GIBB2026

ANNUAL MEETING OF THE ITALIAN GROUP OF BIOMEMBRANES & BIOENERGETICS

UDINE, 11-13 JUNE 2026

Palazzo Garzolini - Di Toppo Wassermann

SCIENTIFIC PROGRAM

https://www.gibb-be.org/

GRUPPO ITALIANO DI BIOMEMBRANE E BIOENERGETICA

ITALIAN GROUP OF BIOMEMBRANES AND BIOENERGETICS

Created in 1973 by a group of Italian Scientists coming from a variety of disciplines (biology, chemistry, biophysics, pathology, pharmacology and physiology), GIBB was born with the idea of promoting Bioenergetics in the Country and to establish an interface with the growing International Bioenergetics Community.

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INVITED SPEAKERS

ERICH GNAIGER
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Innsbruck, Austria

CHRISTIAN LOEW
EMBL Hamburg, Germany

PAOLO BERNARDI
University of Padova, Italy

TOMAS MOROSINOTTO
University of Padova, Italy

LAURA FORMENTINI
University of Madrid, Spain

PROGRAM

THURSDAY, 11 JUNE		
13:00–14:00	Welcome and registration	
14:00–14:15	**Opening remarks**	
MITOCHONDRIA IN CANCER, AGING AND DISEASE CHAIRS: Consiglia Pacelli (University of Foggia), Alessandro Giuffrè (IBPM-CNR, Roma)		
14:15–14:30	Giada Zanini, Post-Doc, University of Modena and Reggio Emilia	*Lonp1 overexpression affects mitochondrial functions, mtDNA localization, and cytokines production in colorectal cancer*
14:30–14:45	Eleonora Martinis, Post-Doc, University of Udine	*Immunophenotypic skewing of B cells toward $IgD^{-}CD27^{-}IgG^{+}$ subtype and metabolic attenuation in colorectal cancer*
14:45–15:00	Ilenia Paoletti, PhD Student, University of Foggia	*ClpP hyperactivation by ONC212 disrupts mitochondrial function and targets cancer stem cells in osteosarcoma and breast cancer*
15:00–15:15	Ali Safarzadeh, PhD Student, University of Padova	*The mitochondrial protein Fis1 but not its interactor Drp1 are essential to sustain Acute Myeloid Leukemia Stem Cells*
15:15–15:30	Elisa Zamprogno, PhD Student, University of Udine	*Investigating mitochondrial dysfunction and autophagic flux in ATP5MC3-associated dystonia*
15:30–15:45	Clara Abobocioae, PhD Student, University of Vienna	*β-hydroxybutyrate restores mitochondrial dysfunctions caused by LETM1 deficiency*
15:45–16:00	Giada Carpi, PhD Student, University of Udine	*Assessing the impact of mitochondrial transplantation on cardiac pericyte viability in vitro*
16:00–16:30	**Coffee break**	
MITOCHONDRIAL STRESS RESPONSES AND PROTEIN QUALITY CONTROL CHAIRS: Anna Signorile (University of Bari), Michele Galluccio (University of Calabria)		
16:30–16:45	Giulia Micheloni, PhD Student, University of Modena and Reggio Emilia	*Mitochondrial effects of a CODAS-associated LONP1 mutation and their modulation by Lonp1 activators*
16:45–17:00	Federica Antonacci, PhD Student, University of Foggia	*A novel ATAD3A variant associated with altered mitochondrial homeostasis*
17:00–17:15	Camila Dib, PhD Student, University of Padova and UCA, Buenos Aires	*Mitochondrial fragmentation induced by the CFTR modulators Lumacaftor and Ivacaftor in immortalized Cystic Fibrosis Cell Lines*
17:15–17:30	Piero Leone, Researcher, University of Bari	*Two novel FLAD1 variants causing UPR in fibroblasts from a paediatric patient*
17:30–17:45	Alessandro Cecconi, PhD Student, University of Padova	*Effects of compartment-specific unfolded mitochondrial protein aggregates on mitochondrial function*

17:45–18:00	Eve Harding, PhD Student, IMOL, Warsaw, Poland	*mtDNA stability alters the mito-nuclear crosstalk through calcium homeostasis*
18:00-18:15	Alice Borsetto, Research Fellow, University of Padova	*Effect of USP14 inhibition, and molecular mechanism of mitophagy induction*
18:15–19:00	**Invited Lecture – Erich Gnaiger, Oroboros** ***Lower mitochondrial calcium uptake capacity than calcium retention capacity in the presence and absence of cyclosporin A***	
19:00	**Welcome cocktail**	

FRIDAY, 12 JUNE		
09:00-10:00	**Invited Lecture – Christian Loew, EMBL Hamburg** ***Nutrient and drug uptake across the membrane*** CHAIRS: Giovanna Lippe (University of Udine), Andrea Rasola (University of Padova)	
STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF CHANNELS AND TRANSPORTERS CHAIRS: Angela Messina (University of Catania), Luca Scorrano (University of Padova)		
10:00–10:15	Luana S. Brunetti, Post-Doc, University of Calabria	*Mechanism of organic cation transport mediated by the OCTN1 human transporter*
10:15–10:30	Raffaella Scanga, Post-Doc, University of Calabria	*Inhibition of the Amino Acid Transporter LAT1 by 1-methyltryptophan reveals a novel therapeutic strategy in cancer*
10:30–10:45	Elena Frigo, Post-Doc, University of Padova	*ATP synthase subunits e and g are essential for Ca^{2+} homeostasis and development in Drosophila melanogaster independent of oxidative phosphorylation*
10:45-11:15	**Coffee break**	
11:15–11:30	Federica Boscolo Nata, PhD Student, University of Padova	*The C-terminus of subunit e is essential to switch the ATP synthase into the mitochondrial permeability transition pore*
11:30–11:45	Stefania Bonusi, PhD Student, University of Brescia	*Targeting mPTP improves mitochondrial bioenergetics and muscle regeneration in Duchenne Muscular Dystrophy*
11:45–12:00	Gabriele Coluccino, Post-Doc, University of Udine	*N-terminal cleavage reshapes Cyclophilin D dynamics and is associated with enhanced F-ATP Synthase binding*
12:00–13:00	***Lectio Magistralis*** **"Medaglia G.F. Azzone" - Paolo Bernardi, University of Padova** ***More doubts than certainties: how I survived in science (still having some fun)***	

13:00–14:00	Light lunch
14:00–15:00	**Invited Lecture – Tomas Morosinotto, University of Padova** ***The impact of mitochondrial respiration on plants metabolism*** CHAIRS: Elena Forte (Sapienza University of Rome), Marco Zancani (University of Udine)

TRANSLATIONAL APPROACHES, DIAGNOSTICS AND BIOTECHNOLOGY

CHAIRS: Valentina Giorgio (University of Bologna), Marcello Pinti (University of Modena)

15:00–15:15	Iolanda R. Infantino, Post-Doc, University of Catania	*NHK1 peptide targeting VDAC1 mitigates mitochondrial impairment in ALS Primary Fibroblasts*
15:15–15:30	Tomas Knedlik, Researcher, University of Padova	*Discovering Small-Molecule Modulators of Mitochondria–ER Contact Sites and their protein targets*
15:30-15:45	Davide Fontana, PhD Student, University of Padova	*A redox cycler-based therapy against mitochondrial diseases*
15:45–16:00	Denis Komarov, PhD Student, University of Padova	*Identification of the mitochaperome as a new tumor-restricted assembly*
16:00–16:15	Antonello Caponio, PhD Student, University of Bari	*CRISPR-based restoration of AGC1 reveals mutation-dependent phenotypic rescue in AGC1-deficient neuronal progenitors*
16:15–16:30	Elettra Leo, Post-Doc, Oroboros	*Challenges in PBMC bioenergetic diagnostics: from cohort-derived reference values to patient interpretation*
16:30–16:45	Dora Scarpin, Post-Doc, University of Udine	*Biotechnological production of extracellular vesicles from* Coffea arabica *L. cell suspension cultures: isolation and proteomic profiling*
16:45–17:00	Aurelia Miscia, PhD Student, University of Padova	*Generation and initial characterization of STACCATO, a family of Split-FAST Mitochondria–ER Contacts probes to measure contact sites of different widths*
17:00–17:15	Beatrice Capita, PhD Student, University of Padova	*FLYMP: a new FRET based sensor to study contact sites between mitochondria and lysosomes*
17:15–17:45	**Coffee break**	

MITOCHONDRIAL BIOGENESIS AND METABOLISM

CHAIRS: Maria Barile (University of Bari), Nazzareno Capitanio (University of Foggia) (University of Udine)

17:45–18:00	Lucrezia Zuccarelli, Researcher, University of Udine	*Exercise prehabilitation combined with nutritional and cognitive interventions during Bed Rest preserves skeletal muscle mitochondrial function in older adults*
18:00–18:15	Giuseppe Giarrusso, PhD Student, University of Catania	*Hexokinase overexpression and its consequences for respiratory parameters*

18:15–18:30	Leila Secchiaroli, PhD Student, University of Bologna	*TMEM65 couples mitochondrial calcium homeostasis to respiratory chain biogenesis*
18:30–18:45	Noemi D'Incau, Research Technician, UCL, UK	*Biallelic variants in PGS1 disrupt cardiolipin biosynthesis and mitochondrial bioenergetics*
19:00-19:30	**Assemblea dei soci**	
20:30	**Social dinner - Ristorante Concordia, Piazza I Maggio, Udine**	

SATURDAY, 13 JUNE

09:30–10:30	**Invited Lecture – Laura Formentini, University of Madrid** ***Nutrient-dependent control of OXPHOS: the role of fatty acid oxidation in ageing*** CHAIRS: Anna Maria Porcelli (University of Bologna), Cesare Indiveri (University of Calabria)
10:30-11:00	**Coffee break**

MITOCHONDRIAL DYNAMICS: FROM BASIC MECHANISMS TO THERAPEUTIC STRATEGIES

CHAIRS: Alessandra Corazza (University of Udine), Vito De Pinto (University of Catania)

11:00–11:15	Cien Zhen, PhD student, University of Padova	*Mitochondrial calcium uptake regulates mitochondrial fission modes*
11:15–11:30	Aytekin Hajighaderi, PhD Student, University of Padova	*Gdap1l1 is a mitochondrial fission factor vicariating Gdap1 in neuronal cells*
11:30–11:45	Federico Magrin, PhD Student, University of Padova	*A chemotypically focused virtual screening identifies novel specific OPA1 inhibitors that enhance cytochrome c release and induce cell death*
11:45–12:00	Kimia Goharian, Research Fellow, University of Padova	*The role of the FIS1-Interactome in Acute Myeloid Leukemia Mitophagy*
12:00–12:15	Promila Lakra, Research Fellow, University of Padova	*Investigating the role of Mitochondria-ER Contact Sites in UV-Induced Melanogenesis*
12:15–12:30	Teresa Valentini, Research Fellow, University of Padova	*Opantimir treatment antagonizing microRNAs that upregulate Opa1: development of a new delivery system*

CLOSURE OF THE GIBB MEETING

12:30-12:40	Final discussion
12:40-12:50	**Award Ceremony – Best Oral Presentation Awards, PhD Students and Post-Docs**
12:50-13:00	Closing remarks and end of meeting

Invited Lectures

Lower mitochondrial calcium uptake capacity than calcium retention capacity in the presence and absence of cyclosporin A

Erich Gnaiger, Cristiane Cecatto, Luiza Helena Daltro Cardoso, Mateus Grings

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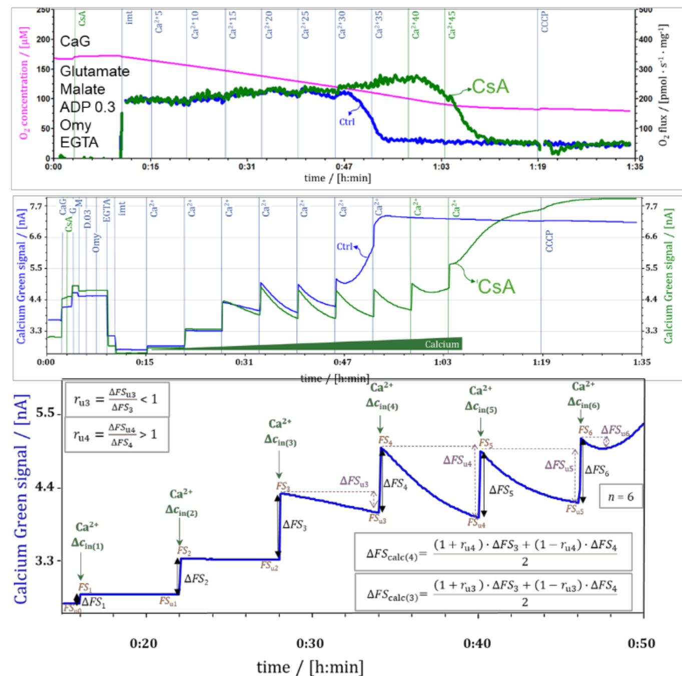

Mitochondrial calcium homeostasis impacts on calcium-dependent cellular bioenergetics and health. Mitochondria contribute to the buffering of cytosolic calcium levels by uptake and release, or storage of osmotically inactive calcium phosphates in the mitochondrial matrix. Defective calcium homeostasis causes bioenergetic stress, cell death, and diseases.

Since the interplay between Ca^{2+} dynamics and oxidative phosphorylation is well recognized, mitochondrial calcium retention capacity is frequently measured as the amount of calcium titrated up to a threshold, when calcium uptake is reversed and mitochondrial permeability transition (mtPT) triggers the release of calcium, disruption of ion balance, dyscoupling, and inhibition of respiration. This is accompanied by leakage into the cytosolic space of signaling molecules such as cytochrome *c*. Developing an integrated assay for real-time monitoring of mitochondrial Ca^{2+} handling and bioenergetics, we measured extramitochondrial [Ca^{2+}] using Calcium Green™-5N (CaG) simultaneously with high-resolution respirometry [1]. The respiration medium MiRCa containing CaG did not impair respiration of isolated mouse liver mitochondria. Ca^{2+} uptake was incomplete as shown by the merely partial decline of [Ca^{2+}] in the medium after initial [Ca^{2+}] titrations and final titrations prior to mtPT.

Consequently, Ca^{2+} uptake capacity, corrected for the increase of [Ca^{2+}] in the medium, was 45 % of the conventionally defined Ca^{2+} retention capacity in controls and 55 % in the presence of cyclosporin A. These results suggest that Ca^{2+} retention capacities depend on experimental mitochondrial concentration, require critical evaluation, and should be replaced by Ca^{2+} uptake capacities properly corrected for increased extramitochondrial calcium concentrations.

In line with most studies on mtPT, ATP was excluded from the incubation medium. However, in the presence of physiological concentrations of ATP, no Ca^{2+} release was observed at Ca^{2+} titrations up to 90 µM, compared to Ca^{2+} release after titration of 35 µM in the absence of ATP. Whereas CsA increased the Ca^{2+}-threshold for mtPT two-fold under the present experimental conditions, physiological concentrations of ATP in the incubation medium exert much more dramatic effects on increasing the Ca^{2+}-threshold. These frequently reported observations should be discussed in relation to the physiological relevance of mtPT and the pharmacological application of CsA.

Reference:

1. Cecatto C, Cardoso LHD, Grings M, Gnaiger E (2025) Mitochondrial Ca^{2+} uptake and high-resolution respirometry. BEC preprints 2025.7. https://doi.org/10.26124/becprep.2025-0007

Nutrient and drug uptake across the membrane

Christian Löw

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Nutrient uptake across the lipid bilayer is essential for life. Out of more than 900 known membrane transport systems in the human body, almost 450 belong to the solute carrier (SLC) family of secondary active transporter. They play a central role in controlling the compartmentalization of metabolism and many members of this superfamily are linked to human disease. Despite being attractive therapeutic targets, many SLCs have been poorly characterized on a functional and structural level.

My research group studies the remarkable substrate promiscuity of peptide transporters (SLC15 family) and tries to understand how vitamins and drugs are recognized and transported (SLC19 family). Furthermore, we use structural biology and biochemical tools to identify substrates of currently orphan SLC transporters (e.g. MFSD1). During this presentation I will highlight our endeavor towards the structural characterization of human SLCs. The resolution revolution in cryo-EM combined with biochemical tools allows us to investigate the functions of SLCs and their relevance in physiology on a molecular level and explore the SLC 'drugability'.

More doubts than certainties: how I survived in science (still having some fun)

Paolo Bernardi
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Lectio Magistralis **"Medaglia G.F. Azzone"**

Individual lives are unique, and yet the lives of individuals have so much in common. Scientists are no exception, and the older I grow the more I learn to appreciate what's unique and what's general in my own experience. The problem is, experience cannot be transmitted. I will therefore narrate some events that, in retrospect, I found to be turning points for my career, hence for my life; and I will try to share some principles that I have honed over the years. While I have no recipe for "success", I may provide some advice in the hope that it can be useful to younger scientists and perhaps to others as well.

