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# 15th WMS World Congress on Targeting Mitochondria

Volkmar Weissig & Marvin Edeas

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WORLD MITOCHONDRIA SOCIETY

15TH CONGRESS

# Targeting Mitochondria



## BOOK OF ABSTRACTS

OCTOBER
29»31 2024
BERLIN- GERMANY

# World Mitochondria Society

## 15th World Congress on

# Targeting Mitochondria

**October 29-31, 2024**

**Berlin, Germany & Online**

**Volkmar Weissig**

President of World Mitochondria Society

**Marvin Edeas**

Chairman of WMS Scientific Committee

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# Welcome to the 15th World Mitochondria Society Meeting

Dear Colleagues,

On behalf of the Scientific Committee of the World Mitochondria Society (WMS), it is a great pleasure to welcome you at the 15th Annual Meeting of WMS on Targeting Mitochondria, which will be held on October 29-31, 2024 at the DoubleTree by Hilton, Berlin Ku'damm, Germany.

Targeting Mitochondria 2024 will discuss breakthroughs in mitochondrial research and their potential clinical applications, marking a significant milestone in the journey towards more effective mitochondrial therapies.

Research into mitochondria is rapidly becoming a foundation of innovative medical treatments. By elucidating the mechanisms of mitochondrial function and dysfunction, Speakers of Targeting mitochondria 2024 are exploring the way for novel therapies that address the root causes of many complex diseases.

The 15th WMS annual meeting will highlight the critical role of mitochondria research in shaping the future of medicine.

We would like to thank all members of scientific committee, speakers and chairpersons of the World Mitochondria society for their contribution. A particular thanks to:

*Ana Andreazza, University of Toronto, Canada*
*Eric Barrey, INRA, France*
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*Carsten Culmsee, University of Marburg, Germany*
*Vladimir Gogvadze, Karolinska Institutet, Sweden*
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*Michael B. Powner, City University of London, United Kingdom*
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*Laurie Sanders, Duke University School of Medicine, USA*
*Eric Schon, Columbia University, USA*
*Stephen Tait, University of Glasgow, United Kingdom*
*Yuma Yamada, Hokkaido University, Japan*
*Yong Zhao, Throne Biotechnologies, USA*
*Hans Zischka, Institute of Molecular Toxicology and Pharmacology, Germany*

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We are grateful for WMS media partners: **CureMito Foundation**, and **Liam Foundation**.

We hope that you will enjoy the Targeting Mitochondria 2024 Congress and that your interactions with colleagues from many countries will stimulate a creative exchange of ideas and challenges

All our best regards,

**Volkmar Weissig**
President of the World Mitochondria Society

# Practical Information

We would like to take the opportunity to give you some additional information about the meeting arrangements.

**The Abstract book contains:**
• Speakers' abstracts (the abstracts of the oral presentations follow the order of the program);
• The abstracts of posters on display.

**Badges**
Upon registration you have received your own personal badge. Please wear this badge during the entire meeting including the coffee breaks and lunch.
The conference secretariat will be located in the area in front of the conference hall.

**Instructions for participants**

**Chairpersons:** The Chairpersons will be seated at the president's table.

**Speakers:** Speakers are invited to give their Power Point presentations for downloading on the computer to the technical team outside and not inside the conference hall. As the schedule is rather tight and to allow sufficient time for discussions, we would be very much obliged if the timing requirements were respected.

**Poster Contributors**: Please ensure that your poster is displayed at the appropriate location, please respect your poster number and day of presentation. Posters will be divided showcased during the breaks. The Poster contributors are invited to stand by their poster during the poster sessions.

**Speakers Dinner**
A dinner is organized on October 30. Please keep updated for the address.
If you registered for this dinner, please join the group at 20h15 at the restaurant.

**Mobile Phones**
As a courtesy to the speakers and other delegates, please turn off your mobile phones or to silent whilst in the conference room. Please do not take pictures of the slides without the consent of the presenting author.

**Questions**
Please state your name and institution or company before asking your question.

**Conference Staff**
Staff at the conference registration desk will be happy to deal with any queries you may have. If we receive any messages for you, they will be announced at the break in the session and can be collected from the desk.

**Personal Belongings**
Please keep your valuables and working materials with you at all times. We would advise you to keep your name on the conference notes, as we may not be able to replace these if lost. DoubleTree by Hilton and the World Mitochondria Society cannot be held responsible for any loss or damage to your property.

## 15th WMS World Congress on

# Targeting Mitochondria

**October 29-31, 2024**

**Berlin, Germany & Online**

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15th WMS World Congress on

# Targeting Mitochondria

## Abstracts of Day 1

## October 30, 2024

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# Mitochondria in alzheimer disease: it's not what you think

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The "amyloid cascade" - the dominant hypothesis of Alzheimer disease (AD) pathogenesis - invokes the accumulation in brain of plaques (containing the amyloid precursor protein [APP] cleavage product β-amyloid [Aβ]) and tangles (containing hyperphosphorylated tau) as drivers of pathogenesis. However, the poor track record of clinical trials based on this hypothesis suggests that the undeniable accumulation of these peptides is not the only cause of the disease.

We propose an alternative hypothesis in which APP-C99 (the substrate of γ-secretase), and not Aβ (the product of γ-secretase), is the main culprit, via C99′s role as a regulator of cholesterol homeostasis.[1] C99 contains a cholesterol binding domain (CBD) that promotes the formation of "mitochondria-associated ER membranes (MAM)", a cholesterol-rich lipid raft subdomain of the ER that communicates with mitochondria. MAM-rafts are dynamic hubs for numerous cell functions, including membrane composition; cholesterol, phospholipid, calcium, and glucose metabolism; mitochondrial bioenergetics and dynamics; and most notably, regulation of Aβ production and tau phosphorylation. Importantly, all of these functions are perturbed in AD.

Of crucial significance, both C99 and γ-secretase are located predominantly in the MAM. Moreover, C99′s CBD is located in the Ab portion of the peptide, such that cleavage of C99 by γ-secretase releases CBD-containing Aβ for export to the extracellular space, thereby preventing further recruitment of cholesterol to the MAM. Thus, MAM-localized C99 and cholesterol (a "C99-cholesterol axis"), and hence MAM raft formation, are co-regulated in a homeostatic loop that is mediated by the amount of full-length C99 present and the degree of its cleavage by γ-secretase. In this way, the cell is able to maintain a MAM "raft setpoint" required for normal function.

In AD, however, this homeostatic loop is disrupted, owing to genetic and biochemical perturbations that ultimately result in abnormally elevated levels of uncut C99 and/or an accumulation of intracellular cholesterol at the MAM. The net result is inexorable MAM raft growth and upregulated MAM function, provoking the symptomatology of AD. Accordingly, the "MAM hypothesis" invokes two interrelated elements - C99 and cholesterol - that converge at the MAM to drive AD pathogenesis. From this perspective, AD is, at bottom, a lipid disorder, and more precisely, a disorder of cholesterol sensing.

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## Micos complex loss governs age-associated murine mitochondrial architecture and metabolism in the liver, while sam50 dictates diet changes

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**Introduction**: The liver, the largest internal organ and a metabolic hub, undergoes significant declines due to aging, affecting mitochondrial function and increasing the risk of systemic liver diseases. How the mitochondrial three-dimensional (3D) structure changes in the liver across aging, and the biological mechanisms regulating such changes confers remain unclear. To find potential regulators of aging-related change, we examined the Mitochondrial Contact Site and Cristae Organizing System (MICOS) complex, which plays a crucial role in maintaining mitochondrial architecture. We also looked at Sam50, which is a component of the sorting and assembly machinery (SAM) complex that acts in tandem with the MICOS complex to modulate cristae morphology.

**Material & Methods**: In this study, we employed Serial Block Face-Scanning Electron Microscopy (SBF-SEM) to achieve high-resolution 3D reconstructions of murine liver mitochondria. We further correlate these findings with human biobank studies, magnetic resonance imaging from human cohorts, as well as the knockout of components of the MICOS complex, alongside Sam50.

**Results**: 3D reconstructions of murine liver mitochondria reveal diverse phenotypes and structural alterations that occur with age, marked by a reduction in size and complexity. We also show concomitant metabolomic and lipidomic changes in aged samples. Aged human samples reflected altered disease risk. We also observe that the MICOS complex is lost during aging, but not Sam50. In murine models subjected to a high-fat diet, there is a marked depletion of the mitochondrial protein SAM50. This reduction in Sam50 expression may heighten the susceptibility to liver disease, as our human biobank studies corroborate that Sam50 plays a genetically regulated role in the predisposition to multiple liver diseases. We further show that changes in mitochondrial calcium dysregulation and oxidative stress accompany the disruption of the MICOS complex.

**Conclusions**: Together, we establish that a decrease in mitochondrial complexity and dysregulated metabolism occurs with murine liver aging. While these changes are partially be regulated by age-related loss of the MICOS complex, the confluence of a murine high-fat diet can also cause loss of Sam50, which contributes to liver diseases. In summary, our study reveals potential regulators that affect age-related changes in mitochondrial structure and metabolism, which can be targeted in future therapeutic techniques.

