induced activation of cell death pathways. Moreover, silencing of cyld-1, the C. elegans CYLD ortholog, fully reverses the extended lifespan of low-TORC1-activity mutant worms. Finally, we find that inactivation of CYLD is associated with hyperactivation of mTORC1 also in skin biopsies from CYLD cutaneous syndrome (CCS) patients. In sum, our findings highlight CYLD as a sentinel of mTOR hyperactivation via direct control of its ubiquitination, and suggest that dysregulated mTOR activity may contribute to the development and progression of CCS tumors.

mTORC1 SIGNALLING IN CELLULAR SENESCENCE AND AGEING

• **Bernadette Carroll** | *University of Bristol, UK*

Cellular senescence is a well-established driver of tissue and organismal ageing. Senescence is defined as an irreversible exit from the cell cycle and occurs in response to potentially transformative cell stress such as oncogene activation and DNA damage. Despite not being able to divide, senescent cells are very metabolically active and are characterised by the paradoxical constitutive activation of mTORC1 signalling and upregulation of the autophagy-lysosome pathway. We are working to explore the mechanistic drivers and functional consequences of this metabolic rewiring in senescence, and more broadly in cellular and organismal ageing. I will focus here on our recent work understanding senescence-associated changes in membrane trafficking pathways and lysosomal health. I will also explore our ideas on whether targeting mTORC1/autophagy has senolytic (senescence killing) potential.

NUTRIENT SENSING BY THE mTORC1 PATHWAY

• **David Sabatini** | *IOCB Prague/IOCB Boston*

We have had a long standing interest in the signaling pathways that sense nutrients and how these pathways control the impact of diet on health and disease in animals. I will discuss recent work on nutrient sensing by the Rag GTPase pathway upstream of the mTOR pathway, particularly its mTORC1 branch. We have uncovered several nutrient sensors for this pathway, such as the Sestrin, CASTOR and SAMTOR proteins, which sense leucine, arginine, and methionine, respectively, and their regulation of the Rag GTPases through their interactions with the large GATOR complexes, GATOR1 and GATOR2. I will discuss new data on the structure of the 1.1 MDa GATOR2 in complex with the sensors and how amino acids regulate these interactions. In addition, I will present work on how Sestrin controls the response of animals, in both mice and flies, to diets low in leucine, which we believe serves as a surrogate for the protein content of a diet. In looking at the expression of Sestrin in the mouse liver we made the unexpected observation that its expression is zonated throughout the liver lobules so that dietary leucine impacts only some hepatocytes but not others and I will discuss the implications of such regulation.

DISCOVERY OF HIGHLY POTENT AND SELECTIVE S6K2 INHIBITORS

• **Matthias Gehringer** | *Department of Medicinal Chemistry, Faculty of Medicine, University of Tübingen*

S6K2 is an understudied p70S6 kinase isoform and a downstream effector of mTOR. While S6K1 has long been considered the dominant isoform, emerging evidence highlights distinct roles for S6K2 in disease biology. Despite this, highly isoform-selective chemical probes remain lacking, limiting detailed pharmacological investigation and validation of S6K2 as a therapeutic target.
Our group has developed the first covalent S6K2 inhibitors, which achieve selectivity over S6K1 by exploiting a cysteine residue unique to S6K2. Through their covalent mode-of-action, these compounds display exquisite potency while maintaining excellent kinome-wide selectivity. In my talk, I will present the discovery and optimization of these inhibitors and discuss their property profiles.

FLASH TALKS

DIVERGENT SYNAPTIC AND TRANSCRIPTOMIC EFFECTS OF BRG1 AND TSC2 LOSS UPON mTOR HYPERACTIVATION

• **Shiwani Kumari** | *International Institute of Molecular and Cell Biology in Warsaw, Poland*

Brg1 is an ATP-dependent catalytic subunit of the BAF chromatin remodeling complex. Through mass spectrometry analysis, we identified Brg1 as one of the nuclear interactors of the mTOR. Dysregulation of mTOR has been implicated in mTORopathies, including TSC and epilepsy. This study explores the nuclear mTOR-Brg1 interaction and its implications for neuronal development and disease. Using in vitro cultured rat neurons, our data confirmed an increased nuclear mTOR-Brg1 interaction following kainic acid (KA) treatment, highlighting mTOR-induced Brg1 phosphorylation. We observed that modulation of mTOR and the proteasome influenced the Brg1 nuclear presence, suggesting proteasome-mediated degradation of Brg1 in the nucleus upon KA treatment. Consistent with these findings, the downregulation of Brg1 expression was noted upon TSC2 loss, resulting in mTOR hyperactivation in neurons. Ca^{2+} imaging and network analysis revealed strong similarities between neurons lacking TSC2 and those deficient in Brg1. However, further investigation demonstrated that their synaptic parameters differed, and the RNA-seq analysis revealed involvement of different gene programs. These observations suggest that although network activity is increased upon TSC2 and Brg1 loss, Brg1 and TSC2 largely regulate distinct transcriptional programs. Collectively, these findings provide new insights into the nuclear functions of mTOR in neurons, particularly in regulating Brg1 stability and activity.

cfDNA LIQUID BIOPSY REVEALS LOW-LEVEL MOSAIC mTOR SOMATIC MUTATIONS IN CEREBROSPINAL FLUID FROM PATIENTS WITH FOCAL CORTICAL DYSPLASIAS

• **Alessia Perciavalle** | *Department of Medical and Surgical Sciences (DIMEC), University of Bologna*

Purpose: Focal Cortical Dysplasias (FCDs) are a leading cause of Drug-resistant Focal Epilepsies (DRFE) frequently associated with brain-only somatic mutations (BSMs) in mTOR pathway genes. These low-VAF (<1–5%) mutations are restricted to dysplastic area and affect few cells, making diagnosis reliant on resected brain tissue and limiting non-invasive or pre-surgical assessment. Cell-free DNA (cfDNA) from cerebrospinal fluid (CSF) is a promising liquid biopsy approach, but has so far failed to detect these low-VAF mutations in DRFE patients.

Method: We collected brain, blood, and intrathecal CSF from 93 DRFE patients. Paired blood-brain ultra-deep sequencing identified BSMs in 22/93 (23.7%). We set a cfDNA cut-off ≥0.5 ng/µL and designed a solid digital PCR assay targeting the recurrent MTOR S2215Y mutation. Seven CSFs met the cut-off: 2/4 (50%) from S2215Y-positive, 4/10 (40%) from S2215Y-negative FCD II patients, and 1/2 (50%) from genetically untested cases.

Results: We detected BSMs at brain-only VAFs of 2.03% and 2.48% in cfDNA of 2/2 CSFs tested (100%). No BSMs were found in 4 CSFs from BSM-negative brains, showing high specificity. S2215Y mutation was also detected in the genetically untested patient, matching FCD II diagnosis.

Conclusions: Despite low CSF volumes (500 µL–1.5 mL), we successfully detected brain-only MTOR mutations in cfDNA. Our findings support liquid biopsy as a non-invasive genetic test for low-VAF BSMs.

A PHOSPHOPROTEOMIC APPROACH IDENTIFIES ER-LYSOSOMAL TARGETS OF THE mTOR/S6 KINASE PATHWAY

• **Vonda Koka** | *Université Paris Cité, INSERM UMR-S1151, CNRS UMR-S8253, Institut Necker Enfants Malades, Paris, France*

mTOR (mammalian Target of Rapamycin) is a serine/threonine kinase activated by nutrients and is a master regulator of cell growth, proliferation, senescence and metabolism. One of the major substrates of mTOR is S6 kinase (S6K). S6K proteins are encoded by two different genes RPS6KB1 (S6K1) and RPS6KB2 (S6K2). S6K1 and S6K2 have more than 80% of sequence homology. S6K phosphorylates proteins involved in protein and nucleotide synthesis, RNA splicing, insulin sensitivity and metabolism. The objective of our study is to identify novel selective substrates of S6K1 and S6K2. For this purpose, we deleted S6K1 and/or S6K2 by genome editing using CRISPR/Cas9 technology in MCF7 cells and IMCD3 cells. In collaboration with Cell Signaling Technology, we performed a phosphoproteomic analysis in these mutant cells. Our phosphoproteome reveals PI-4K2A as a selective S6K1 target. We confirmed a direct phosphorylation of PI4K2A by S6K1 at the serine 462.

We show that S6K1 plays a role in PI4K2A distribution and in PI4P production. mTORC1/S6K1 leads to the repression of lysosomal PI(4)P synthesis through the inhibitory phosphorylation of PI4K2A (1). In addition, we identified several putative candidates involved in ER-lysosome trafficking and lipid transfer proteins. I will present the ongoing strategies for the functional validation of these new S6K substrates.

1:Ebner M, et al. Nutrient-regulated control of lysosome function by signaling lipid conversion. Cell. 2023.Sep.27

ATM-DEPENDENT RHEB PHOSPHORYLATION COUPLES DNA DAMAGE TO LYSOSOMAL mTORC1 SIGNALING TO ORCHESTRATE THE CELLULAR RESPONSE TO GENOTOXIC STRESS

• **Jiyoung Pan** | *Max Planck Institute for Biology of Ageing (MPI-AGE), 50931 Cologne, Germany*

Cells dynamically adapt to environmental stressors by rewiring signaling networks that coordinate growth, metabolism and genome maintenance. The DNA damage response (DDR) and mTORC1 signaling pathways govern DNA repair and cell growth, respectively, but how these pathways intersect remains incompletely understood. Here, we identify RHEB, the most direct mTORC1 activator, as a substrate of the DDR kinase ATM. Strikingly, we find that, although genotoxic stress differentially regulates mTORC1 activity—reducing the phosphorylation of its lysosomal target TFEB, while enhancing phosphorylation of its cytoplasmic target S6K—the DDR-induced phosphorylation of RHEB specifically controls the lysosomal mTORC1 signaling branch. Preventing RHEB phosphorylation impairs TFEB nuclear translocation and lysosome biogenesis upon DNA damage. Functionally, the RHEB phosphorylation-dependent TFEB response is required for proliferative recovery following genotoxic stress. These findings uncover an ATM-RHEB-mTORC1-TFEB signaling axis that links DNA damage to selective mTORC1 outputs, revealing a mechanism which enables cells to adapt to genotoxic cues.