The impact of mitochondrial respiration on plants metabolism

Tomas Morosinotto

Department of Biology, University of Padova (Italy)s
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Photosynthetic organisms use sunlight as an energy source to convert carbon dioxide (CO_2) into biomass, playing a central role in the global carbon cycle while mitigating the accumulation of this greenhouse gas in the atmosphere. Plants also rely on respiration during the night and in non-photosynthetic tissues. However, respiration also occurs in photosynthetically active cells, where it consumes reduced carbon and releases CO_2, effectively offsetting part of the products of photosynthesis.
The biological rationale for this seemingly inefficient cycle remains unclear, largely due to the lack of viable mutants for study. We addressed this limitation developing a strategy to generate lines with altered respiration in the non-vascular plant *Physcomitrium patens*. This approach enabled the isolation of mitochondrial mutants that are typically non-viable in other plants, including mutants lacking respiratory Complex IV (cytochrome c oxidase) or Complex V (ATP synthase). Analysis of these mutants revealed that mitochondrial respiration is essential for supplying ATP to the cytosol under light, demonstrating that respiration-derived ATP is critical even in photosynthetically active cells. Structural analyses further showed that disruption of respiratory complexes leads to pronounced alterations in mitochondrial morphology and ultrastructure. These defects extend beyond mitochondria, severely impacting chloroplast morphology as well.
Together, these findings demonstrate that mitochondrial respiration in plants is not merely a backup system for nighttime energy supply. Instead, they highlight a strong metabolic integration between mitochondria and chloroplasts that is essential for sustaining plants growth.

Nutrient-dependent control of OXPHOS: the role of fatty acid oxidation in ageing.

Laura Formentini

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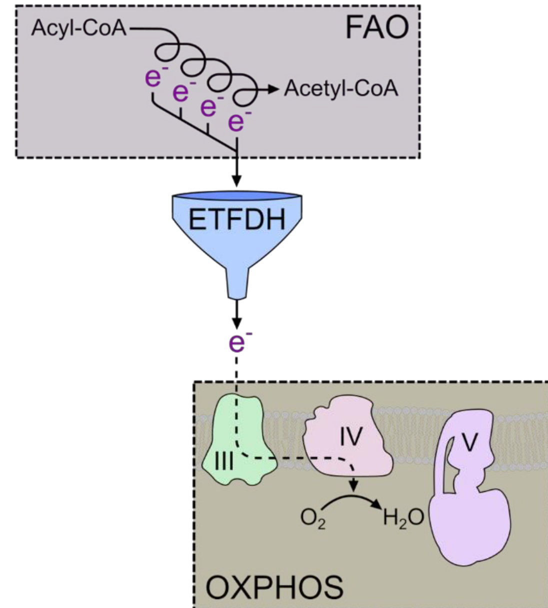

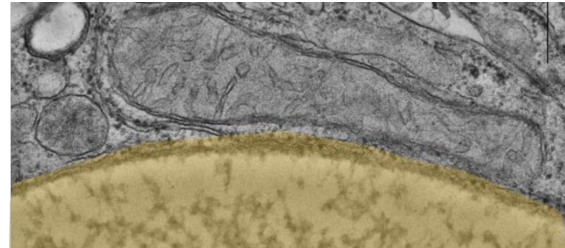

Mitochondrial fatty acid β-oxidation (FAO) decline is increasingly recognized as a hallmark of ageing and age-related disorders. Reduced FAO disrupts lipid signalling and redox homeostasis, promotes adiposity, drives chronic inflammation, and accelerates immunosenescence, collectively increasing the risk of disease. However, current models of FAO inhibition target mitochondrial fatty acid uptake, which mimics short-term physiological regulation (e.g., fed–fasted–refed cycles) but fails to recapitulate the gradual decline in FAO flux and the progressive accumulation of lipid intermediates that occur during ageing, thereby limiting our understanding of disease mechanisms and potential interventions. Building on our discovery that Electron Transfer Flavoprotein Dehydrogenase (ETFDH) acts as final enzyme and master regulator of FAO flux and mitochondrial efficiency [1], we have developed conditional and tissue-specific ETFDH mouse models with partial or complete loss of function in hepatocytes ("LowFAO" mice). These models enable a more pathophysiological simulation of the age-associated decline in hepatic FAO, allowing us to dissect whether and how this process contributes to systemic ageing. Comprehensive profiling of LowFAO animals, including metabolic cage studies and flux analyses, respirometry, mitochondrial and liver homeostasis assessments, systemic multi-omics, and high-dimensional immune profiling, reveals that reduced FAO flux leads to altered lipidoma, intrahepatic cholestasis and liver fibrosis, with secondary effects on pancreatic function, finally reducing metabolic flexibility. Mechanistically, impaired FAO results in the accumulation of metabolic intermediates that alter the post-translational modification (PTM) landscape of mitochondrial proteins, thereby reinforcing mitochondrial dysfunction in a self-amplifying loop. Together, our results identify impaired liver FAO as a key mechanism driving ageing.

References.

1. Herrero Martín JC, Salegi Ansa B, Álvarez-Rivera G, Domínguez-Zorita S, Rodríguez-Pombo P, Pérez B, Calvo E, Paradela A, Miguez DG, Cifuentes A, Cuezva JM, Formentini L. An ETFDH-driven metabolon supports OXPHOS efficiency in skeletal muscle by regulating coenzyme Q homeostasis. **Nat Metab**. 6 (2024) 209-225.

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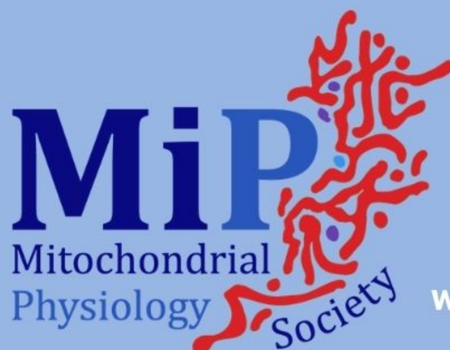
Challenges of mitochondrial diagnostics, prevention, and therapy



December 2-5, 2026
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Short Communications

Lonp1 overexpression affects mitochondrial functions, mtDNA localization, and cytokines production in colorectal cancer

Giada Zanini[1], Valentina Selleri[1,2], Giorgia Sinigaglia[1], Giulia Micheloni[1], Francesca Paone[1], Milena Nasi[3], Marcello Pinti[1]

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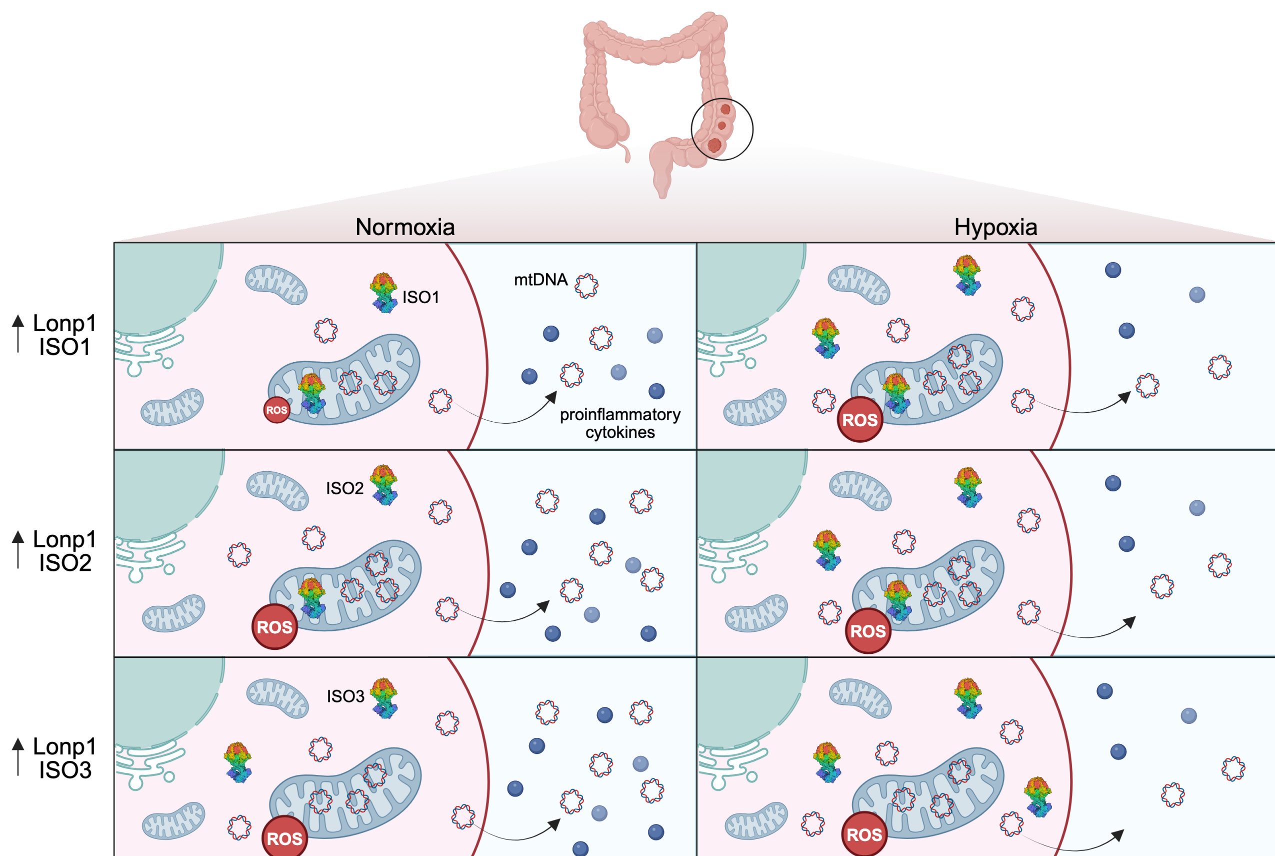
Lonp1 is a mitochondrial protease encoded by nuclear DNA essential for organelle homeostasis and cell response to stress. Lonp1 is induced by several stress stimuli, such as, heat shock, oxidative stress and hypoxia, and could promote cancer cell survival favouring cell resistance to stress. Lonp1 is frequently overexpressed in several tumours, including melanoma, glioma, breast cancer, prostate cancer and colorectal cancer (CRC). Its increased expression is often associated with lower patient survival, although it is not a prognostic marker. To clarify the role of Lonp1 in CRC and, in particular, the impact of isoform 1, 2 or 3 overexpression on the amount and localization of mtDNA, we evaluated the effects of Lonp1 isoforms overexpression in different CRC cell lines. Moreover, since hypoxia is observed in solid tumors, we performed the analysis both in normoxic and hypoxic conditions. Different isoforms overexpression modifies mitochondrial structure and functions, leading to an increase in mitochondrial respiration and ROS levels. Overexpression of Lonp1 isoforms leads also to the release of mtDNA into the extracellular environment and its leakage from mitochondria into the cytosol, both in normoxia and hypoxia. This leakage is directly dependent on the Lonp1 isoform that is overexpressed. Leakage of Lonp1 leads to an increase in the production of proinflammatory cytokines, based on Lonp1 isoform overexpression. These results confirm that Lonp1 plays a key role in modulating mitochondrial functions, with a significant impact on respiration and ROS production. Lonp1 is also crucial not only for mtDNA maintenance, as previously shown, but also for its cellular localization and release, reflected in a different production of proinflammatory cytokines. These changes, together with metabolic changes in CRC cells with different levels of Lonp1, may affect the different levels of lymphocytic infiltrate observed in human CRC tissues.



Immunophenotypic skewing of B cells toward IgD^{-}CD27^{-}IgG^{+} subtype and metabolic attenuation in colorectal cancer

Eleonora Martinis

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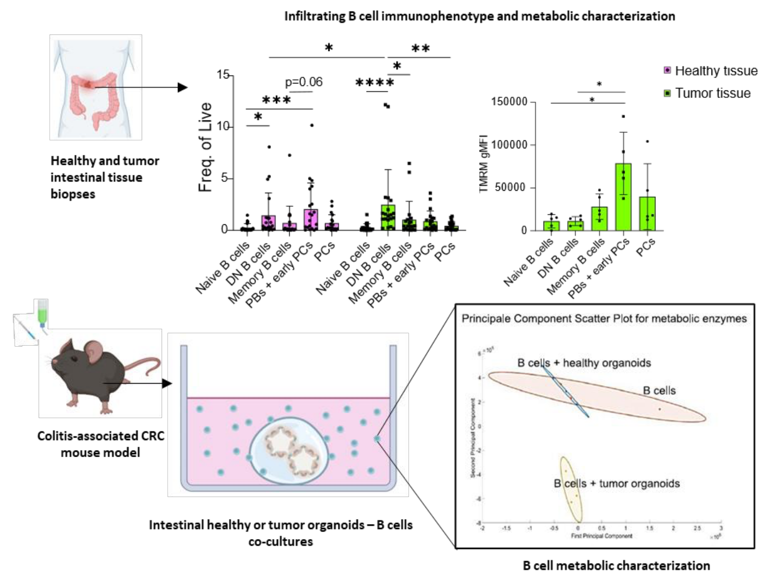

Colorectal cancer (CRC) is the third most prevalent cancer and understanding its tumor microenvironment (TME) is crucial for the development of innovative therapies. Despite the presence of B cells in CRC infiltrate, their clinical significance is poorly understood. In this study, we observed an enrichment of double-negative (DN) B cells, a subset lacking surface IgD and CD27, in CRC biopsies. Typically underrepresented in physiological conditions, DN B cells expand in certain chronic infections, autoimmune diseases, and cancers. Within this subpopulation, low CD21 expression—a phenotypic hallmark of exhaustion—was observed. Consistently, DN B cells displayed low metabolic activity. Accordingly, total B cells infiltrating CRC tissues showed a diminished capacity to differentiate into antibody-secreting cells (ASCs) upon stimulation. In the murine setting, CRC organoids decreased the frequency of ASCs in co-cultured B cells and induced metabolic dysfunction, marked by altered glucose and fatty acid uptake and dysregulated expression of key metabolic proteins. Moreover, B cells displayed reduced glycolysis and mitochondrial respiration, despite increased mitochondrial dependence. This study provides evidence for DN B cell accumulation within CRC infiltrate and metabolic reprogramming of B cells, suggesting that targeting B cell metabolism may represent a promising strategy to potentiate anti-tumor immune responses [1].

1. E. Martinis, S. Tonon, V. Valeri, et al. Immunophenotypic skewing of B cells toward IgD^{-}CD27^{-}IgG^{+} subtype and metabolic attenuation in colorectal cancer. Sci. Rep. 16 (2026) 11403.

ClpP Hyperactivation by ONC212 Disrupts Mitochondrial Function and Targets Cancer Stem Cells in Osteosarcoma and Breast Cancer

Ilenia Paoletti*, Aristide Ferrante*, Francesca Landini*, Mirko Tamma*, Maria Grazia Perrone[$], Antonella Cormio[#], Consiglia Pacelli*, Nazzareno Capitanio*, Claudia Piccoli*
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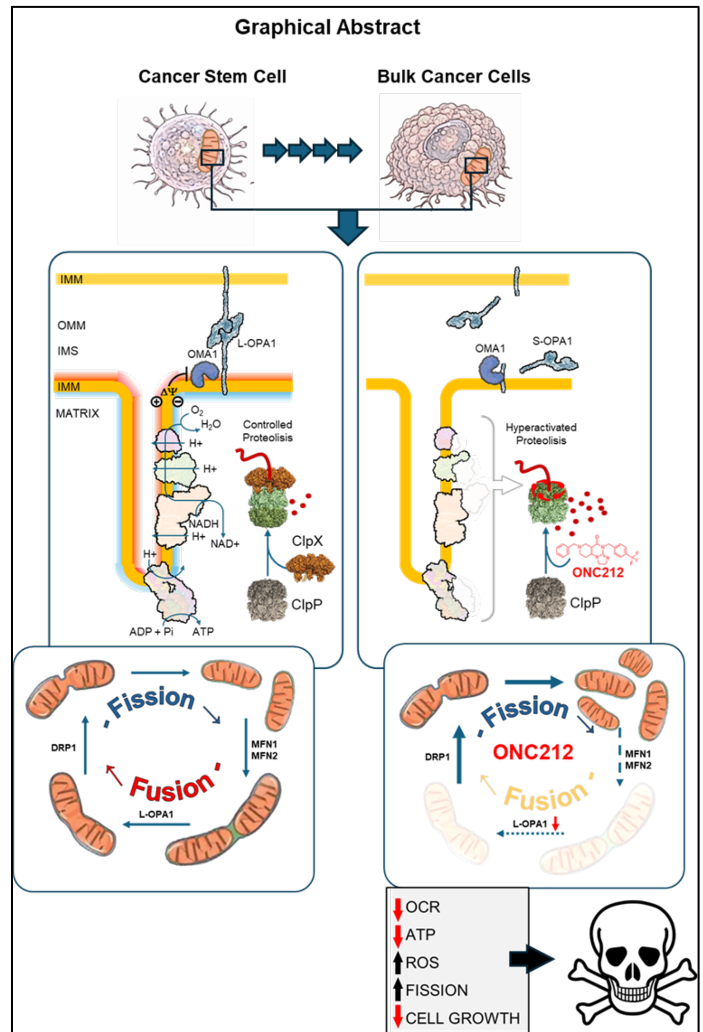
**,Dept. of Clinical and Experimental Medicine, University of Foggia, Foggia (ITA); [$]Dept. Pharmacy-Pharmaceutical Sciences-University of Bari, Bari (ITA); [#]Dept of Precision and Regenerative Medicine and Ionian Area, University of Bari, Bari (ITA);*

Tumor heterogeneity and the persistence of cancer stem cells (CSCs) represent major challenges to effective cancer therapy due to their metabolic plasticity and resistance to conventional treatments. Targeting mitochondrial function has emerged as a promising strategy to overcome these limitations. Here, we investigated the effects of ONC212, a potent agonist of the mitochondrial protease ClpP, on mitochondrial homeostasis, metabolism, and proliferation in osteosarcoma and breast cancer models, including CSC-enriched populations. ONC212 induced a dose-dependent disruption of mitochondrial proteostasis, leading to reduced expression of oxidative phosphorylation (OXPHOS) complexes and impaired mitochondrial respiration, as evidenced by decreased oxygen consumption rates and mitochondrial ATP production. Osteosarcoma cells partially compensated for mitochondrial dysfunction through increased glycolysis, whereas breast cancer cells displayed limited metabolic flexibility, resulting in an overall energetic deficit. Furthermore, ONC212 promoted mitochondrial fragmentation and loss of membrane potential, associated with reduced levels of the long OPA1 isoform, suggesting a shift toward a fission-dominated phenotype. Reactive oxygen species production was cell-type dependent, indicating that oxidative stress is a secondary consequence rather than a primary driver of the observed effects. Importantly, ONC212 significantly inhibited proliferation in both differentiated and CSC-like cells, including chemoresistant populations. These findings identify mitochondrial proteostasis disruption via ClpP hyperactivation as a shared vulnerability in cancer cells, supporting ONC212 as a potential strategy to target resistant tumor populations.