## Dynamics of mitochondria: regulation of nucleoid morphology to protect mitochondrial function

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**Introduction**: Mitochondria are dynamic organelles that continually undergo their double-membrane fusion and fission, processes that are critical for the regulation of mitochondrial functions (ref 1). Mitochondria contain their own DNA, mtDNA, which encodes essential respiratory subunits. MtDNAs are assembled with DNA-binding proteins such as TFAM to form mitochondrial nucleoid structures, and in live imaging, nucleoids dynamically move inside mitochondria and change their distribution and morphology. We previously reported that the dynamic features of nucleoids are regulated in cooperation with mitochondrial membrane dynamics via active fusion and fission (ref 2). However, molecular details and the pathophysiological roles of the nucleoid dynamics had been less well understood.

**Results**: We found that inner membrane AAA-ATPase protein ATAD3A plays critical roles in nucleoid trafficking along mitochondria, and in respiratory complex formation (ref 3). Recently we identified several genes that regulates mtDNA nucleoids, and various cellular processes such as post-translational modifications and composition of inner membrane components are critical for not only the nucleoid morphology but also for respiratory complex formation. In vivo analysis further showed that controlling the nucleoid morphology protect against mitochondrial toxicity.
**Conclusion**: Our data revealed importance of nucleoid dynamics for keeping health and protect from disease formation.

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# Mitochondrial dynamics: updates and perspectives

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**Introduction**: Dynamic mitochondrial (mt) network, existing in cells including the skeletal muscle and heart, facilitates rejuvenation of mitochondrial fragments by a membrane potential check [1]. Mt fragments at delayed mitophagy can be engulfed by early sorting endosomes to enrich exosomes as communicating vesicles with other cell types or organs or tissues. Under the sandwich of outer mt membrane (OMM), inner membrane space, and inner boundary membrane (IBM), typical rich mt cristae lamellae are intercepted by nucleoids of mtDNA. Mitochondrial cristae morphology changes were encountered [1-3], depending on intensity of metabolism and oxidative phosphorylation (OXPHOS). Dynamics of crista lamellae and mobile crista junctions were demonstrated by nanoscopy in living cells [see Refs. in 1]. Glucose-stimulated insulin secretion (IS) in pancreatic β-cells led to cristae narrowing with increasing glucose to promote OXPHOS [2].
**Materials & Methods**: Using focused-ion beam/scanning electron microscopy (FIB/SEM) tomography, transmission electron microscopy (TEM), and 3D superresolution microscopy, we studied INS-1E$^{\Delta e+g}$ cells having ATP-synthases lacking $F_O$-membrane subunits e+g, their mitochondria and IS. Extracellular vesicles released by the cells and pancreatic islets (PI) were inspected by proteomics, also at impaired IS.
**Results**: INS-1E$^{\Delta e+g}$ cells exhibited lower ATP synthesis, profoundly inhibited IS and distinct bi-morphic cristae having a regular part with narrow crista lamellae but with frequently bulky lamellae tips. Analyses of mt and other proteins in exosomes released by PIs or pancreases of mice with impaired glucose-stimulated IS indicated the ability to communicate such a stress response.
**Conclusions**: Regular mitochondrial cristae lamellae or at least their parts can be formed even in the absence of the major membrane $F_O$ subunits e, g of ATP-synthase. Probably, delayed mitophagy in IS-impaired pancreatic β-cells enables formation of exosomes which may signal this pathology.

## Funding

Supported by the GACR grants 21-01205S, 23-05798S and 24-10325S.

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## Cellular dynamics: the synergistic interplay of iron, mitochondria, and ferroptosis in health and disease

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Mitochondria are highly dynamic organelles which undergo constant fusion and fission as part of the mitochondrial quality control. In genetic diseases and age-related neurodegenerative disorders, altered mitochondrial fission-fusion dynamics have been linked to impaired mitochondrial quality control, disrupted organelle integrity and function, thereby promoting neural dysfunction and death. The key enzyme regulating mitochondrial fission is the GTPase Dynamin-related Protein 1 (Drp1), which is also considered as a key player in mitochondrial pathways of regulated cell death. Further, Drp1 acts in close interaction with actin-regulating proteins like cofilin1 thereby determining mitochondrial fusion and fission. Increasing evidence suggests a role for impaired mitochondrial dynamics and integrity in ferroptosis, which is an iron-dependent oxidative cell death pathway with relevance in neurodegeneration, and actin-regulating proteins together with Drp1 are key determinants in these processes.

In our work on mitochondrial dynamics, we demonstrate that Drp1 in close connection with actin-regulating proteins regulate mitochondrial dynamics and mitochondrial integrity in conditions of oxidative stress. Knockout of Drp1 resulted in mitochondrial elongation, attenuated ferroptosis-mediated impairment of mitochondrial membrane potential, and stabilized iron trafficking and intracellular iron storage. In addition, Drp1 deficiency exerted metabolic effects, with reduced basal and maximal mitochondrial respiration and a metabolic shift towards glycolysis. These metabolic effects further alleviated the mitochondrial contribution to detrimental ROS production thereby significantly enhancing neural cell resilience against ferroptosis.

In contrast, cofilin1 deletion increased mitochondrial fission through activated Drp1 under standard culture conditions, but still mediated resilience in neurons against ferroptosis as assessed by quantification of mitochondrial ROS production, mitochondrial membrane potential, and ATP levels. Interestingly, cofilin1-deficient cells also covered their energy demand through enhanced glycolysis, suggesting a key role of metabolic regulation for cellular resilience against oxidative damage. Our findings suggest that cofilin1 acts as a redox sensor in oxidative cell death pathways of ferroptosis, and protective effects by cofilin1 inhibition are particularly attributed to preserved mitochondrial integrity and function.

Taken together, our studies highlight the key role of mitochondrial dynamics regulation in ferroptosis and expose mitochondrial metabolism as a promising therapeutic target in neuronal damage caused by oxidative dysregulation.

## Mitochondria iron metabolism: its role in pathogenesis of diseases

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Iron, an essential cofactor for numerous metalloproteins, plays a pivotal role in mitochondrial function. The involvement in the electron transport chain, heme biosynthesis, and iron-sulfur cluster assembly underscores its indispensability in cellular energy metabolism. Disruptions in mitochondrial iron homeostasis can lead to a variety of human diseases, including mitochondrial myopathies and neurodegenerative disorders. The precise mechanisms regulating iron homeostasis within mitochondria are complex and not fully understood. However, several proteins have been implicated in iron transport and storage within

these organelles. These include the mitochondrial ferritin, which stores excess iron, and the mitochondrial transporters that regulates iron uptake. Dysregulation of these proteins can contribute to mitochondrial iron overload and oxidative stress, which sensitizes cells to ferroptosis. Ferroptosis, a regulated form of cell death characterized by iron-dependent lipid peroxidation, has emerged as a critical mechanism in various diseases. Mitochondria play a central role in ferroptosis, as they are both the site of lipid peroxidation and the major source of ROS. Targeting mitochondrial function has emerged as a promising therapeutic strategy for diseases driven by ferroptosis. For example, PANK2-associated neurodegeneration (PKAN), caused by mutations in the *PANK2*, is characterized by progressive neurodegeneration and iron accumulation in the globus pallidus. Our work on hiPSC-derived astrocytes demonstrated that PKAN cells exhibit alterations in iron metabolism, mitochondrial morphology, respiratory activity, oxidative status, and signs of ferroptosis, thus gaining a neurotoxic phenotype. Analysis of endosomal trafficking and super-resolution microscopy experiments in PKAN astrocytes revealed impaired intracellular iron loading and reduced mitochondrial iron delivery. Additionally, PKAN astrocytes exhibited defects in tubulin acetylation/phosphorylation, suggesting impaired vesicular dynamics and inappropriate iron distribution to mitochondria.

## Funding

These findings suggest that the impairment of iron incorporation into mitochondria is the underlying cause of cytosolic iron overload and mitochondrial iron deficiency, ultimately leading to mitochondrial dysfunction and neurodegeneration. Interestingly, the treatment of PKAN astrocytes with reagent that promote mitochondria health, like leriglitazone, a brain penetrant selective agonist of Peroxisome proliferator activated receptor gamma, reduced iron accumulation, improved viability, and enhanced respiratory activity. Our data suggest that targeting the molecular pathways driving iron accumulation can offer therapeutic alternatives to chelation.

## Reference

Santambrogio P, et al. 2024 May 25. Cell Death Dis. 15(5):361.

# Blood-based biomarker: mitochondrial dna damage in parkinson's disease

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**Introduction**: Parkinson's disease (PD) is the most common neurodegenerative movement disorder, and neuroprotective or disease-modifying interventions remain elusive. High-throughput biomarkers aimed at stratifying patients on the basis of shared etiology are required to ensure the success of disease-modifying therapies in clinical trials. Mitochondrial dysfunction plays a prominent role in the pathogenesis of PD. Previously, we found brain region-specific accumulation of mitochondrial DNA (mtDNA) damage in PD neuronal cultures and animal models, as well as in human PD postmortem brain tissue.

**Materials & Methods**: In this study, to investigate mtDNA damage as a potential blood biomarker for PD, we describe a novel PCR-based assay (Mito $DNA_{DX}$) that allows for the accurate real-time quantification of mtDNA damage in a scalable platform.

**Results**: We found that mtDNA damage was increased in peripheral blood mononuclear cells derived from patients with idiopathic PD and those harboring the PD-associated leucine-rich repeat kinase 2 (*LRRK2*) G2019S mutation in comparison with age-matched controls. In addition, mtDNA damage was elevated in non–disease-manifesting *LRRK2* mutation carriers, demonstrating that mtDNA damage can occur irrespective of a PD diagnosis.