FROM THE TOR PATHWAY TO V-ATPASE REGULATION IN ARABIDOPSIS THALIANA

• **Samuel Laurent** | *IJPB INRAE Versailles France*

V-ATPases are highly conserved complexes acting as proton pumps responsible for the acidification of the endomembrane system in eukaryotes. Their activity can be regulated by reversible dissociation and reassociation. While this mechanism, and the role of TOR complexes in its regulation, has been investigated in yeast and animals, very few studies have focused on the link between V-ATPases regulation and TOR in plants.

In the model plant Arabidopsis, mutant screens have led to the discovery of many actors of the plant TOR pathway. During such a screen, we identified a mutant displaying a peculiar phenotype of ectopic cell clusters developing in the presence of the TOR inhibitor AZD-8055. We located the mutation responsible for this phenotype in a gene encoding a yet undescribed protein. We named this protein LOKI (Localized growth depending on TOR Kinase Inhibition) and identified in its sequence a conserved Rav1 domain, which is known to be central to V-ATPase reassembly.

We found that loki mutants phenocopy a known V-ATPase subunit mutant and that loki mutations lead to increased TGN pH and disturbed intracellular trafficking, which are signs of decreased endosomal V-ATPase activity.

This supports our hypothesis that LOKI could link V-ATPase regulation to the TOR pathway in Arabidopsis. Interestingly, we did not observe any changes in the vacuolar pH of loki mutants, suggesting a compartment-specific function for the LOKI protein.

NEURAL STIMULATION SUPPRESSES mTORC1- MEDIATED PROTEIN SYNTHESIS IN SKELETAL MUSCLE

• **Giorgia Piccoli** | *Vimm institute/ Dipartimento di scienze biomediche of University of di Padova, Padova, Italy*

Skeletal muscle fibers are classified as glycolytic or oxidative, with differing susceptibilities to muscle wasting. However, the intracellular signaling pathways regulating fiber- specific muscle trophism remain unclear because of a lack of experimental models measuring protein synthesis. We developed a mouse model overexpressing a mutated transfer RNA synthetase in muscle fibers, enabling specific protein labeling using an artificial methionine substitute, which can be revealed through click chemistry. This model revealed that denervation increases protein labeling in oxidative muscle fibers through mammalian target of rapamycin complex 1 (mTORC1) activation, while deleting the mTORC1 scaffold protein Raptor reduces labeling in glycolytic fibers. On the other hand, increased
muscle activity acutely decreases protein synthesis, accompanied by reduced mTORC1 signaling, glycogen depletion, and adenosine 5′-monophosphate kinase activation. Our findings identify nerve activity as an inhibitory signal for mTORC1-dependent protein synthesis in skeletal muscle, enhancing the understanding of fiber-specific responses to exercise and pathological conditions.

MODELING FCDII USING INDUCIBLE mTOR CORTICAL ORGANOIDS

• **Ann-Sofie De Meulemeester** | *Institut du Cerveau-Paris Brain Institute-ICM, Team MOSAIC, Sorbonne Université, Inserm, CNRS, Hôpital de la Pitié Salpêtrière, 75013 Paris, France*

Focal cortical dysplasia type II (FCDII) is the most common lesion in children undergoing surgery for drug-resistant epilepsy. FCDII pathogenesis involves mTOR pathway hyperactivation driven by low-frequency somatic mutations that arise during cortical development, creating characteristic mosaic patterns of mutant and wild-type cells. While existing rodent and organoid models demonstrate mTOR pathway effects, they fail to recapitulate the developmental acquisition and mosaic distribution of somatic mutations observed in patients. Somatic mTOR variants account for most FCDII cases, yet no somatic mTOR organoid model has been reported.

We aim to generate an inducible mTOR organoid model that recapitulates somatic mutation acquisition during cortical development. To this end, we will adapt the Brainbow (loxP-Cre) system to link with the pathogenic mTOR S2215F variant. Using PiggyBac, we will co-integrate this construct with an ERT2-Cre plasmid, marked by nuclear BFP. Upon tamoxifen treatment, Cre activation will induce mosaic expression: mTOR-mutant cells (GFP+) or wild-type cells (mScarlet+). Functionality will first be validated in HEK293 and hiPSC lines, then applied to cortical organoids. This system uniquely enables live tracking of mutant versus non-mutant cells, allowing direct analysis of fate, morphology, clonal dynamics, and the emergence of FCDII-specific features such as dysmorphic neurons and balloon cells.

NEUROBIOLOGY AND BRAIN DISORDERS

DIETARY PROTEIN AND APPETITE CONTROL: IS IT ALL ABOUT mTOR ?

• **Clemence Blouet** | *Institute of Metabolic Science, University of Cambridge, UK*

Dietary protein robustly modulates appetite and systemic metabolism, exerting a stronger satiating effect than carbohydrates or fat. Within the brain, neuronal signaling pathways detect and integrate protein-derived cues to regulate feeding behavior and energy balance. The mechanistic target of rapamycin (mTOR) has emerged as a key nutrient sensor in this context. mTOR activity in hypothalamic and brainstem neurons is sensitive to dietary protein intake and amino acid availability, and integrates these nutritional signals with hormonal inputs such as leptin and insulin. Through its actions on anorexigenic and orexigenic neuronal populations, mTOR signaling contributes to the control of appetite, metabolic adaptation, and energy homeostasis. However, emerging data now indicate that mTOR-independent pathways also contribute significantly to protein-driven appetite regulation. For example, deficiency of essential amino acids can activate GCN2-mediated stress signaling, while endocrine mediators such as FGF21 promote protein-specific appetite. In addition, recent studies identify the T-type calcium channel Cav3.1 as a novel player in protein sensing. Cav3.1 influences neuronal excitability and burst firing in hypothalamic and brainstem circuits, thereby modulating the translation of nutrient signals into feeding behavior. Understanding these mechanisms will provide new perspectives for therapeutic strategies targeting obesity and metabolic disease.

UNVEILING NON-CANONICAL mTORC1 SIGNALING IN THE BRAIN

• **Gaia Novarino** | *Institute of Science and Technology Austria, Vienna, Austria*

The mTOR signaling pathway is closely implicated in neurological disorders, including epilepsy and autism, yet limited treatment options suggest important gaps in our understanding of how this pathway is regulated in the brain. Focusing on the autism-associated protein Kaptin (KPTN), a component of the KICSTOR complex that restrains mTORC1, we show that loss of KPTN increases S6 phosphorylation through a mechanism that does not rely on S6K1, and is accompanied by behavioral and structural abnormalities reminiscent of KPTN-related syndromes. Electrophysiological and protein analyses point to altered striatal synaptic function as a contributor to these phenotypes. Guided by the developmental dynamics of mTORC1 signaling, we devised a pharmacological approach that ameliorates neurological symptoms in a KPTN-deficient model without engaging S6K1, and in vitro studies implicate protein phosphatase 1 in S6 regulation. Although the precise molecular steps remain to be defined, these findings indicate a non-canonical mode of mTOR regulation in the developing brain and suggest new therapeutic avenues for mTOR-related conditions.

STRETCHING TO GROW - LOCALLY TRANSLATED mTOR CONTROLS INTERSTITIAL AXONAL ELONGATION

• **Mike Fainzilber** | *Weizmann Institute of Science, Rehovot, Israel*

Mechanical stretch can induce significant acceleration of neuronal growth. Although this process is critical for growth in the maturing nervous system, the underlying molecular mechanisms are largely unknown. We established a system for monitoring stretch-induced neuronal growth in culture and observed marked increases in both growth and axonal protein synthesis rates in response to stretch of mouse sensory neurons. These responses were attenuated in mutants of the retrograde motor dynein, the transport factor importin β1 3'UTR and the RNA-binding protein nucleolin – all components of a previously described length-sensing mechanism that regulates elongating neuron growth. Stretch-induced growth acceleration required translation, but not transcription, and was specifically dependent on axonal translation of mTOR (mechanistic Target of Rapamycin), as shown in a mouse line deficient in axonal localization of mTOR mRNA. Thus, axonal length-sensing and mTOR-controlled local axonal translation regulate stretch-induced neuronal growth.

DISSECTING THE ROLE OF mTOR COMPLEXES IN EPILEPSY

• **Mauro Costa-Mattioli** | *Altos Labs, Inc., Bay Area Institute, Redwood City, California, 94065, USA. 1Department of Neuroscience, Baylor College of Medicine, Houston, TX, 77030, USA*

Epilepsy is a chronic neurological disorder marked by spontaneous seizures, contributing to premature mortality, cognitive impairment, and accelerated brain aging. With seizure disorders increasing globally and about one-third of patients resistant to current antiepileptic drugs, new mechanism-based therapies are urgently needed.

Dysregulation of the mechanistic target of rapamycin (mTOR) pathway—via its complexes mTORC1 and mTORC2—plays a key role in epileptogenesis. Hyperactivation of mTORC1 is a hallmark of mTORopathies, genetic disorders often linked to drug-resistant seizures, memory deficits, and brain abnormalities.

In this presentation, I will share our latest findings on how both mTORC1 and mTORC2 drive seizure development and behavioral changes. I will highlight novel insights into how dysregulated mTOR signaling disrupts neural circuits, induces hyperexcitability, and impairs cognition. Finally, I will discuss our efforts to develop targeted therapies that selectively modulate mTOR components to treat drug-resistant epilepsy, enhance neurocognitive outcomes, and support long-term brain health. These advances offer promising pathways for new treatments that may benefit a broad spectrum of neurological conditions.