Relevant references

- Currie SQW, et al. Molecular mechanisms of mitochondrial AAA+ proteases. *J Biol Chem*. 2026;302(3):111264.
- Ishizawa J, et al. Mitochondrial ClpP-Mediated Proteolysis Induces Selective Cancer Cell Lethality. *Cancer Cell*. 2019;35(5):721-737.
- Armenise D, et al. Mitochondrial Protease ClpP: Cancer Marker and Drug Target. Pharmaceuticals (Basel). 2025;18(10):1443.

The Mitochondrial Protein Fis1 but not Its Interactor Drp1 Are Essential to Sustain Acute Myeloid Leukemia Stem Cells

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The poor survival rate of Acute myeloid leukemia (AML) has been linked to the existence of difficult to eradicate leukemic stem cells that give rise to the leukemic blasts that constitute the bulk of the tumor. Leukemic stem cells (LSCs) rely on oxidative phosphorylation and display increased levels of the outer mitochondrial membrane protein Fission 1 (Fis1) that promotes fission of the organelle depending on Drp1 and ultimately sustains the renewal of LSCs. To address how Fis1 upregulation promotes LSCs self-renewal, we downregulated Fis1, Drp1 in th p53 proficient AML cell lines NB4, MOLM13, OCI-AML3, and in the p53 deficient cells THP-1. These cells in culture are normally composed of a fraction of LSCs and of a larger one of differentiated blasts with feature of myeloid and macrophages lineages. Flow cytometry assessment of the pan myeloid cell differentiation marker CD11b and of the M1 macrophages marker, CD86, revealed that both were increased in all the cell lines following silencing of Fis1, but not of Drp1. Thus, our data indicate that Fis1 but not mitochondrial fission is required for LSCs maintenance and nominate Fis1 as a potential target to deplete AML of LSCs.

ATP5MC3 mutations and dystonia: investigating mitochondrial dysfunction and autophagic flux

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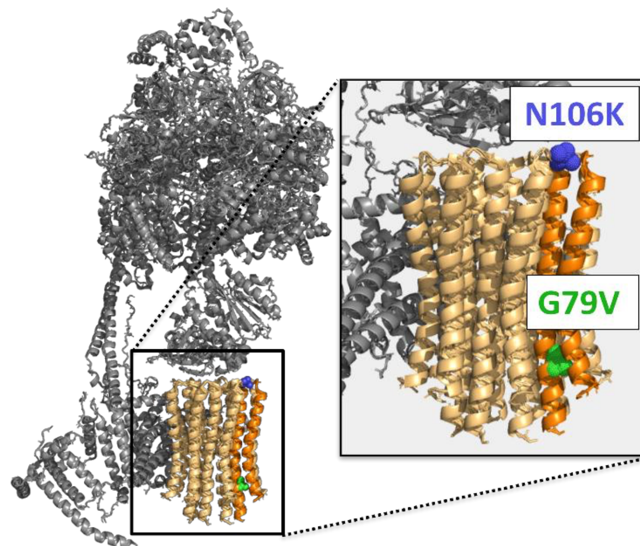
Dystonia is one of the most common motor disorders in childhood, characterized by a wide heterogeneity of disease-causing genes [1]. The multiplicity of genes involved causes frequent errors in diagnosis and often the correct therapeutic strategies are not carried out. Novel pathogenic variants in the *ATP5MC3* gene, which encodes subunit c3 of mitochondrial ATP synthase, have been recently linked to distinct neurological phenotypes: the N106K mutation causes dystonia, whereas the G79V substitution results in psychomotor delay and spasticity without dystonia [2].

This study investigated the differential impacts of these mutations on mitochondrial bioenergetics and autophagy using patient-derived skin fibroblasts. Both N106K (dystonic) and G79V (non-dystonic) fibroblasts displayed significant impairments in mitochondrial respiration and ATP production. However, a phenotypic divergence was observed in cellular clearance: only N106K cells exhibited a marked accumulation of subunit c and of ceroid-lipofuscinosis-like structures.

To clarify the link between subunit c accumulation and dystonia, we also examined fibroblasts from patients with dystonia carrying mutations in *VPS16*, which lead to a block of the autophagosome-lysosome fusion. Interestingly, *VPS16*-mutated fibroblasts similarly showed impaired mitochondrial bioenergetics alongside the accumulation of ceroid-lipofuscinosis-like structures. These findings suggest that while mitochondrial dysfunction is a common denominator of *ATP5MC3* mutations, a concurrent increase of the autophagic pathway may be a critical pathological driver specific to the dystonic phenotype.

[1] M. Thomsen, L.M. Lange, M. Zech, K. Lohmann. Genetics and Pathogenesis of Dystonia. Annu Rev Pathol, 19 (2024) 99-131

[2] M. Zech, R. Kopajtich, K. Steinbrücker, et al., Variants in Mitochondrial ATP Synthase Cause Variable Neurologic Phenotypes, Ann Neurol, 91 (2022) 225



β-hydroxybutyrate restores mitochondrial dysfunctions caused by LETM1 deficiency

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Leucine zipper EF-hand containing transmembrane protein 1 (LETM1) is an essential mitochondrial inner membrane protein that regulates cation homeostasis and mitochondrial volume. Haploinsufficiency or pathogenic variants of LETM1 contribute to Wolf–Hirschhorn syndrome (WHS) or other severe disorders, including to neurological and metabolic impairments ranging from seizures, developmental delay, respiratory chain deficiencies, and mitochondrial structural defects [1,2]. Ketone bodies (KBs), particularly β-hydroxybutyrate (BHB), have emerged as alternative energy substrates that can exert neuroprotective effects [3]. Here, we investigated the effect of BHB in patient-derived fibroblasts carrying LETM1 deficiency. Our preliminary data shows that BHB restores mitochondrial integrity and respiratory functions. These functional improvements are accompanied by changes in the abundance of mitochondrial proteins, which will be further discussed.

References:

1. SEM Mohammed and K Nowikovsky, The mysteries of LETM1 pleiotropy, Pharmacological research vol. 210 (2024): 107485
2. R Kaiyrzhanov et al., Bi-allelic LETM1 variants perturb mitochondrial ion homeostasis leading to a clinical spectrum with predominant nervous system involvement, American journal of human genetics vol. 109,9 (2022) 1692-1712
3. SM Gough et al., Neuroprotection by the Ketogenic Diet: Evidence and Controversies, Frontiers in nutrition vol. 8 (2021) 782657

ASSESSING THE IMPACT OF MITOCHONDRIAL TRANSPLANTATION ON CARDIAC PERICYTES VIABILITY IN VITRO

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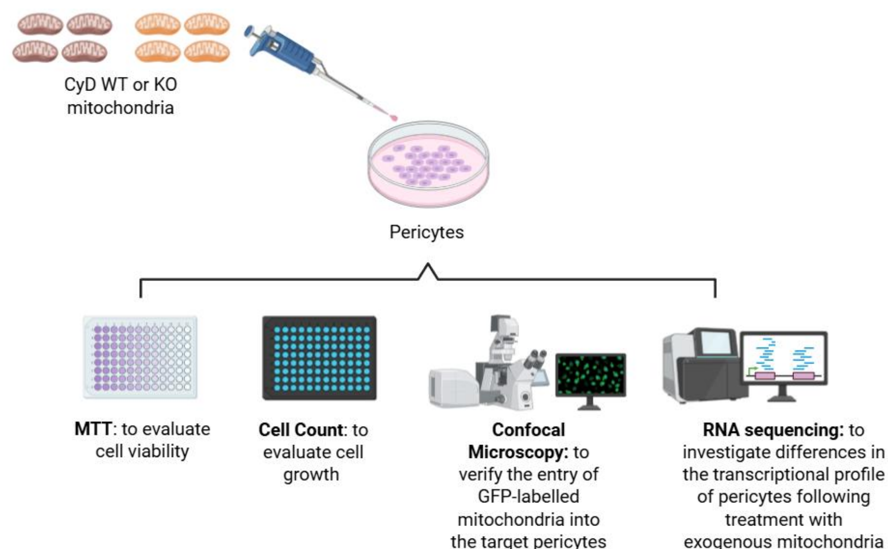
Delivering isolated, healthy mitochondria to cells with bioenergetic defects is an emerging therapeutic strategy for several conditions, including cardiovascular diseases. However, effectively targeting these organelles to specific tissues and enhancing their cellular internalization remain primary challenges. Furthermore, in extracellular fluids or the bloodstream, calcium overload triggers the prolonged opening of the mitochondrial permeability transition pore (PTP), compromising the survival of isolated mitochondria. To address this, our study evaluated the transplantation of calcium-desensitized mitochondria into cultured pericytes, comparing wild-type (WT) HEK293-derived mitochondria with those isolated from Cyclophilin D (CyPD) knock-out HEK293 cells.

Following a 24-hour co-incubation, both fresh WT and KO mitochondria significantly increased pericyte viability and proliferation, as assessed by MTT assay and cell counts. These findings suggest that PTP desensitization plays a minor role in this setting. GFP-labeling confirmed the successful internalization of exogenous mitochondria, while transcriptomic analysis corroborated that mitochondrial transplantation modulates mitochondrial pathways and cell cycle regulation in recipient pericytes.

In conclusion, HEK293-derived mitochondria successfully internalize into recipient pericytes and boost their viability. These results reinforce the therapeutic potential of mitochondrial transplantation strategies.



Mitochondrial effects of a CODAS-associated LONP1 mutation and their modulation by Lonp1 activators

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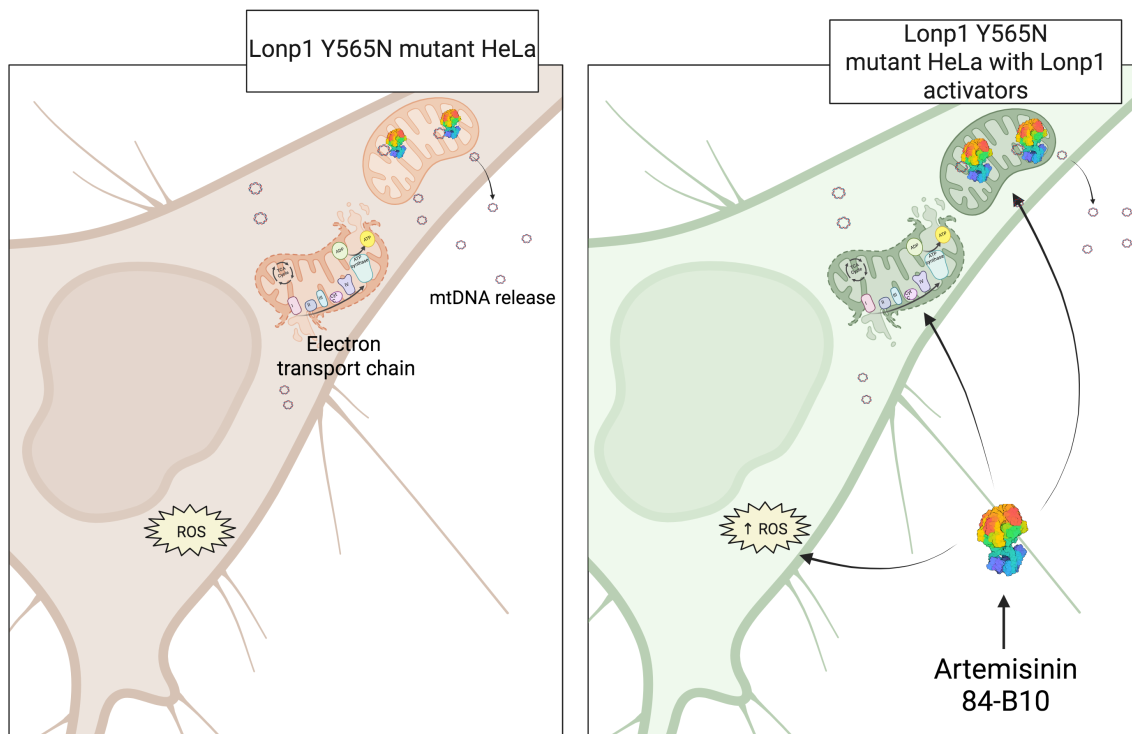
Lonp1 is a mitochondrial protease encoded by nuclear DNA essential for organelle homeostasis and cell response to stress. Pathogenic mutations of Lonp1 can cause CODAS syndrome, characterized by Cerebral, Ocular, Dental, Auricular, and Skeletal anomalies. Reported symptoms show a high degree of variability, all characterized by impaired development of multiple organs and tissues.

In our previous work, we reported the case of a five-year-old girl carrying a novel heterozygous Y565N mutation in the LONP1 gene.

To analyze the impact of the heterozygote mutation and to mimic, as closely as possible, the coexistence of the wild-type and mutant forms, HeLa cells were stably transduced with the same mutation identified in the proband using a viral infection. Mitochondrial morphology and function in these cells were then analyzed and compared them to control cells. Although this variant has not yet been formally classified as causative of CODAS syndrome, our analyses revealed that it compromises key mitochondrial functions, leading to cellular dysfunction. These findings strongly suggest that the mutation is likely pathogenic.

Two molecules known as activators of Lonp1, artemisinin and 84-B10, were tested on HeLa cells to enhance the activity of Lonp1 and counteract, as far as possible, the detrimental effects of the disease associated mutation.

These compounds appear to exert a modulatory influence on several mitochondrial parameters in the mutated HeLa cells, including respiration, superoxide anion production, mitochondrial morphology, and the release of cell-free mitochondrial DNA. These results suggest that these Lonp1 activators may potenrially counteract, at least in part, the detrimental effects associated with the pathogenic Y565N mutation, by influencing mitochondrial homeostasis and stress-response pathways, leading the pathway for a therapeutic strategy.



A novel ATAD3A variant associated with altered mitochondrial homeostasis

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ATAD3A encodes a mitochondrial membrane-spanning ATPase that plays a key role in mitochondrial dynamics, nucleoid organization, and cholesterol metabolism. Pathogenic variants in this gene are linked to mitochondrial disorders. We identified and functionally characterized a de novo heterozygous variant, c.961C>A (p.Arg321Ser). The affected residue is conserved and lies between the transmembrane domain TM2 and the Walker A ATP-binding domain, a region essential for protein function. The substitution is predicted to be pathogenic. Computational modelling of the mutated ATAD3A resulted in a pronounced conformational change of both the monomeric protein and its oligomers suggesting impairment in the formation of functional complexes.

To assess the functional impact of the variant, mitochondrial bioenergetics were analyzed in patient-derived fibroblasts compared with parental controls. Basal respiration remained comparable, while maximal respiratory capacity was significantly elevated, accompanied by increased mitochondrial DNA copy number, consistent with expanded mitochondrial mass. The extent of the respiration-driven mitochondrial membrane potential ($\Delta\Psi m$) was lower in the patient-derived sample. Glycolytic parameters—including basal glycolysis, glycolytic reserve, and glycolytic capacity—were also significantly higher, indicating a metabolic shift toward glycolysis that contributed to elevated total ATP production, predominantly of glycolytic origin. ROS levels were not significantly altered relative to parental controls, and mitochondrial morphology was preserved.