**Conclusion**: Quantifying mtDNA damage using the Mito $DNA_{DX}$ assay may have utility as a candidate biomarker of PD and for measuring the pharmacodynamic response to interventions, such as LRRK2 kinase inhibitors.

## Funding

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# An update on the evaluation and quality control of mitochondrial function: advances in clinical applications

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**Introduction**: This talk will address the measurement and monitoring of mitochondrial oxygenation and oxygen consumption in vivo. The presentation will give an overview of the technique, its current use in clinical studies, and reflect on possibilities to use the technique for screening purposes outside the hospital.
**Material and Methods**: Mitochondrial oxygen tension ($mitoPO_2$) can be measured in humans by oxygen-dependent delayed fluorescence of mitochondrial protoporphyrin IX (PpIX)(1, 2). Application of 5-aminolevulinic acid enhances PpIX production in the mitochondria, increasing PpIX concentration to measurable levels and inducing an intramitochondrial oxygen sensor. Upon excitation with a pulse of green light, PpIX emits red delayed fluorescence of which the lifetime is inversely proportional to $mitoPO_2$.
**Results**: Dynamic measurement of $mitoPO_2$ allows direct assessment of cellular respiration in vivo (3,4). Use of the technique in skin allows for non-invasive monitoring of mitochondrial function. In the presentation results from recent clinical studies and a large-scale screening study in healthy subjects will be shown.
**Conclusion**: Based on the protoporphyrin IX technology a clinical monitor for Cellular Oxygen METabolism (COMET) has been developed (5). Clinical studies with COMET are ongoing in several university medical centers in Europe. COMET has been proven useful as a monitor in perioperative (6) and intensive care (7), but recent results suggest the technology could also be used for screening purposes outside the hospital.

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# Evaluation of mitochondrial dysfunctions: metabolic analysis & respirometry

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Beyond hereditary mitochondrial diseases, mitochondrial dysfunctions are now recognized as a prominent feature in the patho-mechanism of many human diseases, ranging from metabolic (1),

vascular, cardiac (2) and neurodegenerative diseases (3) to cancer (4). Because of their involvement in a myriad of cellular functions and signaling pathways (5), identifying the nature of mitochondrial "dysfunctions" can be challenging. Previously limited to a few specialized laboratories, respirometry has become more widespread since the development of new technologies and is an efficient and easy way to analyze mitochondrial function. In addition, recent technologies can be used to multiplex analyses beyond oxygen consumption alone. Hence, Seahorse® automated plate reader, by coupling the detection of oxygen consumption and proton extrusion rates, allows high-throughput and relatively easy metabolic phenotyping on cultured cells. Last generation of Oroboros® high-resolution oxygraphs coupled to fluorescence detection allow parallel determination of respiration rates and mitochondrial membrane potential or ROS production. Thus, according to the experimental design, information can be gained on multiple processes from cellular metabolism overview to substrate preference/fluxes, oxidative phosphorylation efficiency, proton leak, or mechanistic insight into respiratory chain dysfunction.

Complementary markers used in clinical practice for initial orientation towards mitochondrial dysfunction will also be discussed.

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# Biosensing solutions, super-resolution microscopy and optimized pharmacological inhibitors to counteract mitophagy in cancer cells

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**Introduction**: AURKA is a preferential hit in cancer biology, as it is often found to be overexpressed in epithelial cancers. With their 1500 proteins, mitochondria are key hubs sustaining the cell during its evolving needs. We previously unveiled that AURKA is imported into the mitochondrial matrix. There, AURKA increases ATP production by inducing a PHB2-dependent auto/mitophagy.

**Materials and methods**: In my talk, I will start by describing our cross-interactome approach to understand the complexity of the interactions of AURKA with its mitochondrial partners. These interactions were further refined using Förster's Resonance Energy Transfer (FRET)-based approaches in live cells. Last, I will uncover the development of bioavailable allosteric inhibitors of AURKA to counteract its roles at mitochondria.

**Results**: From our cross-interactome integrated analysis, we isolated 11 preferential targets of AURKA with roles in cristae maintenance and in ATP production. Thanks to FRET in live cells, we observed the functional impact of AURKA on mitochondrial protein distribution at cristae. Last, we developed novel allosteric inhibitors of AURKA that alter its interaction with the mitophagy receptor PHB2 at the structural level. This blocks the capacity of AURKA to induce a mitophagy-induced metabolic switch, which restores mitochondrial functionality.

**Conclusions**: Overall, our data highlight that integrating quantitative fluorescence microscopy approaches is instrumental to uncover how mitochondrial functions change at the nanoscale. New PHB2 ligands pave the way to counteract the mitochondrial consequences of AURKA overexpression in cancer, as well as in alternative pathological conditions where PHB2-related mitophagy is induced.

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## Mitochondrial dysfunction in peripheral mononuclear blood cells (PBMCS) of individuals with mild cognitive impairment – a pilot study

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**Background**: People with amnestic Mild Cognitive Impairment (MCI) are more likely to develop neurodegenerative diseases, which having an impact on life expectancy. The relationship between mitochondrial function in PBMC and neuropsychological tests of individuals with MCI was investigated.

**Methods**: Cross-sectional observational study involving 90 female and male young (YS 24.6±3.24 yr.), normocognitive older (NC 68.9±6.95 yr.), and individuals with MCI (MCI, 71.70±7.92 yr.). Cognitive health or cognitive impairment was evaluated using established psychometric methods (CERAD). Mitochondrial respiratory complex activity, Adenosine triphosphate (ATP), and citrate synthase activity (CS) were determined in cryopreserved PBMC.

**Results**: MCI showed significant deficits in all tested cognitive domains. ATP levels and endogenous mitochondrial respiration in PBMC of MCI were significantly lower than that of controls. Linear Regression Analyses with ATP as a predictor for cognitive performance showed a significant positive relationship between ATP levels and Immediate Recall & Fluency suggesting that higher ATP levels are associated with improved cognitive performance. Subjects who performed poorly on psychological tests also exhibited lower ATP levels.

**Conclusion**: Mitochondrial dysfunction in PBMCs was associated with cognitive impairment. Thus, PBMCs, as easily accessible cells can offer valuable insights into the bioenergetic status of individuals with an increased risk to develop neurodegenerative diseases.

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## Mitochondrial nucleic acid sensing in immune and inflammatory responses to respirable particles

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**Introduction**: Airborne dusts exposure is related to chronic diseases development including pulmonary fibrosis, cancer and autoimmune pathologies. However, the involved mechanisms remain elusive. In murine model, particle instillation triggers pulmonary pro-inflammatory responses including, surprisingly, type-I IFN signature. Such responses are typically induced by innate immune sensors activation upon viral or, under specific circumstances, endogenous nucleic acid (NA) recognition. Recently, by inhibiting double-stranded (ds)RNA sensing, we reduced the pro-inflammatory response to particles in vitro and

in vivo. We hypothesize that particle-damaged mitochondria release mt-dsRNA into the cytosol mimicking viral invasion and triggering innate dsRNA sensors activation.

**Material & Methods**: The presence of mt-dsRNA in the cytosolic fraction of silica-exposed THP1 macrophages was assessed by immunofluorescence and RT-qPCR analyses. Ethidium bromide treatment was used to deplete mt-RNA.

**Results**: Upon particle exposure, an increased presence of mt-dsRNA in the cytosolic fraction was observed while the total mt-RNA amount was unmodified suggesting mt-dsRNA trafficking from silica-impacted mitochondria to the cytosol. Mt-RNA depletion prevented dsRNA sensor activation and abolished pro-inflammatory cytokine release in silica-exposed macrophages.

**Conclusion**: Traffic of mt-dsRNA from damaged mitochondria modulates cell response to cytotoxic particles by triggering RNA-sensing pathways. This mechanism, permanently triggered by bio-persistent particles, might explain the development of chronic inflammatory diseases.

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# Role of mitochondrial dysfunction in the pathogenesis of fabry cardiomyopathy and potential effects of the novel enzyme replacement therapy pegunigalsidase alfa

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**Introduction**: Establish Fabry disease (FD) is a x-linked multiorgan failure and reduced life span lysosomal disease caused by loss of α-galactosidase (a-gal-A) activity. The pathogenesis appears to involve energetic and metabolic, including mitochondrial dysfunction1-3. We explored the involvement of mitochondrial alterations in FD cardiomyopathy, using Pegunigalsidase-alfa (PA), a novel recombinant a-gal-A -homo dimer enzyme replacement therapy.

**Material & Methods**: We employed a humanized mouse model of FD and derived embryonic fibroblasts (MEFs), alongside a-gal-A -KO Hela cells, and patients derived fibroblasts to assess mitochondrial phenotype and the effects of the novel enzyme replacement therapy, PA.

**Results**: Throughout all the FD cellular models, we observed impaired mitochondrial respiration and ATP synthesis, resulting from reduced mitophagy-flux, accumulation of aberrant mitochondria, and compromised PGC-1α-dependent mitochondrial renewal. Similar alterations of mitochondrial function and turnover were detected in GLA-KO mouse hearts, preceding the onset of cardiomyocyte hypertrophy and dysfunction. PA was successfully internalized by FD-cells and Heart. In FD-cells, PA was enzymatically active and able to improve mitochondrial respiration, restoring cellular ATP content.