ASO THERAPEUTICS TARGETING mTORC1-DEPENDENT EPILESPY

• **Paul Dutchak** | *Department of Psychiatry and Neuroscience, University Laval, Quebec, Canada*

Rationale: Pathogenic mutations in GATOR1 (DEPDC5, NPRL2, NPRL3) or TSC1/2 cause persistent mTORC1 activity, leading to abnormal protein expression, altered metabolism, and seizures. As classical mTORC1 inhibitors are short-acting and broadly immunosuppressive, we developed novel antisense oligonucleotides (ASOs) to enable long-term dampening of hyperactive mTORC1 signaling. Leveraging mouse–human sequence conservation, our ASO design supports both rigorous preclinical testing in mice and efficient translation to human mTORC1-driven epilepsies or related disorders. Methods: Conserved RAPTOR regions were identified from transcript alignments. ASOs were screened for potency in MEF and HEK293 cells via qPCR and western blotting. Lead ASOs were tested in GATOR1-deficient cortical neurons using multielectrode array analysis. In vivo efficacy was evaluated in neuronal NPRL2 KO mice using immunohistochemistry, video-EEG, and survival analysis.

Results: ASO treatment reduced RAPTOR mRNA/protein expression, partially restricting mTORC1 without full inhibition and rescuing the electrical differences in primary cultured neurons. Notably, a single ICV dose extended

NPRL2 KO mice survival by >250%, with therapeutic effects lasting >8 weeks—outperforming existing small molecule therapies.

Conclusions: RAPTOR-targeted ASOs offer a potent, long-acting therapeutic alternative for mTORC1-driven GATOR1/TSC epilepsies.

(Provisionally patented @ ULaval)

PHOSPHOPROTEOMICS IDENTIFIES RNA-METABOLISM DEFECTS IN THE BRAIN IN TUBEROUS SCLEROSIS COMPLEX

• **Joseph Bateman** | *King's College London, UK*

Tuberous sclerosis complex (TSC) is a rare disease caused by mutations in the genes TSC1 and TSC2, resulting in activation of mTORC1. Neurological manifestations occur in most TSC patients and include epilepsy, autism and intellectual disability. Two types of brain lesions, cortical tubers and subependymal giant cell astrocytomas (SEGAs), cause the majority of neurological manifestations in TSC. We have limited understanding of the molecular changes that occur in tubers and SEGAs and how these contribute to disease pathogenesis. To understand this we performed proteomic and phosphoproteomic analysis of TSC patient tuber and SEGA tissue. We were unable to detect mTORC1 activation in tubers, likely due to the small number of cells that had lost both copies of TSC2. By contrast, SEGAs showed evidence of strong TORC1 activation and large-scale changes in the proteome and phosphoproteome. At the proteomic level, SEGAs exhibited activation of a neuroinflammatory response. Phosphoproteomics showed that phosphorylation of a multitude of proteins involved in RNA-metabolism, including mRNA splicing, were increased in SEGAs. Consistent with this, we found evidence of large-scale alterations in mRNA transcript splicing the SEGA tissue. Together these data reveal a novel molecular mechanism whereby mTORC1 activation in SEGAs results in mis-regulation of RNA metabolism and mRNA splicing, potentially contributing to the neurological manifestations in TSC.

FLASH TALKS

THE mTOR PATHWAY IN TRANSLATIONAL RESEARCH: FROM MODULATION OF CANCER CELL MORPHOLOGY TO REMODELING OF ISOLATED CARDIAC FIBROBLASTS AFTER LVAD IMPLANTATION

• **Dorota Ciołczyk-Wierzbicka** | *Center for Medical Genomics - OMICRON University, Medical College, Kraków, Poland*

The mTOR pathway plays a central role in regulating proliferation, metabolism, survival, and cellular plasticity. In our earlier studies, we showed that mTOR inhibitors modulate melanoma cell invasiveness, morphology, cytoskeleton, and extracellular matrix organization [Ciołczyk-Wierzbicka et al. 2020, 2023, 2025]. In combination with chloroquine, mTOR inhibition also activated apoptosis and altered lipid membrane organization [Ciołczyk-Wierzbicka et al. 2024], confirming that phenotypic and morphological indicators can serve as sensitive markers of response to targeted therapy. We are now extending this research into translational cardiology. Using primary human cardiac fibroblasts isolated from left ventricular myocardium samples obtained during LVAD implantation, we assess the impact of mTOR pathway modulation on myocardial fibrosis and remodelling. Our results demonstrate that mTOR inhibition induces distinct fibroblast phenotypic changes, including volumetric parameters, cell thickness, and cytoskeletal reorganization. Similar to our cancer studies, morphological alterations in the cardiac model appear to be early and sensitive indicators of disease progression and therapy response. This presentation highlights the continuity of our mTOR research—from oncology to cardiology—and its potential to inspire novel diagnostic and therapeutic strategies for cardiomyopathy and advanced heart failure.

NOVEL INSIGHTS IN mTOR-DRIVEN OVERGROWTH: mTOR ACTIVATION, VARIANT PATHOGENICITY, AND NEUROBEHAVIORAL DEFICITS

• **Julian Martinez-Agosto** | *University of California Los Angeles, Los Angeles, California, USA*

Rare pathogenic variants in mTOR are associated with a spectrum of human phenotypes resulting from somatic mosaicism or germline variants. We explored the spectrum of variant effects on protein function and phenotypic manifestations in patients diagnosed with pathogenic mTOR variants through functional validation and behavioral assessments. In our cohort, novel mTOR missense variants (p.C1390Y, p.K1395R, p.V2406M, p.Q2524K) were identified, with in vitro functional analyses in human embryonic kidney cells confirming hyperactivation of mTOR Complex 1 (mTORC1), while revealing mTORC2 hyperactivation as a novel disease mechanism. Structural analyses demonstrated that pathogenic variants cluster in protein hotspots, particularly within the focal adhesion targeting (FAT) domain, disrupting alpha-helix packing via bulky side-chain insertions. Comprehensive neurobehavioral assessments using standardized measures showed significant impairments in motor coordination, adaptive functioning, social interaction, executive functioning, and behavioral regulation compared to healthy controls, with particularly severe deficits in motor coordination and adaptive functioning. These findings delineate the protein function and neurobehavioral profile of mTOR-driven phenotypes, expand its clinical spectrum, and provide foundations for precision diagnostics, pathogenicity prediction, targeted interventions, and therapeutic development.

NUCLEAR FUNCTIONS OF mTOR IN NEURONS

• **Jacek Jaworski** | *Laboratory of Molecular and Cellular Neurobiolog, International Institute of Molecular and Cell Biology, Warsaw, Poland*

The mechanistic/mammalian target of rapamycin (mTOR) is one of the major metabolic kinases that integrates extracellular instructions with the current metabolic status of the cells. mTOR is regulated by neuronal activity and is essential for neuronal development and plasticity. mTOR acts on many proteins changing their function but occurs mainly in the cytoplasm. However, more research, including our data, indicates that mTOR moves to the cell's nucleus, where it interacts with various proteins involved in epigenetic regulation and RNA metabolism. During the presentation, I will describe conditions needed for the nuclear translocation of mTOR in neurons, its nuclear proteome and the potential contribution of nuclear mTOR activities to neuronal physiology. I will also discuss possibility of nuclear mTOR contribution to neurological symptoms of mTOR-related disorders such as Tuberous Sclerosis Complex.

MECHANISMS COMPROMISING THERAPY OUTCOME OF mTORC1 INHIBITORS IN PANCREATIC NEUROENDOCRINE TUMORS (PANNETS)

• **Andrea Oeckinghaus** | *Lab for Metabolic Signaling, Dept. Metabolism, Senescence and Autophagy, Research Center One Health, Ruhr Alliance and University Hospital Essen, University Duisburg-Essen, Essen, Germany*

PanNETs are a rare tumor entity whose incidence is significantly higher and the time of onset is earlier in mTORopathies than in the general population. PanNETs hyperactivate the metabolic master regulator mTORC1. Its inhibitor Everolimus is standard of care for grade 2 PanNETs and slows tumor progression but is not curative. Grounded on cell cultures, tissues and liquid biopsies from multiple patient cohorts, we identify everolimus-triggered processes that promote therapy resistance and characterize a complex underlying signaling network connecting the TGF-SMAD2/3, PI3K-Akt and MAPK pathways. Our results provide evidence for the relevance of these processes in cancer patients, highlight new targets for combination therapies to be tested in clinical studies and suggest new prognostic markers for disease progression on everolimus and patient stratification.

TUBEROUS SCLEROSIS COMPLEX

NOVEL DISEASE MECHANISMS IN TUBEROUS SCLEROSIS COMPLEX

• **Elizabeth Henske** | *Center for LAM Research and Clinical Care, Brigham and Women's Hospital, Harvard Medical School*

Lymphangioleiomyomatosis (LAM) is a progressive destructive lung disease of women caused by TSC1 or TSC2 mutations Tuberous sclerosis complex (TSC) is a multisystem disease with tumors of brain, heart, and kidney. B7-H3, a PD-L1 homolog, is markedly upregulated in TSC-associated tumors. B7-H3 is upregulated via mTORC1 and is a direct S6K target. Inhibition of B7-H3 has striking efficacy in mouse models of LAM and TSC, by re-activating "exhausted" T cells via IFN gamma-dependent mechanisms (Nat Commun 2023). Single cell RNA sequencing has revealed that M2-like immunosuppressive macrophages expressing TREM2 and MRC1 (mannose receptor 1, also called CD206) are a dominant cell population in TSC tumors (Nat Commun 2022). Inhibition of MRC1-expressing M2-like macrophages decreases disease progression in mouse models of TSC (Eur Resp J 2025). These data highlight the high potential clinical impact of targeting the immunosuppressive microenvironment in TSC and LAM.

In two mouse models of TSC2 (KSP-Cre and CAAG-Cre) genetic knockout of TFEB completely rescues kidney pathology and overall survival (Nat Commun 2024). Unexpectedly, the increased mTORC1 activity in the TSC2 knockout kidneys is normalized by TFEB knockout, even at postnatal day 15 (P15), prior to the development of kidney pathology. These results shift the paradigm of mTORC1 hyperactivation in TSC, suggesting that TFEB is a driver of mTORC1 activity in TSC, likely via increased expression of the RAG-C GTPases.