Taken together, these findings suggest that mitochondrial function is maintained at baseline through compensatory adaptations, including increased mitochondrial content and enhanced glycolytic flux. While these results are compatible with altered mitochondrial quality control or metabolic regulation, further studies are required to assess these pathways and clarify the pathogenic mechanism of this variant.

Mitochondrial Fragmentation Induced by the CFTR Modulators Lumacaftor and Ivacaftor in Immortalized Cystic Fibrosis Cell Lines

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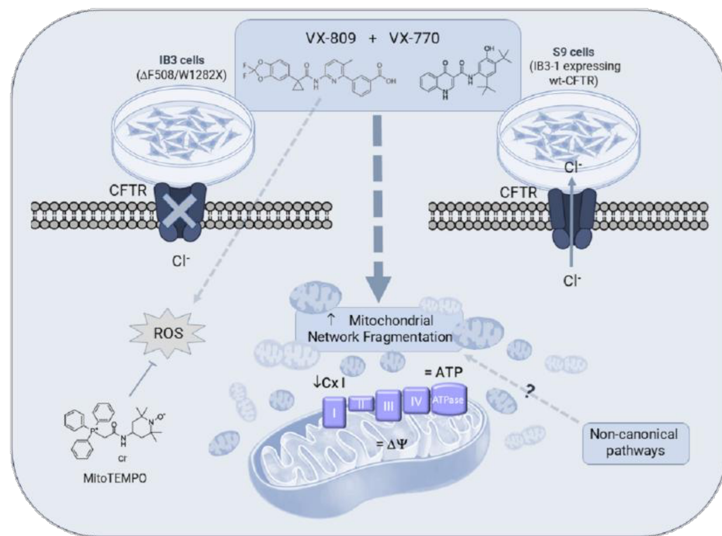

Cystic fibrosis (CF), caused by mutations in the CFTR gene encoding a cAMP-regulated chloride channel, is associated with mitochondrial dysfunction and altered mitochondrial dynamics. Previous studies identified CFTR- and chloride-dependent genes involved in mitochondrial regulation, including *MTND4* and *CISD1*, and demonstrated impaired mitochondrial respiratory chain activity in CF cells. In this study, the effects of the CFTR modulators lumacaftor (VX-809) and ivacaftor (VX-770), alone or combined, were investigated in heterozygous ΔF508/W1282X IB3-1 cells, S9 cells expressing wild-type CFTR, and C38 cells expressing truncated CFTR. After 48 h treatment, mitochondrial morphology, cell viability, mitochondrial membrane potential (ΔΨm), reactive oxygen species (ROS) production, respiratory chain complex I–III activity, ATP levels, CFTR function, and the expression of the mitochondrial dynamics proteins MFN1 and DRP1 were analyzed. Combined VX-809/VX-770 treatment induced significant mitochondrial fragmentation in both CF and wild-type CFTR cells without affecting viability, ATP levels, or ΔΨm. In addition, combined treatment reduced complex I–III activity, whereas MFN1 and DRP1 expression remained unchanged. Although individual treatments differentially modulated ROS production and ΔΨm, these effects were not significant after combined treatment. Importantly, no restoration of CFTR function was observed in heterozygous CF cells. These findings reveal a previously unrecognized impact of CFTR modulators on mitochondrial morphology and function, suggesting that VX-809/VX-770 therapy may negatively affect mitochondrial activity independently of CFTR rescue mechanisms..

Two novel FLAD1 variants causing UPR in fibroblasts from a paediatric patient

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FAD synthase (FADS), coded by FLAD1 gene in humans, catalyzes the last step of biosynthesis of the redox-cofactor FAD. *FLAD1* has been identified as a causative gene of lipid storage myopathy due to FAD synthase deficiency (LSMFLAD, OMIM #255100) [1], a disorder resembling multiple acyl-CoA dehydrogenase deficiency (MADD) and associated with altered mitochondrial metabolism.

Here, we report a case of paediatric patient presenting with hypotonia, developmental delay, and growth deficiency, who carries two FLAD1 missense variants, but lacks classical LSMFLAD plasmatic markers. Genetic analysis in this patient revealed two heterozygous FLAD1 missense variations, c.203A> G (p. D68G) and c.503A>T (N168I), both classified of Uncertain Significance. We characterised a novel recombinant mutant of FADS,

Searching for structure-function relationships we succeeding produced in *E.coli* and purified by IMAC recombinant FADS2 N168I and wt proteins. Results were obtained by comparing the FADS activity of the recombinant N168I mutant with that of the wt enzyme showing a reduction for the synthesis activity but not for the hydrolysis. FADS1 (wt) and its mutant D68G were produced in recombinant form, but their low yield and poor solubility prevented us from characterizing them at functional and molecular level.

We also investigated some biochemical features in patient's fibroblasts where we found the FAD synthesis rate about 50% lower than the control range.

Suspecting a proteostasis derangement in patient's fibroblast, we revealed increased levels of some UPR markers, presumably correlated with a mitochondrial phenotype, further supported by the specific increase of the amount of the mitochondrially encoded subunit I of Complex IV.

Our results support a pathogenic significance of (at last one of) the reported variations in FLAD1 and expand the current understanding of FLAD1-associated disease mechanisms, reinforcing the role of proteostasis derangements and UPR.

References:

[1] RKJ Olsen, et al. Am J Hum Genet. (2016) 98(6):1130-45

Effects of Compartment-Specific Unfolded Mitochondrial Protein Aggregates (UMPA) on Mitochondrial Function

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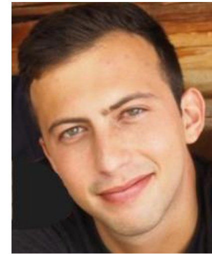

Mitochondria play a fundamental role in energy conversion, metabolism, calcium signalling and cell death. Their function is regulated through quality control mechanisms including chaperones and proteases activation, promoted by the mitochondrial unfolded protein response (UPRmt); and dynamics processes like fission and fusion that contribute to the maintenance of the organelle integrity. When these systems face problems such as protein import issues, metabolic stress, or genetic mutations, it can lead to an accumulation of "Unfolded Mitochondria Protein Aggregates" (UMPA). The accumulation of UMPA within mitochondria have been observed in conditions such as Parkinson's disease [1], Alzheimer's disease [2], and other proteinopathies [3][4], suggesting that uncontrolled UMPA formation may contribute to their pathogenesis. My project aims to study the effects of UMPA accumulation on mitochondrial function and cellular responses, using an inducible expression of self-aggregating protein sensors specifically targeted to the matrix or inter-membrane space. The effect of UMPA on mitochondrial function will be assessed through measuring bioenergetics, ROS production, OXPHOS integrity, and mitochondrial calcium signaling. These studies will be complemented by time-course experiments measuring how long cells can tolerate mitochondrial aggregates before undergoing senescence or apoptosis, defining a window of cellular resistance to mitochondrial proteotoxic stress. Furthermore, I will use a multi-omics approach to profile transcriptomic and cellular proteomic changes associated with matrix- or IMS-specific UMPA formation. These experiments will underline mitochondrial stress pathways and identify adaptation mechanisms activated in response to compartment-specific aggregate formation. My project wishes to define the impact of UMPA on mitochondrial function and cell fate, providing a basis for interrupting the vicious cycle of mitochondrial proteotoxic stress in pathological contexts.

[1] Burtscher, J., Ganjam, G. K., Mehta, B., Devi, L., Outeiro, T. F., & Pértille, F. (2023). Defective mitochondrial proteostasis and Lewy pathology in Parkinson's disease dopaminergic neurons. NPJ Parkinson's Disease, 9, Article 52.

[2] Wang, W., Zhao, F., Ma, X. et al. Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: recent advances. Mol Neurodegeneration 15, 30 (2020).

[3] Oliveira, J. M. (2010). Nature and cause of mitochondrial dysfunction in Huntington's disease: focusing on huntingtin and the striatum. Journal of Neurochemistry*,* 114(1), 1–12.

[4] Vande Velde, C., et al. (2008). Misfolded SOD1 associated with motor neuron mitochondria alters mitochondrial shape and distribution prior to clinical onset. PLoS ONE, 3(7), e2206.

mtDNA stability alters the mito-nuclear crosstalk through calcium homeostasis

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Mitochondria are dynamic organelles crucial for metabolism, ATP production, and stress adaptation. Although they contain over 1,000 proteins, only 13 are encoded by mitochondrial DNA (mtDNA), making them heavily reliant on nuclear genes. Mitochondrial fitness is communicated to the nucleus through retrograde signalling, often via metabolites, but how it changes in mtDNA stability affect this process remains unclear.
Here, we investigated the impact of mtDNA stability on retrograde signalling using of AP-endonuclease 1 (APE1), a key endonuclease required for mtDNA base excision repair as a model. Modulation of APE1 levels resulted in corresponding changes in mtDNA stability.
We found that enhanced mtDNA stability attenuated calcium signalling, reducing the rate of activation of immediate early response genes following rotenone-induced oxidative stress. We validated these findings in an acute myeloid leukaemia (AML) model, using AML cell lines, and by RNA sequencing and proteomic data from primary patient samples from the BloodSpot and PRIDE databases, observing a negative correlation between APE1 expression and calcium signalling signatures.
Collectively, our findings demonstrate that mtDNA stability, modulates calcium-dependent retrograde signalling and influences the kinetics of the oxidative stress response. Linking the mitochondrial genome maintenance and stress signalling pathways, with potential implications for mitochondrial biology and haematological malignancies.

Abstract

Effect of USP14 inhibition and molecular mechanism of mitophagy induction

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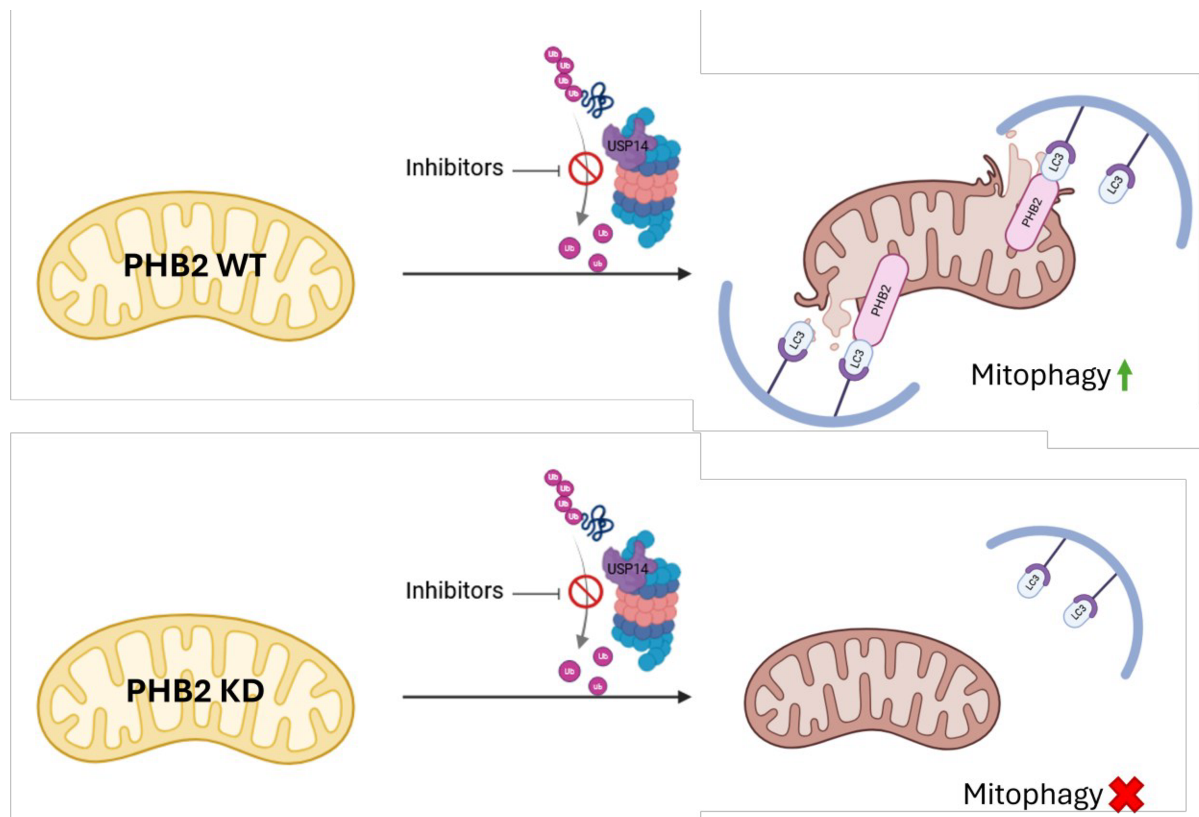
Accumulation of dysfunctional mitochondria is strictly correlated with the onset of neurodegenerative diseases. To counteract this process and maintain homeostasis, cells possessed various mitochondrial quality control mechanisms, including mitophagy, a well-characterized process that select and degrade damaged mitochondria via autophagy. During mitophagy, E3-ubiquitin ligases and deubiquitinating enzymes (DUBs) are antagonist enzymes responsible for tagging damaged mitochondria with ubiquitin, and for promoting or preventing their degradation, respectively. Inhibiting specific DUBs can therefore enhance mitochondrial ubiquitination, and promote mitophagy. We found that inhibition of DUB USP14 enhances mitochondrial ubiquitination and mitophagy. Our data show an accumulation of autophagic markers, particularly in the mitochondrial fraction. We identified MARCH5 as the opposing E3-ubiquitin ligase, and VDAC1 as shared target between USP14 and MARCH5-dependent ubiquitination. We further dissected the mitophagic pathway, and found that VDAC1 ubiquitination leads to efficient exposure of Prohibitin-2 (PHB2), a mitophagy receptor that resides in the inner mitochondrial membrane. Consistent with this, in PHB2 KD cells, the mitophagic effect of USP14 inhibition is diminished.

In conclusion, inhibition of USP14 with specific inhibitors enhances selective mitophagy in a PHB2-depedent fashion.



Mechanism of organic cation transport mediated by the OCTN1 human transporter

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OCTN1 belongs to the SLC22 family of cation transporters. Despite this classification, the list of acknowledged substrates of OCTN1 includes some zwitterions, ergothioneine and carnitine, as well as some organic cations, such as the prototype Tetraethylammonium (TEA) and the physiological substrate Acetylcholine (Ach). While sodium-dependent transport mechanism has been proposed for ergothioneine and carnitine, no clear data are available for the transport mode of organic cations. To address this open question we employed the experimental model of proteoliposomes harbouring hOCTN1, which was obtained through recombinant production in *E. coli*. We measured the uptake of ^{14}[C]-TEA or ^{3}[H]-Ach in proteoliposomes under different pH conditions and in the presence of membrane potential. In particular, we imposed a ΔpH by changing the buffer or used Valinomycin, a ionophore, in the presence of a K^+ gradient. Increasing the concentration of protons in the intraliposomal compartment resulted in a concomitant increase in the uptake of TEA or Ach. A similar increase was detected when Valinomycin was used to generate a membrane potential positive outside. Opposite results were obtained when the concentration of protons was increased in the extraliposomal compartment. We also studied the effect of ΔpH on the efflux of TEA or Ach from proteoliposomes; increasing the concentration of H^+ in the extraliposomal compartment strongly stimulated the efflux. Interestingly, a similar effect was observed when H^+ was substituted by Na^+. The effect of ΔpH on the efflux of TEA in the presence of the protonophore CCCP was significantly decreased. This indicates that OCTN1 functions as a proton/organic cation antiporter. Taken together these data are consistent with the physiological role of OCTN1 in the non-neuronal cholinergic system, in which Ach is exported to modulate the inflammatory response and cell growth.