**Conclusion**: Mitochondrial turnover and energetic alterations participate in FD cardiomyopathy. PA is successfully internalized, stable, and active and shows promising effects on mitochondrial and energetic restoration in FD-cells.

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## Mitochondrial extracellular vesicles: orchestrating brain plasticity and recovery

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Advances in stem cell biology have raised great expectations that diseases of the central nervous system may be ameliorated by the development of non-haematopoietic stem cell medicines. Yet, the application of stem cells as therapeutics is challenging and the interpretation of some of the outcomes ambiguous. The initial idea that stem cell transplants work only via structural cell replacement has been challenged by the observation of consistent intercellular information exchange between the graft and the host. Sustained stem cell graft-to-host exchange of signals has led to remarkable trophic effects on endogenous brain cells and beneficial modulatory actions on innate and adaptive immune responses that ultimately promote the healing of the injured CNS. Among a number of promising candidate stem cell sources, neural stem/precursor cells (NSCs) are being extensively investigated for their capacities to signal to the immune system upon transplantation in experimental CNS diseases.

Here I will focus on defining whether the form of cellular signaling mediated by extracellular membrane vesicles (EVs) exists for NSCs, and on its molecular signature and functional relevance on target cells. I will then share evidence that the EV cargo molecules, including fully functional EV-trafficked extracellular mitochondria, are modulated by extracellular pro- or anti-inflammatory cytokines and determine the key elements responsible for this novel mechanism of EV-mediated intercellular communication. I will finally reflect on the forthcoming challenges related to the translation of some of these exciting experimental proofs into ready-to-use clinical medicines for inflammatory CNS diseases.

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## Mitochondria delivery via extracellular vesicles to the blood-brain barrier

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Extracellular vesicles (EVs) are natural, nano-sized carriers of cellular cargo. EVs play a key role in intercellular communication via transfer of their internal components, including lipids, proteins and nucleic acids. Our works have demonstrated that the medium-to-large EVs (m/lEVs) with particle diameters >200 nm but not the small EVs (sEVs<200 nm) contain mitochondria.[1,2] We isolated EVs from brain endothelial cells (BECs) as BECs carry a five-fold greater mitochondrial load compared to peripherals ECs.[3] (1) m/lEVs isolated from both human and mouse BECs transfer mitochondria into recipient human/

mouse BECs with *homologous* pairs (mouse BEC-derived EVs:recipient mouse BECs or human BEC-derived EVs:recipient human BECs) outperforming heterologous pairs,[2] (2) m/lEVs increase cellular ATP levels and mitochondrial function in the recipient oxygen- glucose deprived (OGD) BECs. Importantly, recipient OGD BECs treated with m/lEVs displayed superior mitochondrial function compared to BECs treated with control EXOs that lack mitochondria.[1, 2, 4] (3) BEC-derived m/lEVs transfer their innate mitochondria that subsequently localized with recipient cell mitochondria in primary human BECs, suggestive of mitochondrial fusion[1] and (4) mice injected with mouse BEC-derived m/lEVs demonstrated a 50% reduction in brain tissue damage and improved neurological functions compared to vehicle- injected mice in a mouse middle cerebral artery occlusion model of ischemia/reperfusion injury (stroke).[2]

In summary, we, for the first time, have demonstrated therapeutic efficacy of the larger, mitochondria-containing EVs in a mouse model of ischemic stroke. Delivery of functional mitochondria is an effective approach to protect the post-ischemic blood-brain barrier—and therefore is a promising strategy to decrease long-term neurological dysfunction post-stroke.[5] In addition to developing EV mitochondria as stroke therapy, we are also developing them as a platform technology that can be used to treat multiple disorders associated with mitochondrial dysfunction.[6]

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# Regulation of mitochondrial calcium uniporter complex in human microglia derived from ipsc of alzheimer's disease patients


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Alzheimer's disease (AD) pathology has been linked to mitochondrial dysfunction and inflammatory processes. Mitochondria, a highly metabolically active organelle, plays a critical role in regulating the brain innate immune function. During AD pathology, microglia undergo metabolic reprogramming to facilitate the energetic needs for cytokine production and phagocytic processes. Mitochondrial Ca2+ uptake via the mitochondrial Ca2+ uniporter (MCU) supports mitochondrial metabolism and it has been suggested that it could bridge the metabolic reprogramming and immune cell function. Therefore, we have hypothesized that MCU activity may impact microglial phagocytosis. Here we evaluated whether the negative modulators of MCU complex can affect phagocytosis in AD microglia and prevent neuronal cell death. Transcriptomic profile of human microglia integrated in AD brain organoids showed that the (mitochondrial) calcium genes and metabolic TCA/glycolytic genes were changed compared to isogenic brain organoids. Moreover, phagocytosis was decreased in human AD microglia compared to isogenic microglia, while negative modulation of MCU complex could prevent the deficits in phagocytosis in AD microglia.

Taken together, our results reveal that negative modulation of MCU complex could prevent neuronal degeneration and increase phagocytosis in human microglia, proposing MCU complex as a potential therapeutic target in diseases linked to increased mitochondrial calcium.

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# Effects of a modified ketogenic diet and glutamine supplementation on the cerebellar metabolome of a mouse model of mitochondrial disease

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Mitochondrial diseases (MD) are disorders caused by dysfunction of the oxidative phosphorylation system. Nutritional therapies are the main treatment for many inherited metabolic disorders. We have assessed the effects of glutamine supplementation (Gln) and a medium chain triglyceride-based ketogenic diet (MITODiet) on the motor function and cerebellar metabolome of the Harlequin mouse model of MD. Male wild-type (WT) and Harlequin (Hq) mice were fed with: i) tap water and standard mice chow; ii) 4% Gln in tap water and standard chow, and iii) tap water and MITODiet, for 5 months. After the interventions animals were sacrificed and cerebella were obtained for analysis of key metabolic enzymes by reverse phase protein microarrays1 and the metabolome by UHPLC-Q/TOF-MS-HILIC. Both interventions attenuated ataxia, Gln prevented strength loss in Hq mice and MITODiet induced higher levels of 18 metabolic enzymes in WT-treated mice. Metabolomic analysis showed 11 metabolites significantly different between groups, of which 6 were higher in Hq than in WT mice, and 4 metabolites were normalized by both interventions. Conclusions: Improvement of the ataxia in the Hq mouse model under Gln supplementation and MITODiet was accompanied by metabolic stabilization in the cerebellum. These results support conducting similar nutritional interventions in clinical studies.

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## Mitochondria to plasma membrane redox signaling is essential for fatty acid-stimulated insulin secretion

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**Introduction**: We have previously shown that palmitic acid stimulates insulin secretion via an intracellular pathway involving the redox-activated mitochondrial phospholipase iPLA2γ, leading to activation of the G-protein-coupled receptor-40 (GPR40) [1, 2]. Here, we tested the hypothesis that β-oxidation of mitochondrial fatty acids (FAs) initiates the redox signal required for fatty acid-stimulated insulin secretion (FASIS).
**Material & Methods**: We have developed methods for the detection and quantification of dynamic insulin secretion together with H2O2 release during FASIS in isolated pancreatic islets and insulinoma cells INS-1E.
**Results**: Using perifusion of isolated mouse pancreatic islets, we demonstrated the ability of fatty acids to stimulate insulin secretion at low glucose concentrations. The increase in insulin secretion was accompanied by an increased release of H2O2. Both insulin secretion and H2O2 release were inhibited by the mitochondrial antioxidant SkQ1, inhibitors of FA β-oxidation, silencing of carnitine palmitoyltransferase 1, and overexpression of catalase. In addition, FASIS was inhibited by a GPR40 antagonist and by pharmacological inhibition or genetic ablation of iPLA2γ.
**Conclusion**: Our results are consistent with a mechanism of an intramitochondrial redox signal derived from β-oxidation of FAs that activates mitochondrial iPLA2γ, causing the intramitochondrial release of FAs that subsequently stimulate GPR40 at the plasma membrane.

### Funding

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## Aging-associated mitochondrial signature in human fibroblasts and corresponding fibroblast-derived neurons

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**Introduction**: Aging is a major risk factor for many prevalent diseases. Understanding the brain's aging process, especially the role of mitochondria, is still underexplored. Finding suitable human models for studying brain aging is challenging. Therefore, advanced neuronal in-vitro models, including neurons derived from induced pluripotent stem cells (N-iPSCs) and directly induced neurons (iNs) from human fibroblasts, are of interest. This study investigated how well these models preserve the aging donor signature, focusing on mitochondria.
**Material & Methods**: We compared the bioenergetic profiles of human fibroblasts from young and aged donors with those of iPSCsN and iNs from the same donors. Parameters assessed included total ATP levels, mitochondrial membrane potential (MMP), reactive oxygen species (ROS), mitochondrial respiration, and glycolysis.

**Results**: Our findings show that aged neurons exhibit decreased ATP, MMP, and OCR and increased ROS and glycolysis, similar to aged fibroblasts. This indicates that iNs and N-iPSCs display an aging-associated mitochondrial phenotype, contrary to the belief that aged iPSCsN would fully rejuvenate.
**Conclusion**: Overall, this work will help us better understand how accurately "aged" iNs and N-iPSCs simulate human brain aging and contribute to the development of cutting-edge methods for discovering pharmacological targets to enhance human health during aging.