CLOSING THE CARE GAP: ADVANCING NEUROPSYCHIATRIC RESEARCH IN TUBEROUS SCLEROSIS COMPLEX THROUGH GLOBAL COLLABORATION AND DIGITAL INNOVATION

• **Anna Jansen** | *Division of Pediatric Neurology, Antwerp University Hospital, Belgium*

Tuberous Sclerosis Complex (TSC) is a multisystem genetic disorder with a high prevalence of TSC-Associated Neuropsychiatric Disorders (TAND)—a constellation of behavioral, psychiatric, intellectual, academic, neuropsychological and psychosocial challenges. Despite their impact, TAND manifestations remain under-identified and under-treated, contributing to a persistent global care gap.

This presentation outlines advances led by the TAND Consortium, a global, multidisciplinary initiative, which has applied a family-centered, data-driven approach to closing the TAND care gap. Key initiatives include the development and validation of the TAND-SQ Checklist, a self-report tool enabling personalized profiling of TAND symptoms, and the TAND Toolkit App, which integrates evidence-based recommendations and empowers families with actionable strategies. The ongoing TANDem-2 project addresses the following objectives: first, to explore longitudinal TAND severity trajectories using the TAND-SQ Checklist; second, to evaluate the association between caregiver wellbeing characteristics, TAND severity and severity trajectories; and third, to evaluate the feasibility, acceptability and limited efficacy of a brief online group-based caregiver wellbeing intervention for TSC family caregivers.

By integrating lived experience, clinical expertise, and digital innovation, the TANDem framework offers a replicable model for neuropsychiatric research in rare diseases.

CHALLENGES IN IDENTIFYING RELEVANT SUBEPENDYMAL LESIONS IN CHILDREN WITH TUBEROUS SCLEROSIS COMPLEX

• **Floor Jansen** | *University Medical Center Utrecht the Netherlands*

Background: This study aimed to (1)analyse growth patterns of SELs, (2)identify potentially hazardous lesions, and (3)evaluate determinants of abnormally growing SELs.

Methods: This retrospective study included children with definite TSC, 2 cerebral MRIs with 5 years of follow-up. A stepwise approach was used to identify pathologically growing SELs: (1)growth plotted as absolute volume over time, (2)identifying outliers with >2SD percentage of growth, (3)unsupervised clustering of nodule characteristics, and (4)K-means clustering based on percentage of growth.

Results: We included 53 TSC children and 500 SELs. Visual inspection and growth thresholding at 2SD did not reliably identify pathological lesions. Unsupervised cluster analysis showed insufficient sensitivity and specificity. K-means clustering identified 5.8% of SELs as abnormally growing. Overall, 18.2% of SELs met literature criteria for SEGA or led to a change in care; 2% were labelled SEGA based solely on clinical care changes. Location at FoM, gadolinium enhancement, baseline volume, percentage of growth and diameter >1 cm were associated with pathological SELs.

Discussion: Defining abnormal SEL growth is difficult and shows limited agreement with clinically diagnosed SEGAs. Pathological SELs are not solely identified by size and growth, but also by location, enhancement and baseline volume.

RAC1 CONTRIBUTIONS TO TUBEROUS SCLEROSIS COMPLEX-ASSOCIATED NEUROPSYCHIATRIC DISORDERS

• **Justyna Zmorzynska** | *Lab of Developmental Neurobiology, International Institute of Molecular Mechanisms and Machines Polish Academy of Sciences, Warsaw, Poland*

Tuberous Sclerosis Complex (TSC) is a genetic disorder caused by mutations in the TSC1 or TSC2 genes, leading to hyperactivation of the mTORC1 pathway and widespread developmental abnormalities. Individuals with TSC often experience TSC-Associated Neuropsychiatric Disorders (TANDs), a spectrum of cognitive, behavioral, and psychiatric symptoms including anxiety. We identified a critical role of Rac1 GTPase in anxiety regulation. Using zebrafish Tsc2-deficient zebrafish model – which display commissural thinning, abnormal tract fasciculation, and increased anxiety-like behaviors – we demonstrated that hyperactive Rac1 contributes to neural dysconnectivity underlying anxiety. Through transcriptome analysis of the Tsc2-deficient brains, we identified upstream regulators of Rac1. Pharmacological inhibition of Rac1 pathwauy successfully rescued anxiety phenotypes in this model, underlining Rac1's mechanistic involvement in neuropsychiatric symptomatology. These findings position Rac1 as a key molecular regulator of anxiety through its impact on neural circuit development and connectivity, offering promising avenues for targeted therapeutic interventions.

TFEB DRIVES RENAL PATHOLOGY IN TUBEROUS SCLEROSIS COMPLEX HUMAN KIDNEY ORGANOIDS

• **Salvatore Gagliotta** | *Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy*

Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder caused by TSC1/2 mutations that constitutively activate mTORC1, driving tumorigenesis across multiple organs, with kidney lesions a major cause of adult mortality. While TFEB has been implicated in mTORC1-driven cystogenesis in TSC2-deficient mouse models, murine systems poorly recapitulate human pathology. To address this, we generated human kidney organoids from induced pluripotent stem cells to examine TFEB's role in TSC renal disease. Two models were developed: a TSC2 knockout and a mosaic, inducible TSC2 knockdown, mimicking second-hit mutations and loss of heterozygosity. Both models showed increased pS6, angiogenesis, epithelial-to-mesenchymal transition, and nuclear TFEB accumulation with target upregulation. The mosaic model displayed a milder phenotype, suggesting dose dependence and closer resemblance to human disease. To dissect the contributions of TFEB and mTORC1, we induced TFEB overexpression in specific cells of wild-type organoids, which reproduced key features of the TSC2-deficient phenotype. Notably, TFEB-overexpressing organoids induced pathological changes in adjacent TFEB-negative regions, and conditioned media from TSC2 organoids triggered TSC-like alterations in wild-type organoids.

These findings identify TFEB as a central driver of TSC kidney pathology, acting through both cell-autonomous and secreted mechanisms, and highlight its potential as a therapeutic target.

GROWTH & DISEASE

THE ANTIDEPRESSANTS SERTRALINE AND INDATRALINE INDUCE TFEB ACTIVATION AND IMMUNOGENIC CELL DEATH

• **Mojgan Djavaheri-Mergny** | *UMRS1138, team metabolism, cancer , immunity, Paris, France*

Sertraline and indatraline are two antidepressants that function as serotonin reuptake inhibitors and have demonstrated promising anticancer potential, although their precise mechanisms of action remain unclear. Here, we uncover a lysosomal-related mechanism by which these two agents combat cancer. Through cell-based drug screening, we identified sertraline and indatraline as potent inducers of the nuclear translocation of transcription factor EB (TFEB). Activation of TFEB was mediated through the autophagy-independent lipidation of microtubule-associated proteins 1A/1B light chain 3B (LC3). Both sertraline and indatraline promoted cholesterol accumulation within lysosomes, resulting in lysosomal membrane permeabilization (LMP) and disruption of autophagy. In cancer cells, sertraline and indatraline elicited immunogenic cell death (ICD), converting dying cells into prophylactic vaccines that were able to confer protection against tumor growth in mice. In a therapeutic setting, a single dose of each compound was sufficient to significantly reduce the outgrowth of established tumors in a T cell-dependent manner. These results identify sertraline and indatraline as immunostimulatory agents that operate through a novel mechanism that links TFEB activation and lysosomal cholesterol transport to lysosomal membrane permeabilization, ultimately leading to immunogenic cell death.

ROLE OF mTOR IN CELLULAR AGEING PHENOTYPES

• **Viktor Korolchuk** | *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*

Naturally aged cells isolated from donor tissues are widely used a model of human ageing, however the mechanisms underpinning cellular ageing phenotypes remain poorly understood. We set ourselves on the mission to extensively characterise human cellular ageing models via a broad characterisation of cellular pathways with the aim to identify drivers of ageing phenotypes. Importantly, by performing a range of analyses in primary cells in culture we aimed to provide a rich resource for the scientific community interested in studying molecular mechanisms driving ageing. The talk will present analyses of dermal fibroblasts with key findings validated in muscle satellite cells. Clear phenotypic differences between cells from young and aged donors allowed us to apply unbiased transcriptomics, proteomics and metabolomics analyses. These visualised a range of processes and pathways affected by ageing. mTOR signalling was found to be unaffected by ageing, however suppression of mTOR robustly rescued age-related perturbation in proteostasis and metabolism by activating autophagy. Overall, our data can serve as a foundation for further mechanistic studies using primary human cells as a model of human ageing.

NON-REDUNDANT FUNCTIONS OF PI3Kγ COMPLEXES IN OBESITY AND METAFLAMMATION

• **Matthias Wymann** | *Department of Biomedicine, University of Basel, Mattenstrasse 28, Basel, Switzerland*

In the last 50 years, obesity has tripled, and ~39% of the adult population is overweight. Obesity promotes insulin resistance, changes in glucose metabolism and lipid accumulation in the liver, and a chronic low-grade inflammation. Thus, the interest in anti-metaflammation drug targets increases.

The phosphoinositide 3-kinase isoform γ (PI3Kγ) is implicated in the regulation of diet-induced obesity and tissue inflammation. However, a specific involvement of its adaptor subunits p84 and p101 is unresolved. Genetically modified mice revealed that the loss of p84 limits weight gain, and prominently protects against obesity-induced hepatic steatosis. In addition, lipid vesicle size in inguinal white adipose tissue was reduced, together with an increase in adipolysis (pHSL) and thermogenesis (UCP1) markers. On the other hand, p101 null mice displayed normal glucose metabolism, despite their obesity. Both adapter subunits also regulate distinct immune cell invasion into adipose tissue, where p84 is required for mast cell recruitment, and p101 for macrophage infiltration. Moreover, the p84-p110γ complex is essential for insulin secretion, potentially explaining observed hepatic and adipose phenotypes in p84 null mice.

In conclusion, we have identified that p84 and p101 play non-redundant roles in glucose and lipid metabolism, and PI3Kγ complexes qualify as targets to modulate underlying causes of metaflammation.