Inhibition of the Amino Acid Transporter LAT1 by 1-Methyltryptophan reveals a novel therapeutic strategy in cancer

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Abstract

L-type amino acid transporter 1 (LAT1) is a plasma membrane antiporter responsible for the uptake of essential amino acids, including tryptophan, mainly expressed in the blood–brain barrier and placenta. [1]

LAT1-mediated tryptophan transport contributes to the regulation of the kynurenine pathway through modulation of indoleamine 2,3-dioxygenase (IDO), a rate-limiting enzyme frequently upregulated in cancer to promote immunosuppression and tumor immune escape, as well as in placenta to prevent rejection of the fetus during pregnancy. [2]

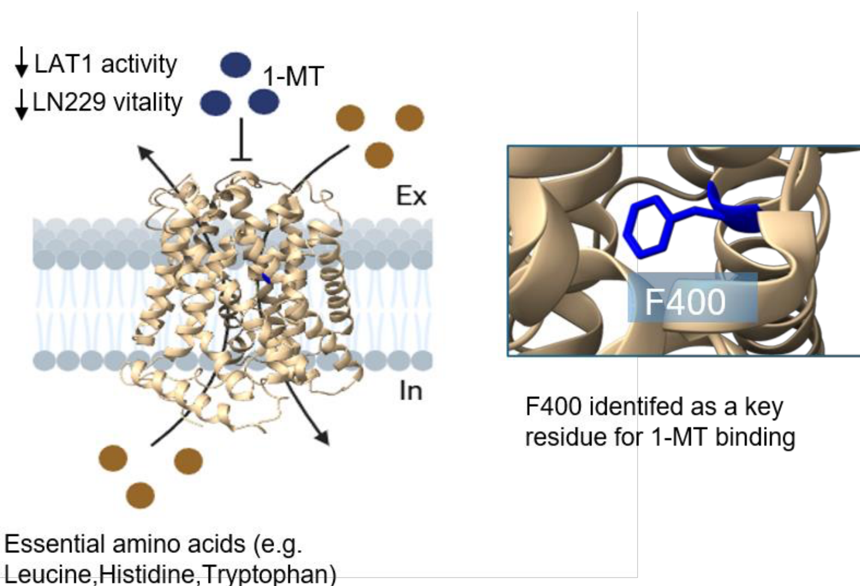
The tryptophan analog 1-methyltryptophan (1-MT) widely described as an IDO inhibitor, was investigated as a potential LAT1 ligand. We demonstrated that 1-MT inhibits LAT1 with IC_{50} value in the micromolar range, using recombinant protein in *in-vitro* and in *ex-vivo* systems. Kinetic analyses indicate a mixed-type of inhibition, suggesting the presence of a 1-MT binding site other than substrate. Then, *in-silico* analyses and site-directed mutagenesis identified F400 as a key residue for the 1-MT binding. Interestingly, 1-MT does not act as a counter-substrate of LAT1 reconstituted in proteoliposomes. Moreover, 1-MT treatment reduced both LAT1 transport activity and the viability of the glioblastoma cell line, LN-229.

Overall, this data identified a novel LAT1 not transported inhibitor suggesting its potential for the treatment of cancer with poor prognosis and a dual effect on LAT1 and IDO.

References:

[1] Scalise M, Scanga R, Console L, Galluccio M, Marzano F, Magrì A, Pochini L, Indiveri C. Crossing the Borders: the amino acid transporter LAT1 (SLC7A5) in the Blood-Brain Barrier. *Biochim. Biophys. Acta* (BBA) – Mol. Cell Res. 2025;191:106070

[2] Terness P, Bauer TM, Röse L, Dufter C, Watzlik A, Simon H, Opelz G. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med.* 2002; 196(4):447–457



ATP synthase subunits e and g are essential for Ca^{2+} homeostasis and development in *Drosophila melanogaster* independent of oxidative phosphorylation

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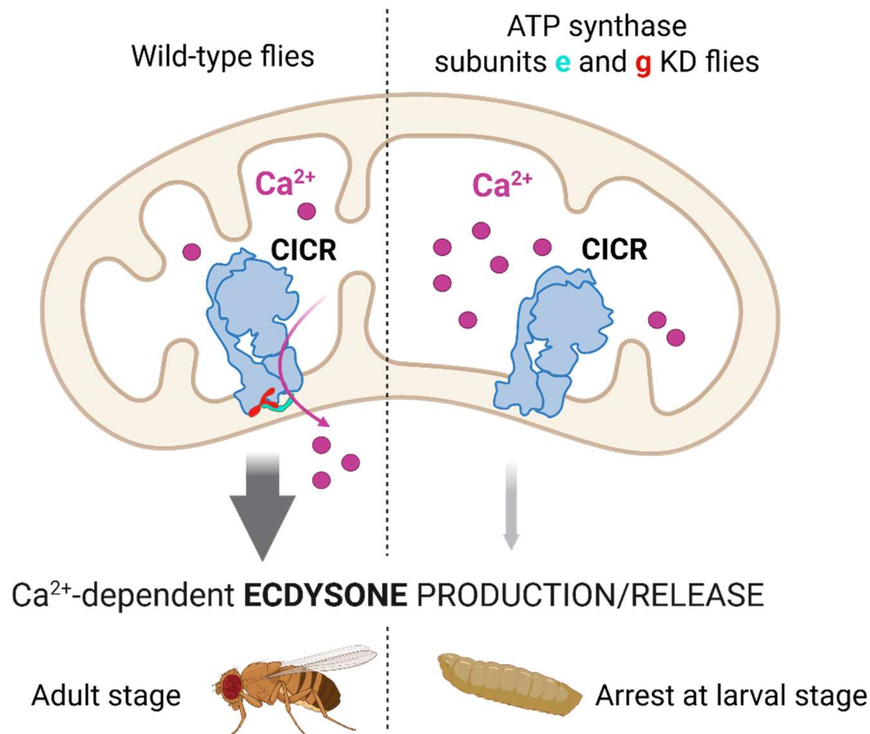

Beyond its roles in ATP production and *cristae* generation, mitochondrial ATP synthase has been implicated in generating the permeability transition pore (PTP), a Ca^{2+}-activated, high-conductance channel that leads to matrix swelling and cell death in mammalian cells. It has been shown that ablation of e and g subunits of ATP synthase affects PT occurrence, suggesting a primary role of these proteins in PTP formation. In *Drosophila melanogaster* the PTP homolog forms a selective Ca^{2+}-induced Ca^{2+}-release (CICR) channel whose physiological relevance at the organism level remains poorly understood. Whether fruit fly ATP synthase mediates the CICR has never been investigated genetically *in vivo*. Thus, *Drosophila* e and g subunits were downregulated ubiquitously and specifically in different tissues. Ubiquitous downregulation causes an arrest in fly development at larval stage, impairs ATP synthase dimerization and oligomerization, decreases *cristae* formation and slightly mitochondrial respiration, yet total ATP is unaltered. Contrarily, although muscle-specific downregulation leads to a deficient climbing ability, to an impaired oligomerization of ATP synthase and *cristae* curvature, mitochondrial respiration is unaffected, as well as ATP levels. Altogether, these results suggest that the observed phenotypes may be caused by the impairment of other functions of ATP synthase, such as its ability to generate the CICR. Outstandingly, mitochondria from both ubiquitous knockdown animals accumulated larger Ca^{2+} loads, consistent with an impaired CICR, which was accompanied by near-complete loss of ecdysone, the Ca^{2+}-dependent master hormone of metamorphosis. In line with these results, neuron-specific knockdown flies displayed defective mitochondrial Ca^{2+} efflux and altered synaptic organization at the neuromuscular junction, consistent with an established role of Ca^{2+} in synaptic growth. Our findings suggest that ATP synthase functions as a CICR channel controlling Ca^{2+} homeostasis, endocrine signaling and development in *Drosophila*.

The C-terminus of subunit e is essential to switch the ATP synthase into the mitochondrial permeability transition pore

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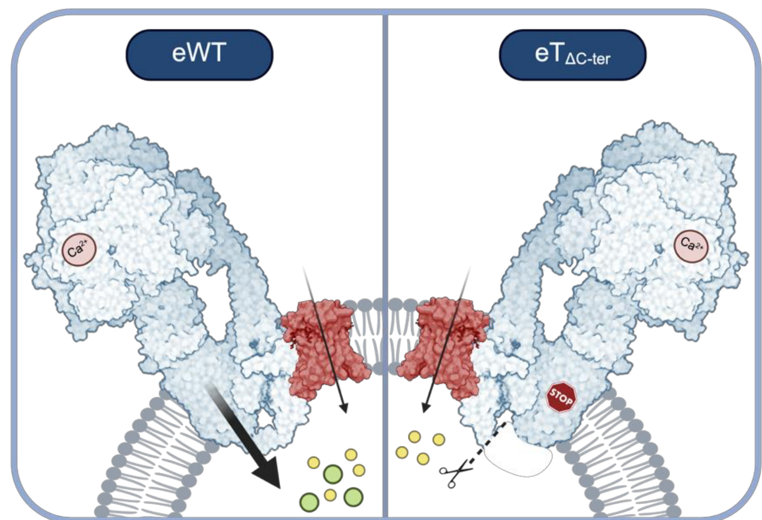
The mitochondrial permeability transition (PT) refers to a sudden increase in the inner mitochondrial membrane permeability caused by the opening of the PT pore (PTP), a Ca^{2+}-activated, high-conductance channel. Recent evidence challenges the long-standing debate on PTP molecular identity, pointing to ATP synthase as a key player. However, in absence of the ATP synthase subunits e and g, that were shown to be crucial for channel formation, an alternative permeability pathway mediated by the adenine nucleotide translocator (ANT) might emerge[1].

Despite the growing understanding of the PT, the mechanism of channel formation by the ATP synthase remains elusive. The "death finger" model proposes that a channel forms within the c-ring after the removal of the lipid plug filling the rotor. This theory is supported by high-resolution structures of ATP synthase which revealed a physical connection between subunit e and lysolipids filling the c-ring. In this model, Ca^{2+} binding to the F_1 domain induces a conformational change transmitted via the peripheral stalk to subunit e, triggering pore formation through lipid extraction.

To test this hypothesis, we combined site-directed mutagenesis with functional analyses of mitochondrial properties in HeLa cells to disentangle ATP synthase catalysis from its channel activity. Using this genetic framework, we show that truncation of the C-terminus of ATP synthase subunit e or the point mutation of its terminal lysin (eK69A), preserves enzyme function while profoundly altering PTP behaviour. Our results identify this region as a critical determinant of pore opening and reveal a previously masked contribution of ANT, highlighting the molecular complexity underlying PTP formation.



[1] A. Carrer, L. Tommasin, J. Šileikytė, F. Ciscato, R. Filadi, A. Urbani, M. Forte, A. Rasola, I. Szabò, M. Carraro, P. Bernardi, Defining the molecular mechanisms of the mitochondrial permeability transition through genetic manipulation of F-ATP synthase, Nature Communications. 12 (2021) 4835–4835.

Targeting mPTP improves mitochondrial bioenergetics and muscle regeneration in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder characterized by progressive myofiber degeneration, calcium imbalance, mitochondrial dysfunction, and chronic inflammation. Mitochondrial alterations, once considered secondary to sarcolemma instability, are now recognized as potential upstream drivers of disease progression, affecting regeneration and immune cell recruitment. We investigated whether inhibition of the mitochondrial permeability transition pore (mPTP) restores mitochondrial function and ameliorates disease features in the *sapje* zebrafish model [1,2].

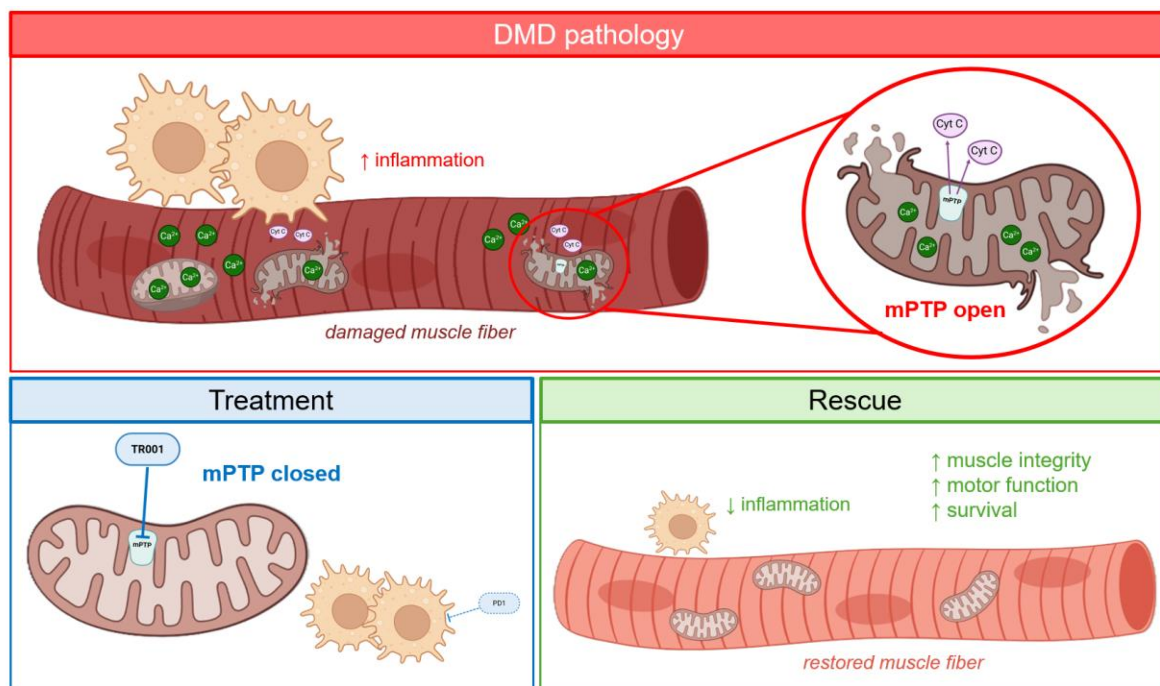
Sapje larvae were treated with the mPTP inhibitor TR001, the anti-inflammatory compound Prednisone Derivative 1 (PD1), or their combination. Effects were assessed on survival, muscle structure, locomotor performance, mitochondrial function, and macrophage infiltration.

TR001 preserved muscle organization, restored mitochondrial membrane potential, and normalized oxidative metabolism, improving motor performance and survival while reducing macrophage infiltration. PD1 showed limited efficacy, with partial structural and functional recovery and incomplete mitochondrial rescue. Combined treatment did not provide an additive benefit, supporting mitochondrial dysfunction as an upstream event in DMD pathogenesis.

Collectively, these findings identify mitochondrial dysfunction as a key driver of disease and highlight mPTP as a promising therapeutic target.

1. A. Stocco, N. Smolina, P. Sabatelli, J. Šileikytė, E. Artusi, V. Mouly, M. Cohen, M. Forte, M. Schiavone, P. Bernardi, Treatment with a triazole inhibitor of the mitochondrial permeability transition pore fully corrects the pathology of sapje zebrafish lacking dystrophin, *Pharmacol. Res.* 165 (2021) 105421

2. T. Briston, D.L. Selwood, G. Szabadkai, M.R. Duchen, Mitochondrial permeability transition: a molecular lesion with multiple drug targets, *Trends Pharmacol. Sci.* 40 (2019) 50–70



N-terminal cleavage reshapes Cyclophilin D dynamics and is associated with enhanced F-ATP Synthase binding

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Protein function emerges from the interplay between structure and dynamics. Cyclophilin D (CyPD) is a central regulator of the mitochondrial permeability transition pore (mPTP), a non-selective channel whose transient opening contributes to mitochondrial Ca^{2+} homeostasis, whereas sustained opening promotes bioenergetic collapse and cell death.

We recently identified two CyPD species in mammalian mitochondria: a full-length form (FL-CyPD) and a truncated form (ΔN-CyPD) generated by proteolytic removal of residues 1–13 (N-terminal tail, NTT), a process putatively mediated by calpain 1. Although Nuclear Magnetic Resonance (NMR) structural analyses revealed no significant differences between FL-CyPD and ΔN-CyPD within their shared folded region, biochemical and functional studies demonstrated that ΔN-CyPD displays enhanced binding to the oligomycin sensitivity-conferring protein (OSCP) subunit of F-ATP Synthase under near-physiological ionic strength conditions, modulating its enzymatic activity. To investigate the molecular basis of these functional differences, we examined the conformational behaviour of FL-CyPD and ΔN-CyPD under varying ionic strength conditions. Combining microsecond-scale molecular dynamics simulations with NMR chemical shift perturbation and line-shape analyses, we compared the two protein forms in the absence and presence of 150 mM KCl. Our results reveal that the N-terminal tail modulates CyPD conformational dynamics and its response to physiologically relevant ionic strength conditions, highlighting a dynamic role for the NTT that may underlie the enhanced interaction of ΔN-CyPD with F-ATP Synthase and possibly its increased propensity to promote mPTP opening.

NHK1 Peptide Targeting VDAC1 Mitigates Mitochondrial Impairment in ALS Primary Fibroblasts

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Amyotrophic lateral sclerosis (ALS) is a progressive and incurable neurodegenerative disorder characterized by the selective loss of spinal motor neurons. Mitochondrial dysfunction is an early and common feature of both sporadic (sALS) and familial (fALS) forms. The accumulation of misfolded proteins such as SOD1, TDP-43, and FUS at mitochondria impairs respiration and disrupts key metabolic exchanges, including ADP/ATP and NAD^+/NADH. Voltage-Dependent Anion Channel 1 (VDAC1), the main protein of the outer mitochondrial membrane, regulates these fluxes and acts as a physiological receptor for hexokinase I (HKI). In ALS, mutant SOD1 competes with hexokinase for binding to VDAC1 [1].

The NHK1 peptide targets this interaction, preventing SOD1-G93A association with mitochondria and restoring mitochondrial function and viability in NSC34 cells [2]. In this study, we evaluated the NHK1 effects in primary fibroblasts from sALS and fALS patients (TDP-43, FUS, C9orf72 mutations) and SOD1-G93A mice using Seahorse and Oroboros respirometry.