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# Increased instability levels of circulating cell-free mitochondrial dna are associated with worse clinical outcomes in late-life depression

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**Introduction**: Late-life depression (LLD) is a common and debilitating mental illness, with growing evidence suggesting that mitochondria play an important role in its development. Dysfunctional mitochondria generate excessive reactive oxygen species, which damage mitochondrial DNA (mtDNA). When released as circulating cell-free mtDNA (ccf-mtDNA), this triggers inflammation and heightens vulnerability to adverse health outcomes.
**Methods**: We included ninety elderly individuals (50 LLD, 40 controls), with a subset of individuals followed up at 30 months (13 LLD, 13 controls). DNA was extracted from plasma and mitochondrial genes MT-ND2 and MT-ND4 were targeted to evaluate ccf-mtDNA levels and deletion using qPCR. Interleukins 1β, 5, and 6 were quantified via immunoassay.
**Results**: LLD is linked to increased ccf-mtDNA instability at baseline (deletion: $F(88,1) = 7.105$, $p = 0.009$; level:$F(88,1) = 6.885$, $p = 0.01$), which was associated with more severe depressive symptoms (deletion: $p = 0.005$; level: $p = 0.002$) and greater medical comorbidity burden (deletion: $p = 0.016$; level: $p = 0.025$). Longitudinal analysis revealed significant effects of diagnosis and time on the ccf-mtDNA levels and deletion rate. Additionally, higher deletion rates at the baseline predicted higher IL-1β levels at 30 months ($p = 0.04$).
**Conclusions**: Our findings indicate that LLD is linked to increased ccf-mtDNA instability, which appears pertinent to clinical features. Further research is needed to validate our findings.

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## MNRR1, a moonlighting druggable target for mitochondrial disease

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Mitochondrial-Nuclear Retrograde Regulator 1 (MNRR1) is a biorganellar regulator of cellular stress response. MELAS disease mitochondria harbor a heteroplasmic genotype with higher mutant levels associated with a neurodegenerative phenotype. We previously showed that MNRR1 levels are reduced in a MELAS cell culture model and enhancing MNRR1 levels significantly reversed heteroplasmy. Successful identification of drugs that stimulate MNRR1 production make it a "druggable" therapeutic target; we showed that one such drug, nitazoxanide, induces homeostatic programs such as mitochondrial biogenesis and mtUPR in MELAS primary patient fibroblasts. Toward the mechanism of MNRR1 reduction, we showed transcriptional downregulation in MELAS cells via competitive inhibition by HIF-2a at a conserved oxygen responsive element (ORE) in the MNRR1 promoter. Our data suggest that HIF-2a levels are transcriptionally induced in MELAS cells and this induction is enhanced by protein stabilization via lower levels of prolyl-4-hydroxylase domain 3 (PHD3). Reducing HIF-2a by either genetic knockdown, specific inhibitors, or overexpression of PHD3 resulted in rescue of mitochondrial function. Towards the mechanism by which overexpressing MNRR1 induces mitophagy and mitochondrial biogenesis to rescue the heteroplasmic defect, we have shown that MNRR1 functions as a transcriptional inducer by interacting with RBPJκ and recruiting p300 in a Sirt1-dependent manner.

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## The micos complex as a therapeutic target that modulates age-dependent mitochondrial metabolic and structural deficits in kidney

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The kidney filters waste and fluids from the bloodstream, concurrently modulating metabolic and hormonal homeostasis. These processes, requiring substantial energy, are reliant on mitochondria, which dynamically maintain function through quality control mechanisms including structural rearrangement. However, aging in the kidney reduces mitochondrial efficiency, impairing kidney function. Mitochondrial dysfunction, including cristae disruption by the mitochondrial contact site and cristae organizing system (MICOS) complex, increased reactive oxygen species (ROS), and metabolic alterations, marks this decline. We hypothesized that the MICOS complex is centrally involved in causing age-dependent mitochondrial dysfunction. To test this hypothesis, we utilized serial block face-scanning electron microscopy (SBF-SEM) and Amira software for 3D visualization of mitochondrial ultrastructure in tubular kidney cells at 3-months and 2-years. Our findings indicate that 2-year-old mitochondria are more fragmented than those at 3-months, with distinct shapes emerging during aging, linked to shifts in ROS, metabolomics, and lipid homeostasis. Additionally, aging impairs the MICOS complex, in turn affecting mitochondrial calcium uptake, retention, and oxidative stress. As MICOS disruption exacerbates mitochondrial dysfunction, creating a cycle of degradation and oxidative stress and potentially contributing to the age-related prevalence of kidney diseases, the MICOS complex may represent a key target to mitigate mitochondrial structural deficits and improve longevity.

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# Defective mitochondrial protein import in secondary mitochondrial damage-related neuromuscular diseases

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**Introduction**: The transport of mitochondrial proteins to the organelle must be error-free for the proper functioning of mitochondria. Abnormal mitochondrial morphology, decreased membrane potential, and ATP production, as well as increased ROS levels have been shown to affect mitochondrial protein import. In addition, our group identified common miRNAs involved in the regulation of mitochondrial damage observed in rare neuromuscular diseases and found that their possible target genes are associated with mitochondrial protein import. For this purpose, the relationship between mitochondrial protein import mechanism and secondary mitochondrial damage in the etiopathogenesis of DMD, Megaconial, and Ullrich CMD was investigated in this study.

**Material & Methods**: Mitochondrial protein import assay was performed using pcDNA3/Mito-GFP plasmid containing the mitochondrial targeting signal sequence of Aconitase 2 in primary myoblast cells. Additionally, LC-MS/MS was performed on pure mitochondria isolated from primary myoblasts.

**Results**: A statistically significant decrease in MitoGFP-TOM20 co-localization was observed in all patients' cells and expression of candidate proteins found to be decreased in the mitochondrial fraction by proteomic studies was confirmed at RNA and protein levels.

**Conclusion**: Mitochondrial protein import defect was identified in rare neuromuscular diseases associated with secondary mitochondrial damage, enabling the identification of common therapeutic targets.

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## Quantifying mitochondrial dynamics and function in primary mitochondrial diseased fibroblasts linked with mitochondrial dna mutations and deletions

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**Introduction**: Primary mitochondrial diseases (PMDs) of varying severity in children are linked to mitochondrial DNA (mtDNA) mutations or deletions impacting the electron transport chain (ETC). Developing better therapies require adequate bench-top models to understand mitochondrial dynamics and function. This study evaluated the feasability of determining mitochondrial dynamics & function as markers in patient-derived fibroblasts for assessing disease severity.

**Material & Methods**: Mitochondria stained with Mitotracker red were analyzed with an ImageJ plugin tool to quantitate mitochondrial dynamics in healthy and diseased cells (1). Mitochondrial function (oxygen consumption rate (OCR) was measured in healthy and diseased fibroblast cells (2). Flow cytometry technique was used to quantitate mitochondrial membrane potential (MMP) with TMRE (1).

**Results and Conclusion**: Mitochondrial fragmentation was associated with PMDs. Interestingly, one PMD line containing mtDNA mutation displayed mitochondrial hyperfusion as a compensatory mechanism. Mitochondrial bioenergetics was significantly reduced in all PMD lines. The exception was, one PMD line displayed higher OCR to compensate for proton leak. MMP was significantly reduced in all the PMD lines. Loss of MMP disrupted mitochondrial fusion and fission pathways, resulting in mitochondrial fragmentation or hyperfusion. Thus, this study demonstrates the sensitivity of mitochondrial dynamics & function as markers in PMDs of varying severity.

### Funding

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## A novel gpcr agonist induces selective mitophagy in a complex iii deficiency model improving bioenergetic function and overall cellular health

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**Introduction**: Mitophagy, the selective degradation of mitochondria by autophagy, is a cellular mechanism used to target and degrade dysfunctional mitochondria1. The accumulation of dysfunctional mitochondria can be a common pathophysiological mechanism in mitochondrial diseases and a target for treatment2,3.

**Material & Methods**: Control and mitochondrial disease patient-derived skin fibroblasts were employed for treatment with CAP-1902. Using confocal microscopy we evaluated: mitophagy by Mito-QC expression; lysosomal and mitochondrial function and distribution. SeaHorse intact respirometry was used

for assessment of bioenergetic function. RNAseq and western blotting of specific markers were used for assessment of autophagy/mitophagy, AMPK and MASR signaling pathways.

**Results**: Here, we report a novel mitophagy inducer, CAP-1902, an agonist of the MAS G-Protein Coupled Receptor (MASR). We find that activation of MASR by CAP-1902 triggers the AMPK/ULK1/FUNDC1 signaling pathway, promoting mitochondrial biogenesis and mitophagy. The combined induction of mitochondrial biogenesis via PGC1α coupled with an increase in mitophagic flux indicates that MASR activation promotes mitochondrial turnover. In studies on human fibroblasts with a BCS1L mutation that disrupts mitochondrial complex III (CIII) assembly, CAP-1902 restored mitophagic flux and improved bioenergetic function, decreasing mitochondrial stress.

**Conclusion**: In summary, we have described the first potential GPCR-mediated treatment of a mitochondrial disease model through mitophagy induction.

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# Structure of the II2-III2-IV2 mitochondrial supercomplex from a parasite reveals a protein inhibitor of complex III

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We report a new type of II2-III2-IV2 supercomplex in Apicomplexa-related parasites. The 1.8 megadalton supercomplex comprises 104 proteins and 114 lipids. The 2.1-Å resolution structure represents the first observation of CII in this type of supercomplex, and activity assays confirmed the presence of a complete electron transfer from succinate to molecular oxygen utilising CII. The distinctive feature is achieved via an apicomplexan subunit, which bridges two copies of CII with CIII dimer and CIV.