CELL-INTRINSIC AND PARACRINE RAG GTPASE SIGNALING IN CANCER

• **Alejo Efeyan** | *Metabolism and Cell Signaling Lab, CNIO, Madrid, Spain*

Cellular nutrients activate mTORC1 via the Rag family of GTPases. This cascade, evolutionarily conserved in all eukaryotes, is key to couple the availability of building blocks and energy to the execution of the energetically onerous processes: protein synthesis, transcription, lipid synthesis and proliferation. Mutations in components of the Rag GTPase pathway are puzzlingly low in human cancer, with the exception of activating mutations in RagC in B-cell lymphomas. We have engineered the mouse genome to knock-in some of these mutations and found that they accelerate lymphomagenesis when bred to the follicular lymphoma prone strain VavP-Bcl2. Surprisingly, without a lymphoma-prone genetic background, RagCmut/+ mice exhibit a paradoxical reduction in the spontaneous tumorigenesis, but a shortened longevity with multiple features of premature aging. We have now found that chronic activation of RagC in innate immune cells fosters inflammation and transformation of the pancreas epithelium. I will discuss some or recent findings and current efforts focused in understanding how autonomous and paracrine active nutrient signaling drives cancer.

mTORC1 SIGNALING LINKS MATERNAL DIET TO CRANIOFACIAL MORPHOGENESIS.

• **Andrei Chagin** | *Institute of Medicine, The University of Gothenburg, Gothenburg, Sweden*

The development of craniofacial skeletal structures is remarkably complex, and elucidating the underlying mechanisms may provide novel biological. We performed genome-wide CAGE-sequencing of human embryonic facial mesenchyme to identify potentially active enhancers and cross-referenced them against GWAS hits for normal-range human facial variation. Several enhancers mapped to components of the PI3K/AKT/mTORC1/autophagy pathway. To probe its functional role, we manipulated mTORC1 signaling genetically and pharmacologically in mice and zebrafish. These experiments revealed that mTORC1 regulates shaping of skeletal mesenchymal condensations, which define the general shape of craniofacial skeletal elements. Later in embryonic development, it further fine-tunes skeletal shapes during clonal intercalation. Given its evolutionary conservation and ability to sense external cues and particularly dietary amino acids, we further checked whether mTORC1 may mediate facial phenotypic plasticity. Supporting this, maternal dietary protein altered fetal mTORC1 activity, modulated skeletogenic clone size, and affected craniofacial geometry. Together, our findings suggest that mTORC1 signaling links environmental conditions to craniofacial morphogenesis.

Posters

ABSTRACTS

1 mTORC1 ACTIVITY LICENSES ITS OWN RELEASE FROM THE LYSOSOMAL SURFACE

• Aishwarya Acharya, Constantinos Demetriades
Max Planck Institute for Biology of Ageing (MPI-AGE), Cologne, Germany

Nutrient signaling converges on mTORC1, which, in turn, orchestrates a physiological cellular response. A key determinant of mTORC1 activity is its shuttling between the lysosomal surface and the cytoplasm, with nutrients promoting its recruitment to lysosomes by the Rag GTPases. Active mTORC1 regulates various cellular functions by phosphorylating distinct substrates at different subcellular locations. Importantly, how mTORC1 that is activated on lysosomes is released to meet its non-lysosomal targets and whether mTORC1 activity itself impacts its localization remain unclear. Here, we show that, in human cells, mTORC1 inhibition prevents its release from lysosomes, even under starvation conditions, which is accompanied by elevated and sustained phosphorylation of its lysosomal substrate TFEB. Mechanistically, ''inactive'' mTORC1 causes persistent Rag activation, underlining its release as another process actively mediated via the Rags. In sum, we describe a mechanism by which mTORC1 controls its own localization, likely to prevent futile cycling on and off lysosomes.

2 ROLE OF PIF1/PIK3CA IN DNA DAMAGE REPAIR, TELOMERES, AND OVERGROWTH SYNDROME

• **Pranavi AMUDA RAVICHANDAR**
Institut Necker Enfants Malades, Université Paris Cité, INSERM

PIF1, a DNA helicase, is described as a negative regulator of telomerase, but also plays a role in maintaining genomic stability. We found a patient with overgrowth syndrome (PROS), a mosaic disorder, who carried two different somatic mutations in the PI3K pathway in his skin and blood.

His father was also suspected of having genetic abnormalities, and this pointed to the possibility of an underlying common mutation between the two. Upon exome sequencing and selecting for genes involved in DNA damage response, it was noted that the patient and his father shared a germline mutation in the PIF1 gene. This same point mutation in PIF1 was also identified in a family of patients with telomeropathies, and some patients in this telomeropathy cohort had a second, somatic activator mutation as well.

My project involves characterising the effects of this PIF1 point mutation broadly, with a particular interest in its impact on DNA damage repair and how it could manifest in patients with overgrowth syndrome (PROS), and investigating whether it could serve as an underlying link in families of patients diagnosed with mosaic disorders.

3 mTOR HYPERACTIVATION DRIVES COLLAGEN DEPOSITION VIA TFEB IN TUBEROUS SCLEROSIS COMPLEX

• **Francesco Avanzi, Mayuri Patel, Nicola Alesi, Elizabeth P. Henske**
Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Tuberous Sclerosis Complex (TSC) is a genetic disorder caused by mutations in TSC1 or TSC2, leading to mTOR hyperactivation and multi-organ involvement. A key mediator of TSC pathophysiology is the transcription factor TFEB, which accumulates in the nucleus of TSC-deficient cells. To investigate TFEB-dependent mechanisms, RNA sequencing and molecular analyses (qPCR, western blotting) were performed in TSC2-/- (621-102) vs TSC2+/+ (621-103) human angiomyolipoma-derived cells and in TSC1-/- vs TSC1+/+ mouse embryonic fibroblasts (MEFs). TSC-null cells showed upregulation of extracellular matrix (ECM)-related genes, including Col1a1, Col1a2, and Periostin (PSTN). Increased Collagen I and PSTN expression was also detected in angiomyolipomas and subependymal giant cell astrocytomas (SEGAs) from TSC patients. Since collagen synthesis is driven by transforming growth factor β (TGFβ), we assessed its role and found Tgfβ1/2/3 upregulated in TSC1-/- MEFs, along with enhanced secretion of Collagen I, PSTN, and TGFβ. Similar Tgfβ induction was observed in kidneys of two mouse models of renal TSC, and was abolished by TFEB knockout or rapamycin treatment. Importantly, TFEB knockdown in 621-102 cells reduced Col1a1/2 and PSTN expression. Altogether, these findings identify collagen deposition and ECM remodeling as key features of TSC driven by mTOR hyperactivation, and suggest a novel role for TFEB in regulating TGFβ signaling, with potential implications for targeted therapies.

4 IDENTIFICATION OF CIRCULATING BIOMARKERS IN CLOVES OVERGROWTH SYNDROME TO EVALUATE THERAPEUTIC RESPONSES

• **Orestes Lopez Ortega, Rubina Cassaca, Guillaume Canaud, Mario Pende**
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Overgrowth syndromes (OS) are genetic disorders characterized by abnormal tissue growth, frequently associated with mutations in the PI3K-mTOR pathway. CLOVES syndrome, caused by mosaic mutations in PI3KCα, is part of this group. PI3KCα mutations lead to uncontrolled activation of the PI3K/AKT/mTOR pathway, driving abnormal cell growth and diverse clinical symptoms. Mouse models and treatments such as the PI3KCα inhibitor Alpelisib have shown promising results. Interestingly, severe overgrowth can occur even with low mosaicism, suggesting a non-cell autonomous influence in the development of the syndrome, where mutant cells affect neighboring wild-type cells through paracrine signaling. Thus, the importance of identifying secreted factors to better understand the microenvironment of OS lesions. In the present study, we explored the hypothesis that paracrine effects amplify the OS phenotype. To do so, we used transduced untransformed human fibroblasts to avoid confounding effects from additional oncogenic mutations found in cancer cell lines. Additionally, we employed other cellular models, including endothelial cells and mouse tissues, and performed a broad analysis of circulating proteins in the serum of PROS patients. We observed that non-cell autonomous factors may lead to hyperactivation in neighboring cells, amplifying the OS phenotype. Hence, identifying these factors offers new therapeutic targets and biomarkers for OS.

5 UNCOVERING CELL-TYPE SPECIFIC CONTRIBUTIONS OF mTOR MUTATED CELLS TO FCDII

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Focal cortical dysplasia type II (FCDII) is a cortical malformation causing refractory epilepsy. FCDII arises during embryonic development, from somatic activating mutations in mTOR pathway genes that result in focal cortical dyslamination and abnormal cytomegalic cells. Mutations were identified in several cell types, including glutamatergic neurons and astrocytes, with only a small fraction exhibiting cytomegalic features. Contrary to the previously existing paradigm, microglia were also shown to carry pathogenic somatic mutations, regardless of them arising from another embryonic lineage as neural cells. Despite these recent findings, the timing at which these mutations arise during embryonic development, and how they affect cell-type-specific transcriptional programs remain poorly understood. Using an approach combining single-nucleus genotyping and transcriptomics in surgically resected tissue from pediatric patients, we aim to uncover the cell-type specific contributions of mutated and non-mutated cells to the epilepsy phenotype. We set out to carry this study out on an extended FCDII cohort, investigating more deeply the contributions of mutated microglia. Furthermore, to uncover the developmental time point at which somatic mutations arise, we will employ a targeted long-read sequencing strategy. Overall, this study aims at revealing cell-autonomous and non-cell-autonomous mechanisms underpinning FCDII pathophysiology that may be leveraged for precision medicine.