The ALS fibroblasts showed altered respiration, with sALS cells displaying the most severe defects, TDP-43 and FUS milder changes, and C9orf72 reduced maximal capacity. All ALS patient fibroblasts showed decreased respiratory reserve. SOD1-G93A mouse fibroblasts exhibited marked uncoupling and reduced fluxes. NHK1 treatment improved mitochondrial function and viability across models. Overall, NHK1 acts as a positive modulator of mitochondrial function, restoring respiratory efficiency in ALS.

[1] A. Israelson et al. Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS, Neuron 2010 Aug 26;67(4):575-87.DOI:10.1016/j.neuron.2010.07.019

[2] A. Magrì et al. Small hexokinase 1 peptide against toxic SOD1 G93A mitochondrial accumulation in ALS rescues the ATP-related respiration, Biomedicines 2021, 9, 948. doi: 10.3390/biomedicines9080948

Discovering Small-Molecule Modulators of Mitochondria–ER Contact Sites and Their Protein Targets

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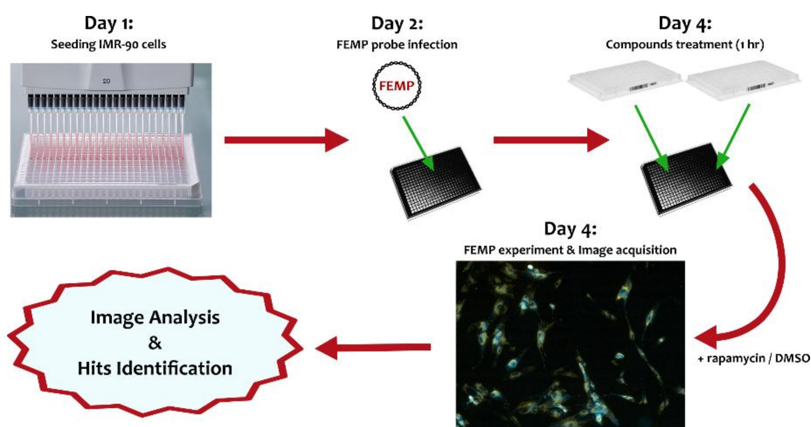

Mitochondria–Endoplasmic Reticulum Contact Sites (MERCs) are specialized subcellular regions where the membranes of the two organelles are closely apposed (10–80 nm). These structures play essential roles in multiple cellular processes, including calcium homeostasis and lipid metabolism, and are increasingly implicated in a variety of pathological conditions. Despite significant progress in identifying the molecular components of MERCs, their precise architecture and the mechanisms governing their plasticity and functional regulation remain incompletely understood.

To address this, we investigated the regulation of MERCs plasticity by small molecules, aiming to identify compounds capable of modulating MERCs, as well as their underlying protein targets. We employed a FRET-based mitochondria–ER proximity probe in combination with high-content imaging to screen the DiscoveryProbe™ FDA-approved drug library. This approach led to the identification of several candidate compounds, from which three bioactive hits were selected and subsequently transformed into affinity probes.

To identify the protein targets of these compounds, we applied a previously developed chemoproteomics approach based on "iBodies". iBodies are hydrophilic copolymer conjugates that recognize target proteins through ligands (i.e., the identified hit molecules) attached to the polymer backbone. Using immunoprecipitation followed by mass spectrometry analysis, we obtained a list of potential protein partners associated with the identified MERCs-regulating small molecules.

This project expands our understanding of the molecular mechanisms regulating MERCs and highlights the importance of MERCs dynamics in inter-organelle communication. Elucidating how small molecules modulate MERCs and identifying their molecular targets may uncover novel regulatory pathways governing organelle function and provide broader insights into the role of membrane contact sites in cellular physiology and disease.

A redox cycler-based therapy against mitcochondrial diseases

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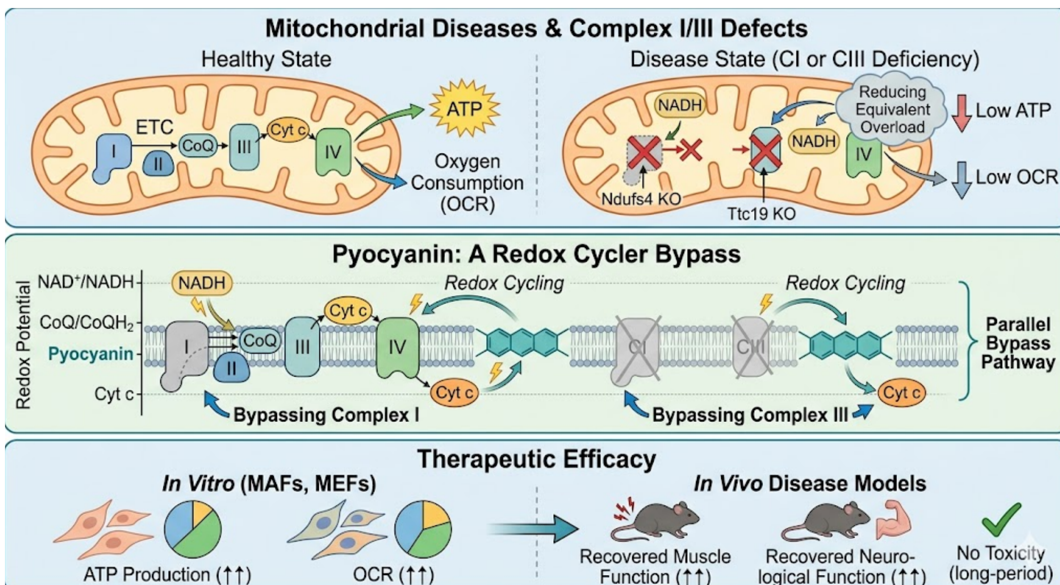
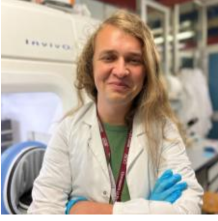
Mitochondrial diseases represent the most common class of genetic inborn diseases related to metabolism, which are characterized by the presence of dysfunctional mitochondria. Furthermore, due to mitochondria being almost ubiquitarian cellular organelles, they are characterized by an highly heterogenous clinical presentation. Gene therapy can be envisioned as the only definitive cure. However, due to the difficulty in targeting the mitochondrial genome which resides inside the mitochondrial matrix, over the last decades several pharmacological approaches have been studied. In this context, we have previously identified a class of small molecules, the so-called redox cyclers, able to undergo cyclic reactions of reduction and oxidation by accepting electrons from donors with lower redox potential and then donating them to acceptors with higher redox potential[1]. In this context, pyocyanin was highlighted as the most promising candidate thanks to its redox potential which is between NAD^+/NADH, CoQ/$CoQH_2$ and cyt c (oxidized)/cyt c (reduced.). *In vitro*, pyocyanin demonstrated, when used in the sub-μM range, to be able to increase mitochondrial ATP production and mitochondrial oxygen consumption in both MAFs Ndufs4 KO cells, a model of complex I (CI) deficiency, and MEFs Ttc19 KO cells, a model of complex III deficiency, suggesting its ability in bypassing CI and CIII and alleviate the reducing equivalents overload caused by the dysfunction of such complexes. Furthermore, pyocyanin proved to be able to bypass such complexes regardless of the underlying mutation and to have beneficial effects on mitochondrial membrane potential. Following, pyocyanin and other redox cyclers were tested *in vivo* in disease models where they proved useful in recovering muscle and neurological functions without inducing any toxicity over long-period treatments.



References

1. Peruzzo R., Corrà S., Costa R., Brischigliaro M., Varanita T., Biasutto L., Rampazzo C., Ghezzi D., Leanza L., Zoratti M., Zeviani M., De Pittà C., Viscomi C., Costa R., Szabò I. (2021) Exploiting pyocyanin to treat mitochondrial disease due to respiratory complex III dysfunction. *Nature Communications* 12, 2103.



Identification of the mitochaperome as a new tumor-restricted assembly

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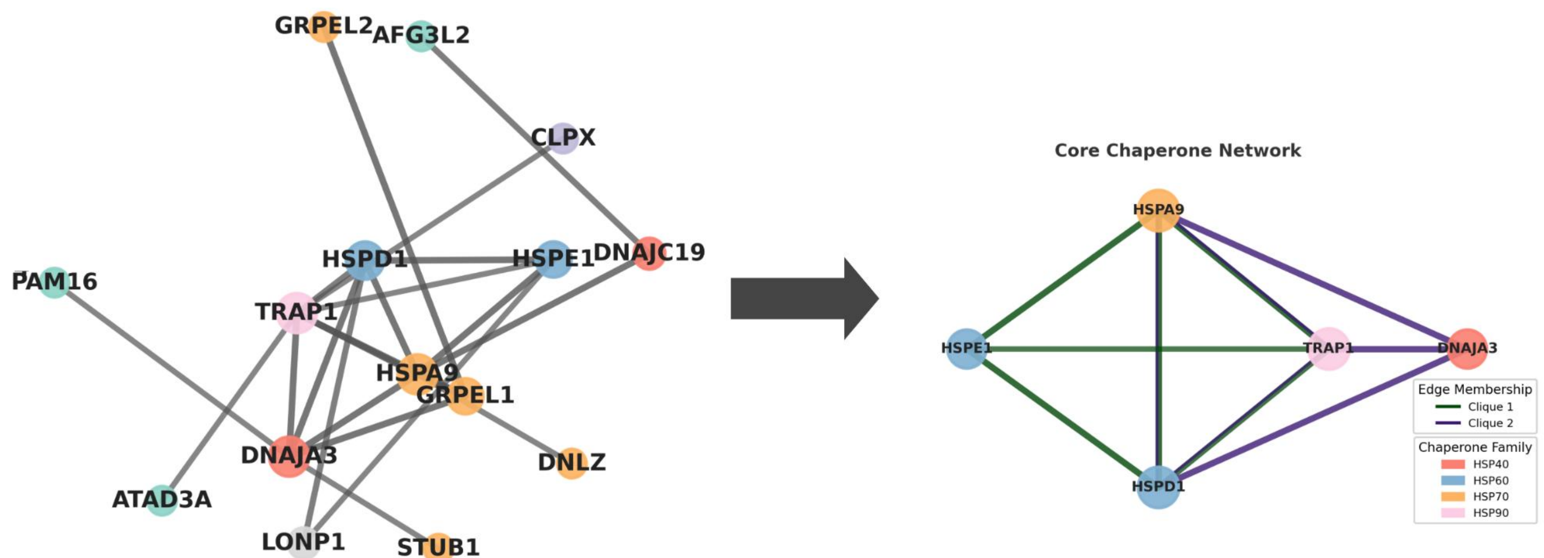
Cells rely on an intricate organization of protein networks distributed across compartments to execute essential biological functions. To maintain a functional proteome, cells utilize molecular chaperones, proteins that coordinate protein folding, stabilize folding intermediates and prevent aberrant protein aggregation. Under physiological conditions, the chaperome operates as a dynamic, interconnected hub in which discrete chaperone and co-chaperone nano-machines carry out specialized functions across multiple cellular pathways. In conditions of persistent stress, like those experienced by cancer cells, this hyperconnectivity drives the formation of epichaperomes, stable, long-lived assemblies composed of tightly associated HSP90 and HSP70 chaperones, co-chaperones, and accessory regulators. To date, the epichaperome has been identified exclusively in the cytoplasm. Nothing is known about analogous assemblies in other subcellular compartments. Among these, mitochondria are of particular relevance as they serve as central hubs for essential metabolic processes. To investigate possible mitochaperome formation, we have first analyzed the expression levels of 51 mitochondrial chaperones across 21 cancer types, linking them to patients' survival. We have then used a supervised machine learning framework to determine functional connections between chaperones and their clients and how they are reorganized following malignant transformation . We show that mitochondrial chaperones form an interaction network, the mitochaperome, that undergoes extensive tumor-specific remodeling and is built around a conserved core of mitochondrial chaperones.



CRISPR-based restoration of AGC1 reveals mutation-dependent phenotypic rescue in AGC1-deficient neuronal progenitors

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AGC1 deficiency is a severe hypomyelinating epileptic encephalopathy caused by mutations in SLC25A12 (AGC1), the neuronal isoform of the mitochondrial aspartate/glutamate carrier and a key component of the malate-aspartate shuttle[1]. Neuronal progenitors (NPs) differentiated from induced pluripotent stem cells (iPSCs) derived from AGC1-deficient patients exhibit glutamine-dependent proliferation and profound metabolic rewiring toward glycolysis, accompanied by impaired mitochondrial oxidation of glucose and pyruvate, and a markedly altered transcriptomic profile.

To restore the defective phenotype, iPSCs from two patients carrying distinct pathogenic mutations were subjected to two CRISPR/Cas9-based gene-editing strategies: (i) insertion of an inducible cassette encoding wild-type(WT) AGC1 cDNA into the AAVS1 safe harbor locus on chromosome 19; and (ii) correction of the endogenous AGC1 mutation using an adenine base editor(ABE).

In iPSCs derived from a patient carrying compound heterozygous mutations causing complete loss of AGC1 expression [2], insertion of the inducible AAVS1 cassette restored WT AGC1 expression and rescued the NP phenotype. Proliferation and bioenergetic parameters became comparable to those of control NPs, but only when AGC1 expression was induced at the onset of differentiation.

Conversely, in iPSCs from a patient harboring a homozygous missense mutation (R353Q) severely impairing AGC1 activity [3], both AAVS1-mediated transgene insertion and ABE restored WT AGC1 expression, yet neither strategy rescued the defective phenotype.

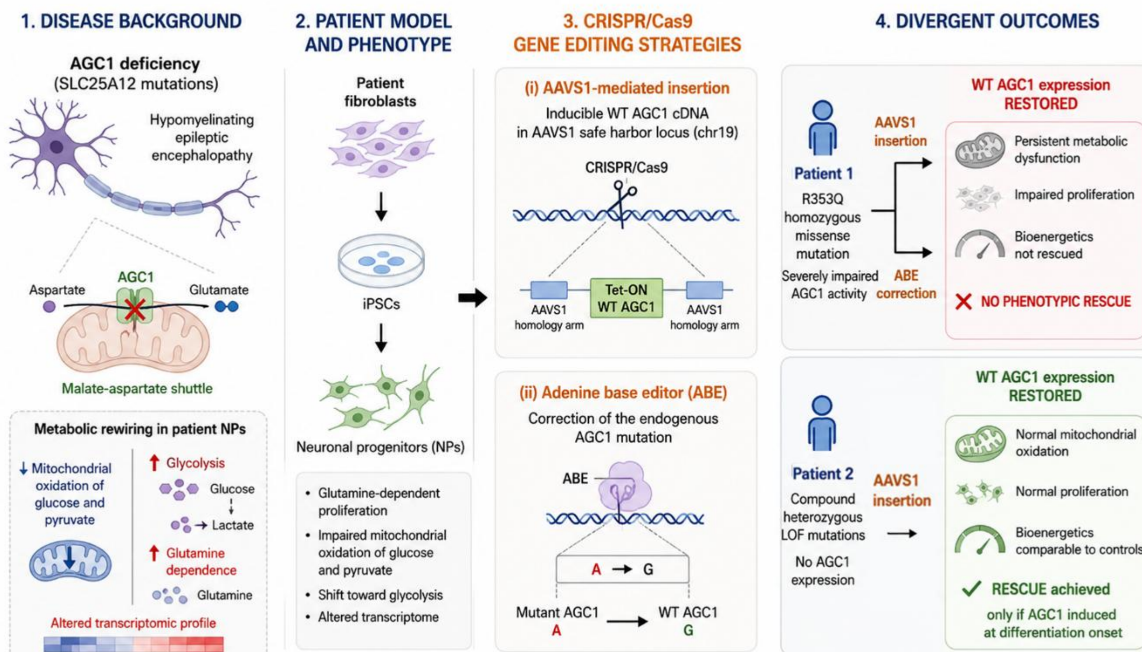
Overall, these findings demonstrate that both genome-editing approaches effectively restore WT AGC1 expression in patient-derived hiPSCs. However, the divergent functional outcomes observed in the two patient cell lines reveal additional layers of disease complexity and underscore the need for personalized strategies to address the heterogeneity of AGC1 deficiency.

References:

[1] Wibom R, Lasorsa FM, Töhönen V, Barbaro M, Sterky FH, Kucinski T, Naess K, et al. AGC1 deficiency associated with global cerebral hypomyelination. N Engl J Med. 2009 Jul 30;361(5):489-95.

[2] Barile SN, Magnifico MC, Poeta E, Distelmaier F, Viggiano L, Balboni N, Protti M, et al. Combined ketone body and glutamine supplementation restores aerobic energy production in AGC1-deficient neuronal progenitors. Cell Death Dis. 2025;17(1):120.

[3] Falk MJ, Li D, Gai X, McCormick E, Place E, Lasorsa FM, Otieno FG, Hou C, Kim CE, et al. AGC1 Deficiency Causes Infantile Epilepsy, Abnormal Myelination, and Reduced N-Acetylaspartate. JIMD Rep. 2014;14:77-85.