The second finding is the identification of a natural negative regulator at the conserved site on CIII. We identified a heterodimer that is a protein binder locking the Rieske Iron-Sulfur Protein head in the c1 state, effectively inactivating electron transfer. Its C-terminus stabilizes the mobile head, while the N-terminus associates the heterodimer in the binding site of the universal electron carrier Cyt c. From an evolutionary perspective, the structure reveals a programmed +2 frameshifts, which illuminate evolutionary adaptations at the gene expression level.

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15th WMS World Congress on

# Targeting Mitochondria

## Abstracts of Day 2

## October 31, 2023

## Mitochondria: more heat than light?

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Mitochondria are not just producers of ATP or clearing houses of intermediary metabolism. Here I will trace the history of how mitochondria have come to be regarded as key contributors to cell and organismal physiology, and how recent findings have opened up new chapters in our thinking (1).
Notably, I will discuss the implications of elevated mitochondrial temperature (2, 3) for evolution, development, cell signaling, immunity and pathology.

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## Mitochondria and photomedicine: advances in infrared light treatment

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**Introduction**: Brain ischemia occurs after a stroke and cardiac arrest leading to neuronal death if left untreated. However, reentry of oxygen into the tissue by removal of the blood clot or resuscitation, respectively, causes additional damage called reperfusion injury due to molecular changes within the mitochondria leading to cell death. Specifically, the reaction of cytochrome *c* and cytochrome *c* oxidase (COX) is increased due to loss of posttranslational modifications which control the activity of the electron transport chain under normal conditions. Loss of these modifications resulting in maximal ETC activity during reperfusion, leading to mitochondrial membrane potential (MMP) hyperpolarization, excessive ROS production, and cell death. An ideal therapy for ischemia-reperfusion injury would normalize mitochondrial hyperactivity non-invasively, act locally where needed, and can be applied early during reperfusion, all of which are major limitations of pharmacological approaches that rely on blood flow.
**Material & Methods**: We have discovered specific wavelengths of near infrared light (IRL) that inhibit the reaction between cytochrome *c* and COX. We here noninvasively applied these IRL wavelengths in small and clinically relevent large animal models of brain ischemia/reperfusion injury to test their therapeutic effect. In addition, we have developed waveguide technology that safely and efficiently delivers IRL into the brain.
**Results**: COX-inhibitory IRL reduces the mitochondrial membrane potential (MMP), suppresses mitochondrial superoxide production, and attenuates neuronal death. In vivo, IRL treatment results in robust neuroprotection whereas untreated animals show a massive loss of neurons. We further show that IRL, applied noninvasively, penetrates deeply into the human brain. Working toward cinical implementation we show that IRL transmission into the human brain can be further boosted using waveguide technology, allowing safe and uniform IRL delivery.
**Conclusion**: We propose that COX-inhibitory IRL is a viable non-pharmacologic and noninvasive therapy for the treatment of cerebral ischemia/reperfusion injury and discuss next steps for clinical implementation.

### Funding

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## Role of light in regulating blood glucose via mitochondrial stimulation

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**Introduction**: Mitochondria regulate metabolism, but visible light influences its rate. Photobiomodulation (PBM) with long wavelength light (~650-900 nm) increases mitochondrial membrane potential, upregulates ATP production and reduces reactive oxygen species [1-5]. Longer wavelength light is absorbed by cytochrome C oxidase, leading to increased electron transport activity that increases mitochondrial membrane potential and ATP production [6]. Evidence also reports that 670 nm reduces the viscosity of nanowater surrounding rotary ATP pumps improving their efficiency [7]. 670 nm PBM increases mitochondrial respiration with systemic effects [8]. The increased ATP production will demand increased cellular glucose uptake. Hence, we test the hypotheses that this may be detectable in changes in plasma glucose levels.

**Materials and Methods**: We address this hypothesis by examining the effect that 670 nm PBM has upon human plasma glucose levels over the course of a standard oral glucose tolerance test (OGTT). Healthy human participants (n = 30) underwent a standard fasting OGTT to spike blood glucose levels, monitoring blood glucose concentration at 15-min intervals over 2 h. Baseline OGTT results were compared against those following a 15-min exposure to 40 $mW/cm^2$ 670nm light over 800$cm^2$ region of upper back (n = 15). This cohort's data was also compared to participants who underwent a placebo exposure (no light) (n = 15).

**Results**: A 15-min exposure to 670 nm light reduced the degree of blood glucose elevation following glucose intake by 27.7 %, integrated over 2 h after the glucose challenge. With the maximum glucose spike during the 2h OGTT reduced by 7.5 %.

**Conclusions**: Photobiomodulation with 670 nm light can be used to reduce blood glucose spikes following meals. This intervention may reduce damaging fluctuations of blood glucose on the body.

### Funding

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## Targeting mitochondrial-driven inflammation to kill cancer

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The only good cancer cell is a dead cancer cell. Indeed, most anti-cancer therapies work by killing cancer cells. Often this occurs through mitochondrial apoptosis, a process whereby mitochondrial outer membrane permeabilisation (MOMP) enables the release of mitochondrial intermembrane space proteins into the cytoplasm, activating caspase proteases and rapid cell death [1]. While engagement of apoptosis is an effective way to eradicate tumour cells, resistance to cell death is commonplace, leading to cancer relapse. Our study's central question was ***can we kill cancer cells more effectively?*** During commitment to cell death, widespread MOMP represents the point-of-no-return, cells die irrespective of caspase activity due to catastrophic loss of mitochondrial function.

We investigated whether engagement of MOMP under caspase inhibited conditions (so-called caspase-independent cell death – CICD) was an effective way to treat cancer[2]. Significantly, in mouse models of cancer, treating tumours to undergo CICD led to dramatic effects – enabling complete tumour regression. These effects were dependent on the ability of CICD to engage anti-tumour immunity. Underpinning this immunogenicity is the ability of permeabilised mitochondria to engage a variety of pro-inflammatory signalling pathways[3,4]. I will discuss the potential to therapeutically harness these mitochondrial-driven inflammatory pathways to improve anti-cancer treatment.

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# The role of mitochondria in zika virus propagation: mechanisms of immune evasion and intercellular transmission

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**Introduction**: Zika virus (ZIKV) remains a significant public health challenge due to the limited availability of preventive measures and treatments. ZIKV is unique among flaviviruses for its ability to be vertically transmitted from mother to fetus, yet the mechanisms underlying this process are not fully understood. Here, we investigated the cellular mechanisms ZIKV employs for vertical transmission and evasion of the host's immune response, with a focus on its interaction with placental trophoblasts.

**Materials & Methods**: The study employed various mammalian cell types, including placental trophoblasts, to investigate the induction of tunneling nanotubes (TNTs) by ZIKV. Both African and Asian lineages of ZIKV were examined, alongside other flaviviruses for comparison. Molecular techniques, including affinity purification-mass spectrometry, were used to identify protein interactions, while a mutant ZIKV strain (ZIKV ΔTNT) was engineered to assess the role of TNTs in viral replication and immune evasion. Additionally, a mouse model of ZIKV vertical transmission was utilized to evaluate the in vivo effects of the TNT-deficient ZIKV strain.

**Results**: ZIKV, unlike other flaviviruses, induced the formation of TNTs in placental trophoblasts and various mammalian cells. These TNTs facilitated the transfer of viral particles, proteins, and RNA to neighboring uninfected cells, aiding in ZIKV cell-to-cell transmission while evading host immune defenses. The N-terminal region of the ZIKV non-structural protein 1 (NS1) was identified as crucial for TNT formation. ZIKV ΔTNT, deficient in TNT formation, triggered a strong antiviral interferon (IFN-λ 1/2/3) response, indicating that TNTs help ZIKV evade immune detection. Furthermore, mitochondrial proteins were identified as major NS1-interacting partners, and ZIKV-infected cells were shown to siphon mitochondria from healthy cells via TNTs, enhancing viral replication.

**Conclusion**: This study uncovers a novel stealth mechanism used by ZIKV for intercellular spread among placental trophoblasts, evasion of antiviral interferon responses, and the hijacking of mitochondria to promote viral propagation and survival. These findings provide a foundation for developing new therapeutic strategies targeting these interactions to limit ZIKV dissemination and its associated fetal adversities.

# Stem cell educator therapy: a new era in mitochondrial transfer and regeneration

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**Introduction**: Over the last 14 years, Throne Biotechnologies (Throne) has developed a unique and novel procedure designated the Stem Cell Educator (SCE) therapy[1]. International multi-center clinical studies have demonstrated its clinical efficacy and safety of SCE therapy that can fundamentally reverse type 1 diabetes (T1D), alopecia areata (AA) and other autoimmune/inflammatory diseases or aging-associated neurodegenerative diseases through the immune education of SCE therapy at the root causes, which are the mitochondrial dysfunctions at the local or systematic levels.