6 MODELING FCDII USING INDUCIBLE mTOR CORTICAL ORGANOIDS

• **Ann-Sofie De Meulemeester, Marco Martins Gama, Constance Lamer, Jean Livet, Stephanie Baulac**

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Focal cortical dysplasia type II (FCDII) is the most common lesion in children undergoing surgery for drug-resistant epilepsy. FCDII pathogenesis involves mTOR pathway hyperactivation driven by low-frequency somatic mutations that arise during cortical development, creating characteristic mosaic patterns of mutant and wild-type cells. While existing rodent and organoid models demonstrate mTOR pathway effects, they fail to recapitulate the developmental acquisition and mosaic distribution of somatic mutations observed in patients. Somatic mTOR variants account for most FCDII cases, yet no somatic mTOR organoid model has been reported.

We aim to generate an inducible mTOR organoid model that recapitulates somatic mutation acquisition during cortical development. To this end, we will adapt the Brainbow (loxP-Cre) system to link with the pathogenic mTOR S2215F variant. Using PiggyBac, we will co-integrate this construct with an ERT2-Cre plasmid, marked by nuclear BFP. Upon tamoxifen treatment, Cre activation will induce mosaic expression: mTOR-mutant cells (GFP+) or wild-type cells (mScarlet+). Functionality will first be validated in HEK293 and hiPSC lines, then applied to cortical organoids. This system uniquely enables live tracking of mutant versus non-mutant cells, allowing direct analysis of fate, morphology, clonal dynamics, and the emergence of FCDII-specific features such as dysmorphic neurons and balloon cells.

7 ROLE OF GLYCEROL KINASE IN SENESCENCE PHYSIOPATHOLOGY.

• de Villeneuve Delphine, Khaled Tighanimine, Margarita Papaxanthis, Emmanuelle Enee, Bastien AZNAR, Stefano Fumagalli and Pende Mario
INSERM U1151 INEM

Cellular senescence is a stable cell cycle arrest which is associated with dramatic changes in cell morphology, inflammatory secretory phenotype and metabolic reprogramming. Mostly due to altered secretion phenotype, senescent cells strongly influence surrounding tissue contributing to the development of age-related pathologies, including cancer. Furthermore, senescence would also participate in healing. We identify by in vitro approaches Glycerol kinase as a new potential senescent biomarker. We found Glycerol kinase accumulation in oncogenic KRAS and PIK3CA induced tumors and also in wound healing mouse skin. Interestingly, many of these conditions are related to the appearance of senescent cells but the direct connection is not yet well established. The aim of this project is i) to generate new conditional mouse models with Glycerol kinase knock-out in oncogenic induced tumors or overexpression in specific tissue in physiological or wound healing skin contexts ii) to score senescence program iii) to validate Glycerol kinase contribution in pathological or physiological process. These new mouse models will also help us to understand the contribution of GK in senescence program in vivo.

8 PI3K/AKT PATHWAY ACTIVATION IN CAJAL-RETZIUS CELLS ALTERS THEIR SURVIVAL IN HIPPOCAMPUS TOGETHER WITH MEMORY IMPAIRMENTS AND SOCIAL INTERACTIONS

• **Driss El Ouardi, Stephanie Moriceau, Patrick Azzam, Nasim Ramezanidoraki, Guillaume Canaud, Nadia-Bahi Buisson, Alessandra Pierani, Pierre Billuart**
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Cajal–Retzius cells (CRs) are a class of transient neurons present in the mammalian cortex that play a role in cortical development. We previously reported that activation of AKT in CRs increases their survival in neocortex at juvenile and adult stages and leads to an increase susceptibility of females to kainite-induced seizures.

Here, we investigate the role of AKT activation in the survival of hippocampal CRs and also the consequences of their persistence on the behaviors related to hippocampus. We used two complementary conditional approaches in mouse, either Pten loss- or PIK3ca gain- of-functions to activate AKT in hippocampal CRs.

We found an increase of the CRs densities in the hippocampal fissure of juvenile animal in both models but only PIK3ca adults maintained this difference. Whereas CRs persist in the cortex of Pten model, they disappear in adult hippocampus suggesting that PI3K/AKT pathways are differentially controlled according to brain regions.

Finally, these CRs persistence led to variable episodic and spatial memories alterations according to sex and model together with an increase of sociability in females.

9 INVESTING ROLES FOR MEMBRANE MICRODOMAINS IN TORC1 SIGNALLING

• **Max Gardner, Kenji Muneshige and Riko Hatakeyama**
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TORC1 functions on the lysosomal (vacuolar in yeast) membrane. The yeast vacuolar membrane visibly segregates into a sterol-rich and sterol-poor region in starved cells (Toulmay & Prinz, 2013). Most vacuolar proteins localise to the sterol-poor region, but multiple TORC1 pathway components, such as Tco89 (yeast TORC1 subunit), Gtr1 and Gtr2 (yeast Rag GTPases), localise to the sterol-rich regions (Toulmay & Prinz, 2013) (Murley et al., 2017). We are interested in vacuolar microdomains for two reasons. First, microdomains are implicated in TORC1 regulation (Murley et al., 2017), with mechanisms poorly understood. Second, yeast TORC1 forms a functionally distinct pool on so-called signalling endosomes (Hatakeyama et al., 2019). Signalling endosomes form from the vacuolar membrane, but not all vacuolar proteins are transferred to signalling endosomes (Muneshige & Hatakeyama, 2025). We hypothesize that microdomains explain this selectivity, specifically, signalling endosomes may selectively form from the sterol-rich vacuolar microdomain. To assess this idea, I am examining how signalling endosome proteins localise to vacuolar microdomains in starved cells. I am also examining actively growing cells, as Airyscan high-resolution microscopy revealed uneven, clustered TORC1 localization on the vacuolar membrane, potentially representing microdomains. My work will improve our understanding of the roles for membrane microdomains in TORC1 regulation and signalling endosome biogenesis.

10 mTORC1 HYPERACTIVATION IN MUTANT MICE REVEALS CRITICAL ROLES IN CORTICAL AND HIPPOCAMPAL DEVELOPMENT

• **Alice Gilbert, Marion Doladilhe, Delphine Roussel & Stéphanie Baulac**
MOSAIC team, Paris Brain Institute (ICM) /CNRS UMR 7225/INSERM U 1127/Sorbonne Université, Hôpital de la Pitié Salpêtrière, F-75013 Paris, France

Focal cortical dysplasia type II (FCDII) is a malformation associated with epilepsy, developmental delay, and autism. It arises from somatic mutations in mTOR pathway genes, most often mTOR (57% of patients), including hotspots S2215F and T1977K. Previous in vitro functional assays have shown that the two FCDII hotspot mutations induce variable levels of mTORC1 activity. We generated two knock-in mouse lines with conditional expression of these variants in Emx1 cortical neurons to study mechanisms of FCDII.

Both models reproduced hallmarks of the disease: cortical delamination, increased cortical thickness, dysmorphic neurons with mTORC1 hyperactivity, spontaneous seizures (~5seizures/week), and social deficits. They also showed hippocampal abnormalities, including a double CA1 layer, CA3 neuron dispersion, and ring heterotopias at dentate gyrus boundaries. Using MERSCOPE spatial transcriptomics with a 450-gene panel, we confirmed preserved cell identity in both normal and ectopic neurons from cortex and hippocampus. Additionally, we observed persistency of Cajal Retzius cells, suggesting sustained Reelin secretion may underlie cortical and hippocampal migration defects.

These new mouse models hold promises for elucidating the pathophysiological mechanisms of FCDII and identifying potential therapeutic targets, as well as informing on the role of mTOR pathway in neuronal migration, particularly within the hippocampus.

11 INHIBITION OF THE POTASSIUM CHANNEL TREK1 BY mTOR: A MECHANISM FOR ABERRANT NEURONAL EXCITABILITY IN TSC

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Neurological disorders, such as epilepsy and autism, affect millions of people worldwide with the underlying causes ranging from genetic mutations to developmental issues. Among these neurological disorders are mTORopathies, a group of genetic diseases characterized by the dysregulation of the mechanistic Target Of Rapamycin (mTOR) signaling pathway. The most common mTORopathy, Tuberous Sclerosis Complex (TSC), leads to significant health challenges, however, the precise mechanisms by which it causes neurological hyperexcitability remain unclear. To identify potential novel targets downstream of mTOR responsible for these neurological disorders, we performed the first phospho-proteomic analysis of the TSC brain and identified TREK1, a potassium channel highly expressed in the brain. Amino acid stimulation, leading to an increase in TREK1-S333 phosphorylation, closes and deactivates the potassium channel as shown by patch clamp recordings in HEK293 cells. Notably, this was rescued by Rapamycin treatment, linking TREK1 channel activity to the nutritional regulation of mTOR. To further expand our understanding of TREK1 regulation, we generated three different mouse models with a particular focus on its intracellular C-terminal domain (TREK1-S333X, TREK1-S300A/E306A, and TREK1-S333A). These mice with modulated TREK1 activity will enable us to characterize the role of mTOR in TREK1 regulation, offering novel mechanistic insights into the role of mTOR dysregulation in TSC.

12 CONTRIBUTION OF NEURONAL SUBTYPES TO ICTOGENESIS IN A mTOR-BASED MOUSE MODEL OF FOCAL CORTICAL DYSPLASIA II

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Somatic mutations of mTOR pathway in neural progenitors may cause aberrations in metabolism and migration of neurons causing focal cortical dysplasia II. We used optogenetic stimulation of neurons expressing either mutated or WT mTOR or neurons in the vicinity of mutated cells to elucidate their role. We also aimed to decrease seizure frequency by chemogenetic modulation of the mutated neurons. ChR2-induced activity of neurons within FCD lesion expressing mutated mTOR leads to seizure onset. Stimulation of non-mutated neurons in FCD animals resulted in seizure-like activity with different characteristics including seizure duration and relative seizure power. Stimulation of mutated neurons by 3 Hz and 8 Hz light trains induced more seizures compared to stimulation of non-mutated neurons. We observed overall low efficiency of seizure induction in control animals expressing WT mTOR. In spontaneously seizing animals, the chemogenetic modulation of mutated neurons resulted in a mild decrease in seizures. Our data show that excitability of neurons expressing mutated mTOR is one of the key factors in ictogenesis in the model of FCD II. Mutated neurons seem to be part of the neuronal network within epileptic tissue that also includes neurons without the causal mutation. This suggests that selective modulation of distinct cell-types within the lesion could have different therapeutic potential for drug resistant FCD.