Challenges in PBMC bioenergetic diagnostics: from cohort-derived reference values to patient interpretation.

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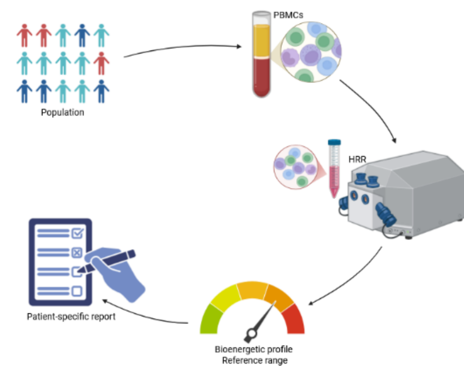
Assessment of the bioenergetic profile of human peripheral blood mononuclear cells (PBMCs) is gaining increasing interest as a minimally invasive approach for investigating mitochondrial function in clinical settings. Methodological standardization - including cell isolation, cryopreservation, and respirometric analysis - is essential to ensure reproducibility [1]. Current practices rely largely on reference ranges derived from statistically defined cohorts. However, translating these group-based values to single-patient interpretations is complex. Biological variability, overlapping distributions between healthy and diseased populations, and sensitivity to pre-analytical factors complicate the classification of individual bioenergetic phenotypes.



In this study, we present high-resolution respirometry (HRR) data from PBMCs of healthy donors used to establish reference boundaries, alongside datasets from patients with pathologies with suspected mitochondrial involvement. Selected case studies illustrate scenarios where individual results fall near or within reference limits yet potentially indicate clinical relevance. These examples highlight the limitations of rigid threshold-based interpretations and underscore the need for integrative approaches that incorporate statistical, biological, and clinical contexts and follow-up analyses.

Our findings emphasize the importance of refining interpretative frameworks to improve diagnostic confidence in mitochondrial medicine. Further studies are required to meet the challenge of defining age- and sex- matched values for the healthy reference population.

Reference:

1. A. Romero, A Timón-Gómez A, J.R. Willis, A. Perfler, T. Lindlbauer, M. Knoflach, E. Gnaiger, Isolation and cryopreservation of human peripheral blood mononuclear cells for high-resolution respirometry, BEC preprints (2026) 2026.2.v2. https://doi.org/10.26124/becprep.2026-0002.v2

2. Figure created with BioRender.com

Biotechnological production of extracellular vesicles from *Coffea arabica* L. cell suspension cultures: isolation and proteomic profiling

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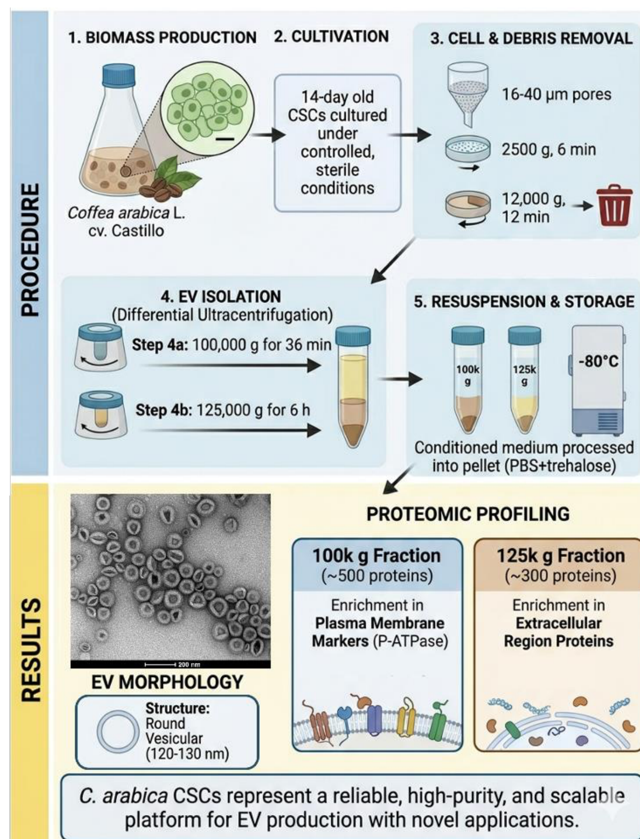
Extracellular vesicles (EVs) are emerging as a transformative category of biotherapeutics and biotechnological tools due to their pivotal role in intercellular communication and the transport of bioactive molecules, including proteins and nucleic acids. While EVs can be derived from several biological sources, plant-based systems offer significant advantages by minimizing risks related to immunogenicity and pathogen contamination. Specifically, plant cell suspension cultures (CSCs) provide a sterile, controlled, and scalable environment for high-purity EV recovery. In this study, we investigated the potential of *Coffea arabica* L. (cv. Castillo) CSCs as a reliable source of EVs. The vesicles were isolated from the conditioned culture medium of 14-day-old cells through a series of differential ultracentrifugation steps at 100,000 *g* (100k *g* for 36 min) and 125,000 *g* (100k *g* for 6 h). Morphological characterization via Transmission Electron Microscopy (TEM) confirmed the presence of round-shaped vesicles with an average diameter of approximately 120-130 nm. Proteomic profiling identified roughly 500 and 300 proteins in the 100k *g* and 125k *g* fractions, respectively. The 100k *g* fraction was primarily enriched with plasma membrane and cell periphery proteins, whereas the 125k *g* fraction contained proteins mostly associated with the extracellular region. Our findings demonstrate that coffee CSCs actively secrete EVs, likely through plasma membrane budding or unconventional secretory pathways. These results prove that *C. arabica* CSCs represent a highly efficient and scalable system for obtaining EVs without mechanical cell disruption. Such a platform opens new opportunities for downstream functional studies and innovative applications in the biotechnological and pharmaceutical sectors.



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References:

1. Wang & Kong, 2025 - doi: 10.1111/pbi.70074

2. Kırbaş et al., 2025 - doi: 10.1002/biof.2090

Generation and initial characterization of STACCATO, a family of Split-FAST Mitochondria-ER Contacts probes to measure contact sites of different widths.

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In eukaryotic cells, membrane contact sites are established between organelles, forming specialized subcellular compartments characterized by distinct functions and proteomes. Among these, the contact sites between the endoplasmic reticulum (ER) and mitochondria, known as mitochondria–ER contact sites (MERCs), regulate several essential physiological processes. Alterations in MERC composition and morphology have therefore been associated with a variety of diseases. MERC formation and dynamics can be visualized using bimolecular fluorescent probes combined with confocal microscopy. However, currently available probes often suffer from important limitations: they may produce weak fluorescence signals, artificially induce tethering between organelles, or require complex imaging procedures that are incompatible with standard microscopy setups commonly available in most laboratories. An ideal probe should instead provide reversible complementation, be easy to use, and display a high signal-to-noise ratio. To address these limitations, we developed STACCATO, a bimolecular fluorescent probe for the detection of MERCs based on split Fluorescence-Activating and absorption-Shifting Tag (FAST) technology. The C-terminus of STACCATO is targeted to mitochondria through the AKAP1 targeting sequence, whereas the N-terminus is directed to the ER via the Sac1 targeting signal. Flexible linkers connecting the two moieties allow probe reconstitution across MERCs of varying widths following the addition of an exogenous fluorogen. In this study, we demonstrate the efficiency of STACCATO in detecting changes in MERCs using inducible U2OS STACCATO cell lines. After confirming the correct localization of STACCATO at MERCs, we showed that the probe responds to both artificially induced tethering and to the overexpression of endogenous factors known to modulate MERCs, including ERMIT, the MAM-localized alternative splicing variant of MFN2. Furthermore, STACCATO was able to detect reductions in contact site number following metabolic treatments such as palmitic acid exposure.

FLYMP: a new FRET based sensor to study contact sites between mitochondria and lysosomes

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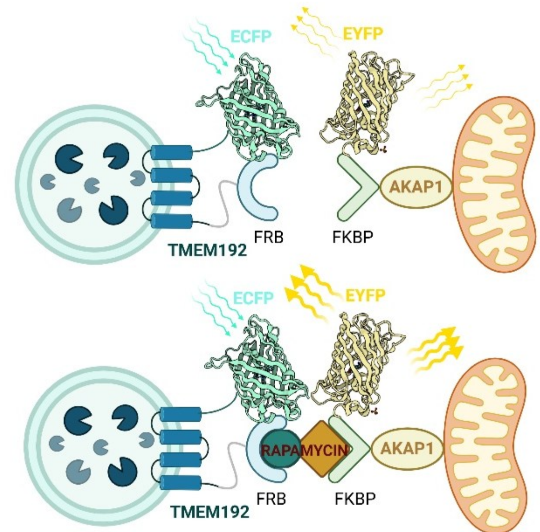

Mitochondria and lysosomes are key organelles for cell homeostasis, with their dysfunction linked to diseases like Parkinson's and Gaucher's. Recently, beyond the already known functional interdependence, scientists have discovered physical membrane contact sites, regulated by Rab7-GTP (tethering) and TBC1D15 (untethering via Fis1) [1]. These contacts mark fission sites and regulate Ca2+ transfer, to name a few processes. To study mitochondria-lysosomes interactions, we developed FLYMP (FRET-based sensor for Lysosome-Mitochondria Proximity). The probe consists of two moieties: AKAP1-FKBP-EYFP (mitochondria-targeted) and TMEM192-FRB-ECFP (lysosome-targeted), equimolarly expressed via T2A sequence in between and cloned into a pSIK-CMV backbone. Moreover, FLYMP exploits FKBP-FRB module, that after rapamycin treatment dimerizes, therefore increasing mitochondria-lysosome juxtaposition. Basal and maximum FRET signals were measured at Operetta CLS, with the best rapamycin concentration assessed at 400 nM. Time-course experiments confirmed rapamycin capacity of maintaining the maximum tether over time. To further validate FLYMP, we co-transfected MS1 cells with Rab7 mutants and measured both basal and maximum FRET compared to WT. The Rab7Q67L (permanent tether) seems to reduce a bit the difference between maximum and basal FRET, while Rab7T22N has the opposite trend. Moreover, we are interested in confirming the complete reversibility of the probe after rapamycin addition. Our goal is to establish FLYMP as a tool for studying mitochondria-lysosome contact sites in both physiological and pathological contexts.

1. Y. C. Wong, S. Kim, W. Peng, D. Krainc, Regulation and Function of Mitochondria-Lysosome Membrane Contact Sites in Cellular Homeostasis, Trends in cell biology vol. 29,6 (2019) 500-513

Exercise Prehabilitation Combined with Nutritional and Cognitive Interventions During Bed Rest Preserves Skeletal Muscle Mitochondrial Function in Older Adults

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Ageing is associated with a progressive decline in skeletal muscle oxidative metabolism, exacerbated by physical inactivity. Bed rest (BR) impairs oxidative metabolism in older adults. However, the potential protective role of prehabilitation remains unclear. This study investigated whether a multimodal intervention combining exercise prehabilitation, nutritional support, and cognitive training could preserve mitochondrial function and oxidative status during inactivity.

Ten healthy older males (≥65 years) completed 30 days of supervised exercise prehabilitation before 10 days of horizontal BR, combined with a leucine-rich high-protein diet (1.6 g/kg/day) and daily cognitive training during BR. Maximal oxygen uptake (V'O_2peak) was assessed before and after BR, while vastus lateralis biopsies were analyzed for mitochondrial respiration, hydrogen peroxide (H_2O_2) emission, and citrate synthase activity.

BR reduced V'O_2peak, indicating a decline in whole-body oxidative capacity. However, mitochondrial content was preserved, with unchanged citrate synthase activity. Mitochondrial function improved, as shown by increased maximal oxidative phosphorylation and electron transport system capacity. H_2O_2 emission increased under both LEAK and ADP-stimulated conditions, but when it was normalized to oxygen flux no differences were observed.

These findings indicate that the proposed multimodal countermeasures intervention (exercise prehabilitation, nutritional support [leucine-rich high-protein diet], cognitive training) preserves mitochondrial content and enhances intrinsic oxidative capacity during inactivity in older adults, although it does not prevent declines in whole-body V'O_2peak. This dissociation highlights the complexity of physiological adaptations to disuse. Overall, the proposed multimodal intervention may represent a promising strategy to partially counteract disuse-induced impairments in oxidative metabolism and to support muscle function in ageing populations.

Hexokinase overexpression and its consequences for respiratory parameters

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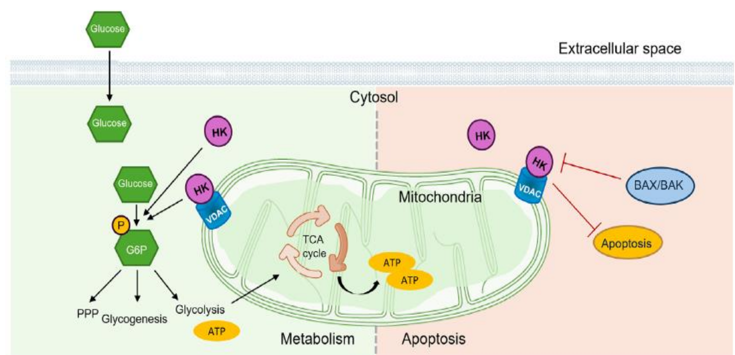
Hexokinase 1 is a key glycolytic enzyme that can be found in the cytosol or interact with the outer mitochondrial membrane, particularly via the voltage-dependent anion channel (VDAC). HK-VDAC binding has metabolic and anti-apoptotic implications, and plays a pivotal role in several illnesses, including cancer and neurodegenerative diseases [1]. While the exact mechanism of this interaction is still debated, it is clear that this bond could be a therapeutic target for various diseases. In this study, we aimed to evaluate how and whether the overexpression of HK1 modulates cellular metabolism and apoptosis in two phenotypically distinct cell lines. Stable HK1-GFP-overexpressing clones were established in HeLa and HEK 293T cells, which were then characterised using flow cytometry, image scanning microscopy, real-time live-cell imaging (IncuCyte) and high-resolution respirometry (Oroboros O2k). Preliminary data showed that HK1 overexpression led to a significant reduction in mitochondrial respiratory activity in both HEK293 and HeLa cells, albeit to different degrees, indicating a shift in cellular energy metabolism. Furthermore, when the enzyme is overexpressed, it affects apoptotic responses, particularly under Staurosporine-induced stress, which supports its anti-apoptotic role. Further investigations are required to elucidate the specific pathways activated in response to HK1 overexpression in each cell type. Overall, these findings provide new insights into how HK1 modulates apoptosis and metabolism, with potential implications for therapeutic strategies targeting related diseases.



References:

1.N. A.Pesce, G.Seminara, G.Giarrusso, and M. F.Tomasello, HK1 and HK2 Beyond Glycolysis: Mitochondrial Interactions and Dual Roles in Metabolism and Cell Fate, Advanced Biology10, no. 1 (2026): e00472.

Title: **TMEM65 Couples Mitochondrial Calcium Homeostasis to Respiratory Chain Biogenesis**

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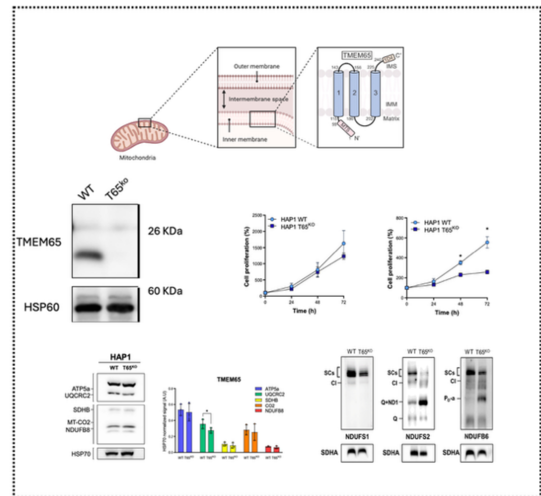
Abstract

TMEM65 is an inner mitochondrial membrane protein essential for Na^+ dependent Ca^{2+} extrusion, either acting as a Na^+/Ca^{2+}exchanger itself or as a functional partner of NCLX [1,2]. Loss of TMEM65 causes mitochondrial Ca^{2+} overload, bioenergetic dysfunction, and severe neuromuscular phenotypes [3,4] and has been associated with recessive mitochondrial disorder [5]. Since Ca^{2+} homeostasis regulates oxidative phosphorylation (OXPHOS) and cristae organization, we investigated the role of TMEM65 in the biogenesis, stability and function of OXPHOS complexes.



Cells lacking TMEM65 (T65KO) showed impaired proliferation in galactose and reduced maximal respiratory capacity, indicating a bioenergetic defect. Decreased abundance of complex III and accumulation of early complex I (CI) assembly intermediates were found, consistent with altered respiratory complex biogenesis.

Complexome profiling supported a delay in CI assembly and altered supercomplexes organization in T65KO cells. TMEM65 interactome analysis revealed enrichment in assembly factors and components linked to OXA1L and TIM23 machinery, suggesting a role at early stages of OXPHOS biogenesis.