**Material & Methods**: Patients with T1D, AA, or other autoimmune/inflammatory diseases were recruited and received one treatment with Educator therapy through the US FDA-approved phase 2/3 clinical trials and Right-to-Try Act. A patient's blood is circulated through a blood cell separator (apheresis), wherein the collected patient's immune cells are co-cultured with adherent cord-blood-derived multipotent stem cells (CB-SC) in vitro, after which "educated" immune cells are returned to the patient's circulation. The safety and clinical efficacy were evaluted during 1-year follow-ups and autoimmune/inflammatory markers were examined by flow cytometry analysis.

**Results**: Using human C-peptide (a by-product of insulin biosynthesis) as an indicator for the restoration of endogenous islet β cell function, the levels of C-peptide were monitored after the treatment with Stem Cell Educator therapy in recent onset T1D subjects (N =10). Clinical data demonstrated that T1D subjects markedly increased their C-peptide levels relative their baseline levels in the 1st month post treatment with Stem Cell Educator therapy (P = 0.028), with the improved sugar control and the reduction of total insulin dosages. Some of T1D patients became insulin-free. Additional clinical data showed the long-lasting improvement of hair regrowth in subjects with moderate or severe AA, without safety issues post SCE therapy. Mechanistic studies revealed that SCE therapy could fundamentally correct the autoimmunity and induce immune tolerance through multiple molecular and cellular mechanisms such as the expression of a master transcriptional factor autoimmune regulator (AIRE) in CB-SC for T-cell modulation[1], an expression of Galectin-9 on CB-SC to suppress activated B cells[2], secretion of CB-SC-derived exosomes to polarize human blood monocytes/macrophages into type 2 macrophages (M2)[3], and releasing mitochondria contributing the immune modulation and tissue regeneration.

**Conclusion**: Stem Cell Educator therapy has the potential to revolutionize the treatment of T1D and eliminate the need for lifelong insulin therapy, without the safety and ethical concerns associated with conventional immune and stem cell-based approaches. SCE therapy is the leading technology in immunotherapy and mitochondrial transfer to date to safely and efficiently repair and correct the mitochondrial dysfunctions in multiple human diseases caused by the autoimmunity, chronic inflammation and/or aging.

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## Mitochondria-transfer based therapies reduce the morbidity and mortality of leigh syndrome

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**Introduction**: Mitochondria transfer is a recently described phenomenon in which donor cells deliver their mitochondria to acceptor cells. One function of mitochondria transfer is to provide energetic support to neighboring cells, as evidenced by studies demonstrating that exogenous healthy mitochondria can rescue cell-intrinsic defects in mitochondrial metabolism in $\rho^0$ cells or Ndufs4$^{-/-}$ macrophages. Exposing hematopoietic stem cells (HSC) to purified mitochondria before autologous HSC transplantation was used to treat anemia in patients with large-scale mtDNA mutations, and mitochondria transplantation minimizes ischemic damage to the heart, brain, and limbs. However, the therapeutic potential of using mitochondria transfer-based therapies to treat inherited mitochondrial diseases is unclear.

**Material & Methods**: We used bone marrow and mitochondria transplants in Ndufs4$^{-/-}$ mice and assessed disease severity using true survival, rotarod testing, grip strength testing, and metabolic cage analyses.

**Results**: We show that wildtype (WT) bone marrow (BM) transplantation improves the morbidity and mortality of the Ndufs4$^{-/-}$ mouse model of Leigh Syndrome (LS), effects associated with release of hematopoietic cell-derived extracellular mitochondria into circulation and transfer of mitochondria to host cells in multiple organs. Administering isolated WT mitochondria also extended lifespan, improved neurologic function, and increased energy expenditure of Ndufs4$^{-/-}$ mice. Mitochondria isolated from Ndufs4$^{-/-}$ mice had impaired respiratory capacity and failed to ameliorate LS, suggesting a potential bioenergetic mechanism of mitochondria transplantation. Furthermore, cross-species administration of human mitochondria into Ndufs4$^{-/-}$ mice ameliorated LS.

**Conclusion**: These data suggest that mitochondria transfer-related pathways can be harnessed to treat mitochondrial diseases such as LS.

## Mitochondrial transplantation mediates improved cellular energy metabolism, restoration of mitochondrial function and prevention of cell death

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**Objectives**: 1. Describe the role of MOT in TBI; 2. Discuss the importance of mitochondria in TBI and related disorders; 3. Identify the potential targeted approaches for the treatment of TBI.

**Introduction**: Mitochondrial diseases are a class of acquired or inherited metabolic disorders caused by mitochondrial dysfunction which may affect almost all the organs in the body and present at any age. Currently, there are no adequate therapeutic approaches available for mitochondrial diseases. Mitochondrial transplantation is a promising approach for treatment of mitochondrial diseases by rescue of dysfunctional mitochondria in cells using isolated functional mitochondria. Mitochondrial dysfunction is associated with traumatic brain injury (TBI), Gulf War illness (GWI), neurodegenerative diseases, such as amyotrophic lateral sclerosis

(ALS), aging and senescence. But also is critical in diseases like Friedriech's Ataxia, AIF mutations, (mitochondrial encephalomyopathy, X-linked Charcot–Marie–Tooth disease-4), among others.

**Methods and Materials**: We have developed a novel approach to the isolation and administration of mitochondria using mitochondrial organelle transplantation (MOT™) to replace, repair and boost mitochondrial health in *in vitro* and *in vivo*.

**Results**: Isolation, characterization and application of mitochondria from primary cells and cell lines as well as tissue specific mitochondria (TSM) demonstrated that the mitochondria are viable and functional and using intracerebralventricular (ICV) injections, tail vein injections or nasal administration, the mitochondria localized to the site of injury and remain functional for several weeks. In the mouse CCI, model, we can attenuate the lesion progression, reduce inflammation (microglial and astrocytic activation), cathepsin B and oxidative stress as well as improve behavioral outcomes in the animals. Moreover, the application of exogenous mitochondria significantly reduced cellular apoptosis, promoted angiogenesis and alleviated brain edema and blood-brain barrier leakage in mice subjected to TBI. In addition, MOT therapy improved ATP and mitochondrial function, BDNF and NGF levels.

**Conclusions**: The results suggest that MOT might be a translational approach for the treatment of mitochondrial diseases, including TBI, both early and late, and provide benefit to individuals not only with TBI, but other neurological or neurodegenerative disorders. In addition, we have evidence of MOT impact in mouse models of AD, ALS, stroke, and AIFM1 mutations, both in human cells and mice. We are scaling up to potentially execute clinical trials with MOT at the James A. Haley VA.

## The intersection of mitochondrial function and psychiatric disorders: biomarkers, gene variants, and patient stratification

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**Introduction**: Mood and metabolic disorders, such as bipolar disorder (BD) and schizophrenia (SCZ), are increasingly recognized as co-occurring diseases with shared pathophysiological mechanisms, particularly mitochondrial dysfunction. This study investigates the bidirectional relationship between mitochondrial function and these psychiatric conditions, focusing on two key areas: mitochondrial blood-based biomarkers (lactate and circulating cell-free mitochondrial DNA, ccf-mtDNA) and the association of nuclear-encoded mitochondrial gene (NEMG) variants with psychiatric disorders. The aim is to enhance patient stratification and therapeutic targeting, providing insights into personalized treatment strategies.

**Materials & Methods**: The study involved two French cohorts (FACE-BD & I-GIVE) with BD, some of whom also had metabolic syndrome (MetS). Lactate and ccf-mtDNA levels were measured and analyzed for their association with MetS and mood disorders. Additionally, a random-effects meta-analysis of single nucleotide polymorphisms (SNPs) in NEMG, based on MitoCarta v3.0, was conducted using data from large psychiatric consortia (SCZ-PGCw3 and BD-PGCw3). Mitochondrial polygenic risk scores (mitoPRS) were generated to assess the genetic susceptibility to BD and SCZ.

**Results**: Lactate levels were found to be significantly associated with MetS in the FACE-BD cohort. High lactate levels also facilitated patient stratification in mitochondrial-targeted metabolomics. No significant associations were found with ccf-mtDNA. In the genetic analysis, positive associations were observed between NEMG variants and BD and SCZ. The mitoPRS for BD and SCZ showed strong associations with these disorders in a held-out cohort, explaining a notable proportion of phenotypic variance.

**Conclusion**: These findings underscore the potential of lactate as a robust biomarker for mood and metabolic disorders, particularly in patient stratification and targeted therapies. The evidence of nuclear-encoded mitochondrial genetics' involvement in BD and SCZ susceptibility further highlights the importance of mitochondrial function in psychiatric disorders. Future studies should explore the role of mitochondrial metabolites in larger, longitudinal cohorts and investigate the potential of dietary

interventions, including ketogenic diets, to modulate these biomarkers. The integration of mitochondrial biomarkers and genetic risk scores could pave the way for more personalized treatment approaches in psychiatric disorders.

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# State of the science mitochondria specefic-targets, therapeutics and biomarker investigations following traumatic brain injury in the us military

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**Introduction**: In a preclinical model of penetrating traumatic brain injury (PTBI), our post-injury time-course of mitochondrial function observed a biphasic response of bioenergetic failure, together with calcium and redox homeostasis dysregulation between 30 minutes to 7 days post-injury period (1-3). Our results imply that targeted therapy directed at mitochondrial functions could be an effective neuroprotection approach following PTBI. Additionally, the presence of such mitochondria-specific markers in biofluids may offer new theragnostic and prognostic values to alleviate TBI pathology. The invited talk will present mitochondria-centric state of the science updates on preclinical military medicine approaches undertaken to mitigate TBI pathology.