13 mTOR-MUTANT ASTROCYTES IN FCDII: INVESTIGATING SELECTIVE ENRICHMENT MECHANISMS USING HUMAN PRIMARY CELL CULTURES

• **Marco Martins Gama, Constance Lamer, Ann-Sofie De Meulemeester, Stéphanie Baulac**
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Hemimegalencephaly patients often present high variant allele fractions (VAFs) of somatic mTOR-pathway mutations in resected brain tissue, while these variants remain undetectable in other tissues. This selective enrichment suggests that mTOR-mutant cells may possess a proliferative advantage specifically within the neuronal lineage.

To investigate this hypothesis, we derived primary astrocyte cultures from epilepsy surgical resections harboring mTOR variants. Fresh tissue samples from six patients were enzymatically dissociated and grown successfully in an astrocyte-specific medium. Astrocyte identity was confirmed by SOX9, GFAP, and S-100β immunostaining. For each patient and across successive passages, we evaluated proliferative capacity through: (1) VAF quantification at each passage using targeted deep sequencing and digital droplet PCR to detect changes in mutant cell proportions; (2) mTOR pathway hyperactivation monitoring via phospho-S6 (pS6) immunofluorescence intensity measurements; and (3) proliferation rate assessment using Ki67 staining.

This strategy provides mechanistic insights into FCDII pathogenesis and may explain why mTOR mutations show tissue-specific enrichment patterns. Understanding the proliferative advantage could inform targeted therapeutic approaches and provide a platform for testing mTOR inhibitors in a patient-specific context.

14 THE TSC-RHEB-mTORC1 SIGNALING PATHWAY FORMS A NOVEL AUTOREGULATORY FEEDBACK LOOP

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mTORC1 lies at the center of an intricate signaling network that allows cells to homeostatically respond to a multitude of intra- and extra-cellular cues. Although this network is dynamically rewired to avoid excessive mTORC1 activation under permissive growth conditions, the signaling events that facilitate the fine-tuning of its activity are not fully understood. Here, we show that TSC1, a component of the TSC complex, is a novel lysosomal substrate of mTORC1. TSC1 phosphorylation on mTORC1-dependent sites promotes its stability. Despite the general role of the TSC complex in downregulating global mTORC1 activity, TSC1 phosphorylation specifically regulates the lysosomal branch of mTORC1 signaling. Preventing TSC1 phosphorylation is associated with lower levels of TFEB phosphorylation, increased nuclear TFE3 translocation, and enhanced lysosomal biogenesis, while phosphorylation of the non-lysosomal canonical substrates, such as S6K1, remains largely unaffected. Based on these findings, our work sheds light on the shortest feedback loop within the mTOR pathway that orchestrates compartmentalized signaling to selectively promote the processes downstream of lysosomal mTORC1 signaling.

15 UNDERSTANDING mTORC1 ASSEMBLY AND MATURATION BY THE HSP90-R2TP CHAPERONE SYSTEM

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mTOR (mammalian target of rapamycin) is a large PIKK (phosphatidylinositol 3-kinase-related kinase) which controls cell growth and proliferation upon integrating nutrient and growth factor signals. mTOR is only active as part of two complexes, termed mTORC1 and mTORC2, with distinct subunit compositions and biological functions. Dysregulated mTORC1 activity disrupts cellular homeostasis, and it has been linked to cancer progression and ageing, among other pathologies. Importantly, mTORC1 assembly is essential for its activation, requiring the HSP90 (heat shock protein 90) chaperone working in concert with the TELO2–TTI1–TTI2 (TTT) and the RUVBL1–RUVBL2–RPAP3–PIH1D1 (R2TP) co-chaperone complexes. However, the molecular mechanism and regulation of these processes remain obscure due to the lack of structural information.

We are aiming to elucidate how HSP90 stabilizes mTOR to promote its dimerization and interaction with mLST8 and RAPTOR. Preliminary pull-down assays suggest that independently purified HSP90 and mTOR interact upon in vitro reconstitution. In parallel, we are optimizing the recombinant co-expression of the HSP90–mTOR/mTOR-mLST8 complexes for their isolation directly from insect cells. Structural analyses of the purified complexes will then be performed by cryo-EM. Eventually, adding TTT and R2TP complexes by in vitro reconstitution or co-expression will help us to uncover the sequence of intermediate states of mTORC1 assembly and activation.

16 DYSREGULATION OF mTORC1 SIGNALING IN LISSENCEPHALY SPECTRUM DISORDERS

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Lissencephaly ('smooth brain') spectrum disorders comprise a group of rare, genetically heterogeneous congenital brain malformations commonly associated with epilepsy and intellectual disability. In previous work, we demonstrated that cerebral organoid models, generated from patient-derived induced pluripotent stem cells with two genetic subtypes of lissencephaly spectrum disorders, exhibit a thickened cortical plate, resembling clinical presentations of lissencephaly. Quantitative proteomics revealed mTOR pathway hypoactivation and a brain-selective and highly specific activator of mTORC1 prevented and, importantly, reversed cellular and molecular defects in the lissencephaly organoids.

We have now generated and characterized organoids from additional lissencephaly spectrum disorder genetic subtypes. Cellular and molecular analyses revealed mTORC1 hypoactivation and downstream processes that depend on mTORC1 signaling, evidenced by significantly lower levels of phosphorylated S6 protein as well a global reduction in translation.

We are characterizing the effects of pharmacological modulation of mTORC1 aiming to define the molecular mediators of mTORC1 activation in human neural development. Our work identifies a convergent molecular mechanism in lissencephaly, extending the continuum of mTORopathies beyond disorders characterized by mTOR pathway hyperactivity, and could help elucidate disease pathogenesis while also providing valuable insights into the molecular mechanisms of human cortical development.

17 DIVERGENT REGULATION OF mTOR AND RAPTOR IN MATURE HIPPOCAMPAL NEURONS DURING NUTRIENT STRESS AND SYNAPTIC ACTIVATION.

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International Institute of Molecular and Cell Biology in Warsaw

mTOR is a protein complex that helps neurons respond to nutrient levels and external signals, regulating cell growth, metabolism, and synaptic plasticity. While well studied in developing neurons, its behavior in mature neurons under metabolic stress—particularly within the nucleus—remains poorly understood.
In this study, we used mature rat hippocampal neurons cultured in vitro to investigate mTOR signaling during nutrient deprivation. Neurons were incubated in nutrient-poor Neurobasal medium (NB) for 2 hours (short-term deprivation) or 6 hours (long-term deprivation). In some conditions, full medium was reintroduced for 20 minutes.

After 2 hours in NB, the strongest activation of mTOR and its phosphorylated form (P-mTOR) occurred upon refeeding, indicating nutrient-sensitive activation. After 6 hours, mTOR and P-mTOR were already elevated and did not increase further with refeeding, suggesting a shift in regulatory mechanisms during prolonged stress.

We also examined Raptor, a key mTORC1 component. In control neurons, Raptor formed nuclear puncta, while kainic acid (KA) stimulation caused it to become more diffusely distributed in the nucleus. Raptor consistently localized to the nucleolus (co-labeled with nucleolin), whereas mTOR was excluded from this region under all conditions.

These results reveal a time-dependent mTOR response to nutrient stress and suggest that Raptor may have spatially distinct functions from mTOR in mature neurons.

18 SENESCENCE CONTROL BY REWIRING LIPID METABOLISM

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Senescent cells accumulate with age and contribute to the pathophysiology of chronic diseases, but the underlying mechanisms remain poorly understood. Using dynamic transcriptome and metabolome profiling in human fibroblasts, we uncovered a homeostatic switch leading to glycerol-3-phosphate (G3P) and phosphoethanolamine (PEtn) accumulation, linking lipid metabolism to the senescence gene expression program. Functional modulation of key lipid metabolism enzymes, activation of glycerol kinase (GK) and inactivation of phosphoethanolamine cytidylyltransferase 2 (PCYT2), resulted in senescence induction and lipid droplet accumulation (Tighanimine et al. Nat Metab. 2024). Building on these findings, we show that GK overexpression and PCYT2 depletion enhance DNA damage signaling and trigger nuclear translocation of phosphorylated Akt, suggesting a link between lipid metabolism, DNA damage response, and pro-survival signaling. Altogether, our work highlights lipid metabolic enzymes as central nodes integrating metabolic rewiring, cell cycle arrest, and stress signaling in senescence, pointing to potential therapeutic strategies for aging and cancer.

19 UNCOVERING RSK2 DOWNSTREAM EFFECTORS IN NEURONAL REGENERATION: INSIGHTS FROM DRG PHOSPHO-PROTEOMICS

• **Colline Sanchez[1], Albrecht Fröhlich[2], Charlotte Decourt[2], Vonda Koka[1], Elena Pasho[3], Lamine Touré[1], Edor Kabashi[3], Stéphane Belin[2], Mario Pende[1]**

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Central nervous system (CNS) injuries cause irreversible cognitive, sensory, and motor deficits due to the limited regenerative capacity of mature CNS neurons, unlike peripheral nervous system (PNS) neurons that readily regrow and restore function. Neurons are key drivers of regeneration, largely through growth-associated signaling such as mTOR activation and phosphorylation of ribosomal protein S6 (RPS6). Following sciatic nerve injury in mice, we previously observed strong upregulation of the p90S6 kinase RSK2 in dorsal root ganglion (DRG) neurons, widely used as PNS/CNS injury model for regeneration. RSK2 is the major kinase phosphorylating RPS6 in mouse DRG, and its activation correlates with regenerative responses, including axonal growth and spinal cord plasticity. Our findings indicated that regenerative effects of RSK2 are only partly explained by RPS6 phosphorylation and translational regulation, pointing to additional substrates and mechanisms. To address this, we performed phospho-proteomic analysis of DRG neurons after sciatic nerve injury. Our preliminary data reveal a robust RSK2-dependent phosphorylation signature, suggesting broad molecular programs potentially supporting neuronal survival and regeneration. As RSK2 is an unsuitable direct therapeutic target due to its oncogenic potential, mapping its downstream effectors may provide promising directions and open perspectives for identifying candidates to promote neural repair.