Ongoing studies are assessing respiratory complexes assembly kinetics, ATP synthesis and recapitulating the impact of TMEM65 loss in neuronal-like SH-SY5Y.

References

[1] J.L. Zhang, et al., TMEM65 functions as the mitochondrial Na+/Ca2+ exchanger, Nat Cell Biol 27 (2025) 1301–1310.

[2] M. Vetralla, et al., TMEM65-dependent Ca2+ extrusion safeguards mitochondrial homeostasis, Nat Commun 17 (2025) 923.

[3] J.F. Garbincius, et al., TMEM65 regulates and is required for NCLX-dependent mitochondrial calcium efflux, Nat Metab 7 (2025) 714–729.

[4] Y. Zhang, et al., Loss of TMEM65 in mice causes mitochondrial disease mediated by mitochondrial Ca2, Nat Commun (2026).

[5] A. Nazli, etal., A mutation in the TMEM65 gene results in mitochondrial myopathy with severe neurological manifestations, Eur J Hum Genet 25 (2017) 744–751.

Biallelic variants in PGS1 disrupt cardiolipin biosynthesis and mitochondrial bioenergetics

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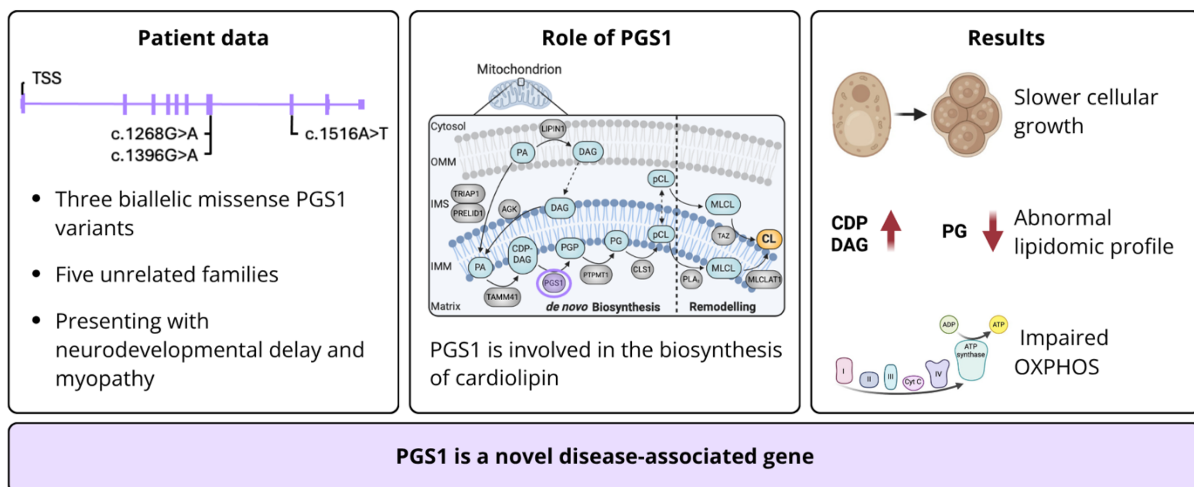
Cardiolipin (CL) is a signature phospholipid of the inner mitochondrial membrane and plays a central role in maintaining cristae architecture, supporting oxidative phosphorylation, and stabilising respiratory chain complexes and supercomplexes [1]. Defects in CL biosynthesis or remodelling impair mitochondrial membrane organisation and bioenergetics and are increasingly recognised as causes of human disease. Pathogenic variants in TAZ [2], which impair CL remodelling, cause Barth syndrome, while defects in CL biosynthetic genes such as TAMM41 [3], CRLS1 [4], and PTPMT1 [5] have been associated with mitochondrial diseases and neurodevelopmental syndromes, highlighting the importance of CL metabolism for mitochondrial function and human health.

Despite this, the role of phosphatidylglycerol phosphate synthase 1 (PGS1) in human disease remains poorly understood. PGS1 catalyses the conversion of CDP-diacylglycerol to phosphatidylglycerol phosphate, an early and essential step in the CL biosynthetic pathway. Although PGS1 has been characterised in yeast and mammalian systems [6], pathogenic variants in PGS1 had not previously been identified in patients.

Here, we report three novel homozygous missense variants in PGS1 in five independent families presenting with neurodevelopmental delay and myopathy. Functional studies were performed in patient-derived fibroblasts, a *PGS1*-knockdown H4 neuroglioma cell model, and yeast models engineered to harbour the patient-specific variants. The latter showed growth defects and abnormal lipid profiles, including accumulation of CDP-DAG and reduced phosphatidylglycerol levels, consistent with impaired PGS1 enzymatic function. In parallel, *PGS1* depletion in H4 cells impaired mitochondrial bioenergetics and reduced OXPHOS complex activity and assembly.

Together, these findings identify PGS1 as a novel disease-associated gene, show that impaired PGS1 function disrupts cardiolipin metabolism and mitochondrial bioenergetics, and expand the genetic spectrum of cardiolipin-related mitochondrial disorders.



References:

1. M. Falabella et al. Cardiolipin, Mitochondria, and Neurological Disease. *Trends Endocrinol Metab.* (2021)
2. S. Bione et al. A novel X-linked gene, G4.5. is responsible for Barth syndrome. *Nat Genet.* (1996)
3. K. Thompson et al. Biallelic variants in TAMM41 are associated with low muscle cardiolipin levels, leading to neonatal mitochondrial disease. *HGG Adv.* (2022)
4. R.G. Lee et al. Deleterious variants in CRLS1 lead to cardiolipin deficiency and cause an autosomal recessive multi-system mitochondrial disease. *Hum Mol Genet.* (2022)
5. M. Falabella et al. Biallelic PTPMT1 variants disrupt cardiolipin metabolism and lead to a neurodevelopmental syndrome. *Brain* (2025)
6. T. Ohtsuka et al. A somatic cell mutant defective in phosphatidylglycerol phosphate synthase, with impaired phosphatidylglycerol and cardiolipin biosynthesis. *J Biol Chem.* (1993)

Mitochondrial calcium uptake regulates mitochondrial fission modes

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Mitochondrial fission is an essential and finely regulated process that can be further classified into "midzone" and "peripheral" according to the position of the mitochondrion where it occurs. Mitochondrial calcium (Ca^{2+}) influx is associated with inner membrane constriction as priming event of fission and cristae reorganization. The smaller daughter mitochondria originating from peripheral division are preceded by significant increase of Ca^{2+}, whereas Ca^{2+} level increased mildly in bigger peripheral daughter mitochondria or midzone daughter mitochondria. However, the relationship between mitochondrial Ca^{2+} and mitochondrial fission modes remains unclear. In order to address these questions, we used ionomycin, an ionophore that raises the intracellular Ca^{2+} level and induces mitochondrial Ca^{2+} uptake, to explore the mitochondrial fission modes. Treatment with ionomycin caused an increase in the rate of peripheral fissions. Interestingly, Ru360, a specific mitochondrial calcium uptake inhibitor, reduced ionomycin-induced peripheral fission, whereas per se it induced an increase in mitochondrial branching and elongation. Consistently, compared with control cells, peripheral fission, but not midzone fission, was blocked in cells knockout for the mitochondrial Ca^{2+} uniporter (MCU) undergoing ionomycin treatment. Upon histamine stimulation, 4mtGCaMP6s-indicated mitochondrial Ca^{2+} levels are heterogeneously distributed in the organelles originating from peripheral but not midzone fission. Data from increased 4mtGCaMP6s intensity normalized by mt-mScarlet showing that in peripheral fission, Ca^{2+} uptake is lower in the smaller daughter mitochondria and higher Ca^{2+} uptake in the larger daughter mitochondria. Our data suggest that mitochondrial calcium uptake participates in peripheral mitochondrial fission.

Gdap1l1 is a mitochondrial fission factor vicariating Gdap1 in neuronal cells

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Ganglioside-induced differentiation–associated protein 1-like 1 (Gdap1l1) is a paralogue of the mitochondrial fission factor Gdap1, whose loss of function is known to cause Charcot–Marie–Tooth disease (CMT) [1–3]. In the context of CMT, Gdap1l1 has been proposed to compensate for the absence of Gdap1 within the central nervous system (CNS) [2]. In a genome-wide screen for components of the mitochondrial fission machinery conducted in our laboratory, Gdap1l1 emerged as one of the strongest candidates, warranting further investigation of its role in mitochondrial dynamics.

To assess whether Gdap1l1 influences mitochondrial morphology, we employed both gain- and loss-of-function approaches, followed by quantitative confocal microscopy. Subcellular localization of Gdap1l1 was examined using biochemical fractionation. To evaluate whether Gdap1l1 functions as a recruitment factor for Drp1, we performed co-immunoprecipitation assays.

Silencing of Gdap1l1 and Gdap1 in combination, but not individually, resulted in mitochondrial elongation, resembling the phenotype observed upon Drp1 depletion. Conversely, overexpression of Gdap1l1 induced increased mitochondrial fragmentation irrespective of Gdap1 presence. Subcellular fractionation analyses confirmed the mitochondrial localization of Gdap1l1, and additional data suggest a direct or close interaction between Gdap1l1 and Dnm1.

Collectively, these results support a regulatory role for Gdap1l1 in the mitochondrial fission machinery.

1. Pedrola L, Espert A, Wu X, Claramunt R, Shy ME, Palau F. GDAP1, the protein causing Charcot-Marie-Tooth disease type 4A, is expressed in neurons and is associated with mitochondria. Hum Mol Genet. 2005 Apr 15;14(8):1087-94. doi: 10.1093/hmg/ddi121. Epub 2005 Mar 16. PMID: 15772096.

2. Niemann A, Wagner KM, Ruegg M, Suter U. GDAP1 mutations differ in their effects on mitochondrial dynamics and apoptosis depending on the mode of inheritance. Neurobiol Dis. 2009 Dec;36(3):509-20. doi: 10.1016/j.nbd.2009.09.011. Epub 2009 Sep 25. PMID: 19782751.

3. Niemann A, Huber N, Wagner KM, Somandin C, Horn M, Lebrun-Julien F, Angst B, Pereira JA, Halfter H, Welzl H, Feltri ML, Wrabetz L, Young P, Wessig C, Toyka KV, Suter U. The Gdap1 knockout mouse mechanistically links redox control to Charcot-Marie-Tooth disease. Brain. 2014 Mar;137(Pt 3):668-82. doi: 10.1093/brain/awt371. Epub 2014 Jan 29. PMID: 24480485; PMCID: PMC3927703.

A chemotypically focused virtual screening identifies novel specific OPA1 inhibitors that enhance cytochrome c release and induce cell death

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The mitochondrial Dynamin-like protein Optic Atrophy 1 (OPA1) is a large GTPase involved in mitochondrial fusion, cristae remodeling, cytochrome c release and apoptosis. OPA1 upregulation has been increasingly identified as an exploitable vulnerability in cancer cells. A previous screening of >10,000 drug-like molecules identified MYLS22 as a promising hit for the inhibition of OPA1 GTPase activity. MYLS22 is not mitochondriotoxic but causes mitochondrial fragmentation and cristae remodeling, enhancing cytochrome c release following proapoptotic stimuli, recapitulating the effect of OPA1 downregulation in cells. A SAR analysis of MYLS22 identified several derivatives with increased water solubility. Three derivatives enhanced OPA1 GTPase inhibition in vitro and induced mitochondrial fragmentation in cells. To identify hits with further enhanced potency, solubility and overall availability of the leads, we generated a virtual, combinatorial library of ≈60,000 compounds based on the main chemotype of MYLS22. The library was then docked to the GTPase domain of the currently available structure of OPA1 (PDB: 6JTG). Based on the results, 37 compounds have been selected and are currently being synthesized. Their potency as OPA1 inhibitors will be analyzed both in vitro and in cells. Our work identifies specific and potent OPA1 inhibitors with the potential to treat cancers where OPA1 is upregulated.

The role of the FIS1-Interactome in Acute Myeloid Leukemia Mitophagy

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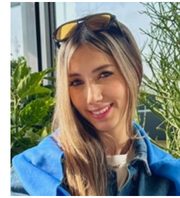

Acute myeloid leukemia (AML) is driven by leukemia stem cells that depend on mitochondrial homeostasis to sustain survival and therapy resistance. Increasing evidence links AML progression to mitochondrial regulation and mitophagy, which supports leukemic cell fitness by maintaining mitochondrial quality. The mitochondrial fission protein Fission 1 (FIS1) is required for mitophagy and leukemic stem cell maintenance in AML; however, the molecular mechanism through which FIS1 regulates mitophagy remains unclear. To address this gap, we applied bioinformatic approaches to define the FIS1 interactome and assess whether FIS1 associated protein networks are involved in AML related mitophagy pathways. These analyses identified BNIP3L (NIX)**,** a mitophagy receptor, as a candidate FIS1 associated protein, suggesting a potential mechanistic link between mitochondrial fission and mitophagy execution. Because hypoxia is a defining feature of the bone marrow microenvironment and is known to induce BNIP3L (NIX) dependent mitophagy, FIS1 centered interaction networks is analyzing under Normoxic and Hypoxic conditions to identify context specific regulatory interactions relevant to AML mitochondrial adaptation**.**

Title: Investigating the Role of Mitochondria–ER Contact Sites in UV-Induced Melanogenesis

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Abstract:

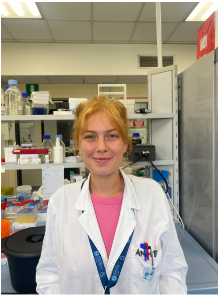
Ultraviolet (UV) radiation is a major environmental factor that induces melanogenesis while simultaneously triggering cellular stress responses. Although mitochondria–endoplasmic reticulum contact sites (MERCs) are critical regulators of inter-organelle communication and stress signaling, their involvement in UV-induced melanogenesis remains poorly understood.

We exposed normal human epidermal melanocytes (NHEM) to increasing doses of UV-B (from 10mJ/cm^2 to 300mJ/cm^2) to investigate the relationship between melanogenesis, and MERCs dynamics The dose-dependent increase in melanin production that followed UV exposure confirmed activation of melanogenesis. MERCs changes were measured by means of a FRET-based assay: UV-treated cells exhibited enhanced ER–mitochondria interactions suggesting that MERCs may participate to the UV-induced stress response.

Confocal microscopy experiments further revealed that mitochondrial elongation occurs at higher UV doses, whereas changes in MERCs are detectable at lower exposure levels, indicating that MERC and mitochondria remodeling may occur independently.

Together, our results indicate that MERCs take part to the cell response to UV exposure and suggest a previously unrecognized link between organelle crosstalk and pigmentation. This study provides a framework to further explore the molecular mechanisms underlying UV-induced skin responses and their potential relevance to pigmentation disorders and melanoma.

Keywords: MERCs, Mitochondria, melanogenesis, UV-B



Opantimir treatment antagonizing microRNAs that upregulate Opa1: development of a new delivery system.

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Mitochondrial myopathies manifest with weakness, motor deficiency and exercise intolerance, impairing the prognosis of these already devastating disorders that lack treatments. Optic Atrophy 1 protein (OPA1), a mitochondrial inner membrane protein modulating mitochondrial dynamics, is downregulated in mitochondrial myopathies. We have found that OPA1 is downregulated by the miR148 family in skeletal myocytes in different mitochondrial myopathies. Opantimirs, miRNA antagonists targeting the miR148 family, rescue muscle function in two models of mitochondrial myopathy caused by deletion of the complex IV assembly factor Cox15 and by ischiatic nerve resection (Djalalvandi et al., 2025). We are now developing liponanoparticles loaded with Opantimirs that target skeletal muscle. We will present the primary data on the efficiency of these new delivery systems in different cell lines, with the aim of improving the efficiency and specificity of drug administration and ultimately facilitating the clinical translation of this therapeutic strategy.

Investigating the Role of MFF in organelle remodelling

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The Mitochondrial fission factor (MFF) is a well established regulator of mitochondrial and peroxisomal fission. Recent findings from our laboratory indicate that MFF also modulates melanosome size and maturation, suggesting a potential broader role in organelle biology. We are therefore investigating whether MFF plays a conserved function in shaping organelle morphology across distinct cellular compartments.

To address this possibility, we used two approaches.

First, we firstly focused on another subcellular compartment, e.g. lipid droplets (LD). We chose these organelles because they are known to undergo a dynamic life cycle encompassing biogenesis, maturation, and turnover. We have performed stable MFF knockdown in 3T3-L1–derived adipocytes, which are characterized by robust LD formation. Following adipogenic differentiation, we assessed the number, size and spatial distribution of LD using confocal microscopy, high content imaging, and Oil Red (ORO) staining. Our data show that stable Mff silencing leads to an increase in the total lipid content along with a tendency towards changes in LD size, though these were not statistically significant.

Then, as a proof of concept experiment, we have designed chimeric MFF constructs to target it to specific subcellular compartments, including LDand melanosomes.

Altogether, these approaches will enable us provide insights on a potential general role of MFF in shaping organelle membrane dynamics.