20 TOR-MEDIATED TRANSLATIONAL CONTROL OF GENOTOXIC STRESS RESPONSE IN PLANTS

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Ultraviolet (UV) radiation is an important stress factor for land plants routinely exposed to sunlight. Although UV stress leads to significant defects in growth and development, eucaryotes have evolved the precise mechanism to regulate gene expression at both the transcriptional and translational levels to protect cells from the detrimental effects of UV-induced damage. Mechanistically, UV radiation causes bulky DNA lesions, activating the DNA damage response (DDR). This response is primarily mediated by two checkpoint kinases, Ataxia-Telangiectasia Mutated (ATM) and Rad3-related (ATR), as well as by DNA-dependent protein kinase (DNA-PKc), all members of the PI3KK family. Beyond its canonical function in genome stability, DNA-PK drives translation reprogramming under genotoxic stress in yeast and mammals. However, DNA-PK is absent in angiosperms, prompting us to investigate alternative mechanisms of UV-induced translational control in plants. While ATM and ATR coordinate cell cycle progression, their direct role in translational control remains undefined. This gap provides a unique opportunity to examine the role of another PI3KK family member–Target of Rapamycin (TOR) kinase–in translational control under UV stress.

We are currently investigating the interplay between ATM, ATR and TOR kinases in translational control of genotoxic stress response in land plants.

21 CYSTEINE-DEPENDENT BINDING OF GATOR2 TO ENDOSOMAL/LYSOSOMAL PROTEINS

• **Marcel Seibert[1,2], Nina Kurrle[1,2,3], Leon Börner[1], Heike Nürnberger[1], Frank Schnütgen[1,2,3], and Hubert Serve[1,2,3]**

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The GATOR2 complex is a critical upstream regulator of mechanistic target of rapamycin complex 1 (mTORC1) signaling, which controls cellular growth and metabolism. While its role in amino acid-dependent mTORC1 activation is well documented, its broader functions under amino acid deprivation remain incompletely understood. Structurally, GATOR2 comprises flexible protein domains that enable interactions with diverse partners, particularly those involved in amino acid sensing, suggesting potential roles in multiple signaling pathways.

To investigate binding partners and functions, we performed a comprehensive interactome analysis using endogenously GFP-tagged GATOR2 subunits combined with immunoprecipitation and mass spectrometry. This endogenous approach preserved the native conformation of GATOR2, allowing identification of interactions that may have been missed previously. Our data revealed several novel interactors, including proteins of the endomembrane system and miRNA processing machinery. Notably, these interactions were modulated by cysteine (Cys) availability, a condition known to influence mTORC1 signaling, ferroptosis, autophagy, and the amino acid starvation response. The identification of Cys-dependent GATOR2 interactions highlights a previously unrecognized mechanism for sensing Cys depletion. Together, our findings suggest that GATOR2 functions as a cellular Cys sensor that selectively recruits downstream mediators in response to fluctuations in Cys availability.

22 THE V-ATPASE ASSEMBLY FACTOR RAVE IS CRITICAL FOR LYSOSOMAL FUNCTION BUT DISPENSABLE FOR mTORC1 ACTIVITY

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V-ATPases are ATP-driven proton pumps that acidify lysosomes, endosomes and the Golgi. V-ATPase-mediated acidification has been implicated in various organellar functions such as lysosomal catabolism, trafficking, and nutrient-sensing by mTORC1. Studies in yeast identified the V-ATPase assembly factor RAVE as a critical regulator of vacuolar V-ATPases activity through reversible assembly. In mammalian cells, however, the relevance of RAVE at different organelles remains unclear. Here, we set out to systematically investigate the role of mammalian RAVE in V-ATPase assembly and organelle functions. We show that RAVE plays a critical role in V-ATPase assembly at lysosomes, endosomes and the Golgi. Loss of RAVE leads to a strong decrease in assembled active V-ATPases at these different organelles. At lysosomes, loss of RAVE results in elevated pH, loss of mature catabolic enzymes and decreased degradative activity. Lysosomal enzymes are instead secreted from cells through enhanced lysosomal exocytosis. However, loss of RAVE does not disrupt mTORC1 recruitment to lysosomes in response to amino acid stimulation and resulting activation of downstream targets. Thus, RAVE-mediated assembly of lysosomal V-ATPases is critical for lysosomal acidification and catabolic activity but dispensable for mTORC1 signaling.

23 RAPAMYCIN-RESISTANT PHANEROCHAETE CHRYSOSPORIUM MUTANTS AS TOOLS TO EXPLORE STREPTOMYCES METABOLITE DIVERSITY

• Cintia Blanco Nouche, Delphine Noël, Duy Vuong Nguyen, Antonio Fernández-González, Thomas Roret, Eric Gelhaye, Mélanie Morel-Rouhier, Cyril Bontemps, Rodnay Sormani

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Rapamycin, produced by Streptomyces hygroscopicus, was first identified as an antifungal compound and is now widely used in medicine for its immunosuppressive, anti-inflammatory, and anticancer properties. Its mode of action involves forming a complex with FKBP12 that binds and inhibits the TOR kinase, a central regulator of eukaryotic growth. Other Streptomyces-derived molecules such as FK506 or FK520 also target FKBP12 but act independently of TOR, highlighting this protein as a key target in antagonistic interactions. The wood-decaying fungus Phanerochaete chrysosporium offers a relevant model to study such mechanisms due to its ecology and reliance on TOR pathway regulation. Using the homokaryotic RP78 strain, a collection of rapamycin-resistant mutants (rap) was generated by UV mutagenesis, including strains carrying TOR missense mutations, a nonsense mutation in FKBP12, and one uncharacterized mutant. These mutants were confronted with various Streptomyces isolates to identify novel antifungal molecules. Four possible outcomes were anticipated to classify compounds based on their dependence on FKBP12, TOR, both or alternative targets. Screening revealed molecules acting like rapamycin and others acting independently of TOR/FKBP12. Purification and characterization of these compounds are ongoing. Our results suggest that Streptomyces remains an underexplored source of antifungal agents and potential rapamycin analogues.

24 METABOLIC SHIFTS IN ANGIOMYOLIPOMA OF TUBEROUS SCLEROSIS COMPLEX PATIENTS

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Tuberous sclerosis complex (TSC) is an autosomal dominant multi-organ disorder that is driven by mutations in the genes encoding the tuberous sclerosis proteins TSC1 or TSC2. Constitutive activity of the mechanistic target of rapamycin complex 1 (mTORC1) kinase results in metabolic rewiring and causes tumor growth and aberrant neuronal activity. Kidney tumors occur in the form of angiomyolipomas (AML). The metabolic alterations leading to their formation are incompletely understood. We profiled and mechanistically validated metabolic changes in patient-derived AML cultures and a cohort of TSC patients by mass spectrometry and NMR. Our data reveal metabolic pathways that may contribute to kidney manifestations in TSC, opening new therapeutic avenues.

25 MOLECULAR MECHANISMS IN RHEB REGULATION

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The activation of mTORC1 is dependent on the nucleotide loading status of the small GTPase Rheb. In the GTP-bound form Rheb activates mTORC1 to regulate cellular growth. The Tuberous Sclerosis Complex (TSC) protein complex is the GTPase activating protein (GAP) for Rheb and inactivates Rheb, which results in attenuated mTORC1 signalling. We are working towards resolving the structure of Rheb bound to the TSC complex by cryo EM. Our preliminary structural model suggests multiple non-equivalent interaction sites for Rheb. Opposing GAPs, the activation of small GTPases by exchange of GDP to GTP is usually mediated by guanine nucleotide exchange factors (GEFs). The identity of a GEF for Rheb has long been elusive, but recently two new GEF candidates were proposed for Rheb: the C-terminal domain of the V-ATPase subunit ATP6AP1 (Feng et al. 2024) and the kinase domain of the epidermal growth factor receptor (EGFR) (He et al. 2025). However, using in vitro GEF assays we have not been able to show exchange activity of ATP6AP1 or EGFR towards Rheb. Furthermore, we have also performed in vitro characterizations of Rheb variants reported in patients to investigate their functional consequences. Interestingly, the variants show variable effects on intrinsic and TSC protein complex stimulated GTP hydrolysis activity, indicating that distinct mechanism may contribute to disease. Collectively, our data provide new insight into the molecular basis of Rheb-dependent TORopathies.

26 GATOR1 COMPLEX CONTROLS CISPLATIN SENSITIVITY

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Cisplatin administration is the primary chemotherapy approach for various carcinomas, yet resistance to this drug significantly impedes its clinical efficacy. Low expression of NPRL2 was previously associated with cisplatin resistance. NPRL2, alongside NPRL3 and DEPDC5, forms the GATOR1 complex, an upstream regulator of mTORC1. We compared GATOR1-depleted non-cancerous bronchial epithelial BEAS-2B cells, serving as a model for cisplatin resistance, with non-small cell lung cancer cell lines that developed cisplatin resistance. We found that deletion of any GATOR1 component, not solely NPRL2, promotes cisplatin resistance, whereas their overexpression re-sensitizes cells to the drug. Cells with GATOR1 depletion displayed elevated expression of the cisplatin efflux transporter ATP7A, while expression of influx transporters CTR2 and LRRC8A was downregulated, especially after treatment with the drug. Consequently, drug accumulation and formation of cisplatin-DNA adducts were reduced, coinciding with enhanced DNA damage response and mTORC1 activity. Transcriptomic analysis of GATOR1-depleted BEAS-2B cells, treated or not with cisplatin, identified unique signatures associated with GATOR1 deletion and drug response. Thus, GATOR1 not only participates in the cellular response to amino acid availability, but also contributes resistance to DNA-damaging anticancer drugs. This novel function of GATOR1 should be taken into account when developing new strategies to combat chemoresistance.

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