**Material & Methods**: We used both Sprague-Dawley rats and Yorkshire swine to evaluate post-injury pathophysiological responses after TBI. Assessments conducted in isolated mitochondria from injury core, CSF, and biofluids following TBI. Mitochondrial functions such as bioenergetics, calcium, redox, and cell death analysis were performed during acute post-injury period. Statistical comparisons were analyzed using either unpaired t-test or ANOVA ($N = 6\text{-}8$, $^*p < 0.05$).

**Results**: Our results indicated that mitochondria-targeted neuroprotection therapeutics have potential to mitigate secondary injury responses after TBI. Additionally, mitochondrial markers are detectable in biofluids.

**Conclusion**: Mitochondria-centric mechanisms may serve as novel therapeutic targets for TBI, which further contribute to the assessment of neuroprotection therapies for TBI.

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# Targeting giant mitochondria (megamitochondria) in human liver disease: potential diagnostic and therapeutic interventions

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**Introduction**: The first report about megamitochondria, disproportionately enlarged organelles, in liver parenchymal cells dates back to the 1960s[1]. Interestingly, besides their unusual size, giant mitochondria also present a very peculiar ultrastructure, showing regular paracrystalline inclusions. Despite their presence being detected in specimens from various liver pathologies, megamitochondria have been mostly recognised as a hallmark of alcohol-related liver disease (ALD)[2]. The significance of megamitochondria is still under debate, but their detection has strikingly been linked to improved survival among ALD patients[3], rendering megamitochondria immensely valuable both clinically and prognostically. Furthermore, previous studies from us and others have highlighted a possible role of Drp1 in the formation of megamitochondria[4, 5, 6]. However, their precise characterisation, including the observed inclusions, and molecular role in ALD pathogenesis remain uncertain and constitute an intriguing phenomenon in liver pathology which requires further investigations.

**Material & Methods**: While enlarged organelles have been induced experimentally, those containing paracrystalline inclusions have been challenging to produce due to the lack of suitable human-relevant experimental models. Precision-cut liver slices (PCLS) are 250µm-thick slices derived from human fresh tissue, which can be cultured *ex vivo* maintaining viability for several days. The key characteristics of PCLS include the preservation of multiple cell types (parenchymal and non-parenchymal) and non-cellular (extracellular matrix) components in their native environment, retainment of functional and metabolic activities, ability to respond to hepatotoxic insults. PCLS cultures constitute an organotypic and multicellular model suitable for the investigation of clinically relevant processes in liver disease, including ALD.

**Results**: Alcohol exposure of PCLS induced hepatocyte death, exacerbated by fatty acids and lipopolysaccharide supplementation, which triggered a significant increase in cytokine levels associated with severe ALD inflammation. Furthermore, profibrogenic activation was observed in the slices and investigated in the context of slice preparation[7]. Finally, we have demonstrated that the exposure of PCLS to increasing doses of alcohol was able to induce the formation of megamitochondria in 24-72 hours and electron microscopy analysis clearly showed the presence of paracrystalline inclusions in the giant mitochondria detected in the slices.

**Conclusion**: In conclusion, our findings underscore the validity of the PCLS model for exploring crucial disease processes in ALD and the alcohol-induced mitochondrial adaptations, particularly emphasising the remarkable occurrence of megamitochondria, opening the path for further investigations aimed at elucidating this intriguing phenomenon.

## Funding

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## Frozen xenogeneic human mitochondria transplantation prevents ischemic reperfusion injury in small and large animal models of acute kidney injury

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Transplanted kidneys experience ischemic reperfusion injury (IRI), causing delayed graft function, and poor long-term prognosis. Kidneys donated after cardiac death (DCD) experience significantly longer warm ischemia times that those donated after brain death (DBD), and display higher rates of DGF. Improving engraftment by countering the damage cause by IRI will improve long term outcomes and increase the potential donor pool.

Purified mitochondria reduce ischemia induced damage when administered at the time of reperfusion – known as Therapeutic Mitochondria Transplantation (TMT). Initial approaches to TMT used freshly prepared autologous mitochondria, and efforts to store therapeutically efficacious mitochondria have been difficult. Overcoming this storage challenge will enable the development of a clinically and commercially viable product for use in multiple indications connected to ischemic reperfusion injury.

We showed that frozen human cell-derived mitochondria can be stored, transported, and show significant benefit in small and large animal models of acute kidney injury (AKI) associated with extended warm ischemia times, such as those experienced during DCD donation. In a mouse model of acute kidney injury caused by bilateral renal clamping for 35 mins, a single dose of frozen human mitochondria significantly reduced the damage-induced increase in Serum Creatinine (SCr) and Blood Urea Nitrogen (BUN) values. Additionally, inflammation and acute tubular necrosis were reduced in a dose dependent manner. In a porcine model of AKI caused by a bilateral 90 minute warm ischemia, we observed a significant reduction of SCr, BUN, and kidney necrosis.

Collectively our data demonstrate that infusion of a single dose of therapeutic mitochondria can protect kidneys from the effects of acute kidney injury brought about by extended warm ischemic reperfusion, suggesting that therapeutic mitochondria could reduce delayed graft function and improve long term outcomes following kidney transplant in the clinic. Our ability to isolate large amounts of mitochondria that can be frozen, stored, and shipped whilst retaining efficacy is a key step in the pre-clinical development of mitochondria and paves the way for our future first in human proof of concept trials in kidney transplant patients.

## Mitochondrial transplantation improves skeletal muscle bioenergetics in aging mice and rat models

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Mitochondrial dysfunction, often caused by aging, genetic issues, or deconditioning, impacts muscle function and mobility. Exercise is not always possible for those with limited mobility, and systemic

treatments like nutraceuticals lack precision. Mitochondrial transplantation supplements native damaged mitochondria with respiration-competent mitochondria and offers a localized therapy to boost energy in compromised muscle tissue. We hypothesize this approach can effectively alleviate mitochondrial dysfunction.

Our study is the first to examine the efficacy of mitochondrial transplantation in two aged rodent models with congenital skeletal muscle dysfunction: 1) in aged mice1 and 2) the aged low-capacity-runner inbred rat model (LCR)2. In both models, rodents were split into placebo and transplant groups, receiving mitochondria from age-matched donors injected into different types of muscles. Aged mice that received mitochondrial transplants showed significant increases in cytochrome-c-oxidase activity, ATP levels, mitochondrial-protein expression, and improved exercise tolerance. LCR-aged rats demonstrated enhanced mitochondrial marker expression in glycolytic and mixed muscle tissue and beneficial epigenetic changes.

We conclude that mitochondrial transplantation has the potential to improve muscle bioenergetics and performance. It offers a targeted treatment for age-related and genetic mitochondrial dysfunction, thus addressing mobility challenges associated with these conditions.

## Funding

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# Optimization of artificial mitochondrial transfer into human pancreatic islets

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**Introduction**: Artificial mitochondrial transfer (AMT) could be beneficial in regenerative medicine approaches involving cell and organ transplantation to reduce oxidative stress, and promote cellular repair. As AMT for islets (spheroids) has not been tested, we aim to compare three methods for mitochondrial transfer into isolated human islets.

**Materials & Methods**: Healthy mitochondria were isolated from human cardiac fibroblasts (CFCs).

- AMT1: Islets were centrifuged at 1500xG, 15min, 4°C, resuspended in HBSS in a 96-well plate for 4h at 37°C, then centrifuged again.
- AMT2: like in AMT1 but with centrifugation at 300xG.
- AMT3: Islets were resuspended in HBSS with/without mitochondria in a 96-well plate, then placed on a orbital shaker for 4h at 37°C.

Endpoints were: islet viability (Syto13-PI), glucose stimulated insulin secretion (GSIS) and mitochondria internalization (confocal microscopy/western blot).

**Conclusion**: AMT1 and AMT2 significantly reduced cell viability (57.1±4.5% and 63.9±4.1%, $p<0.05$) compared to AMT3 and control (88.1±1.1% and 90.4±1.1%). There was no difference in GSIS index between AMT3 and control (2.27±0.10 vs. 2.34±0.22). Mitochondrial internalization was observed and an increase in the COX4/GADPH signal (1-fold for control, vs. 1.6 for ATM3 from 0.9x106 CFCs, vs. 6.3 for ATM3 from 13.6×106 CFCs). AMT3 is the optimal method.

## Funding

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## Trans-cellular mitochondrial transfer: mediator of macrophage-myocyte cross talk and labor onset

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**Introduction**: Progesterone (P4) is vital for maintaining pregnancy, its withdrawal initiates labor. We demonstrated that despite elevated peripheral P4 in humans, P4 withdrawal occurs at myocyte (MYO) level by M1 macrophages (M1-MAC)-induced 20α-Hydroxysteroid Dehydrogenase (20α-HSD). This study investigates the mechanism of cross-talk between uterine MYO and MACs.
**Methods**: In vitro, Human monocytes were differentiated into M1- or M2-MACs. MYO were co-cultured with MACs directly, or treated with MAC-conditioned media, or with isolated MAC-mitochondria. In vivo, monocytes with fluorescent-tagged mitochondria (MD) were injected in pregnant mice, followed by immuno-assessment of monocyte tissue infiltration, polarization, and mitochondrial transfer.
**Results**: Calcein-AM dye-transfer revealed enhanced connectivity between M1-MACs and MYO, not observed with M2-MACs. M1-MAC-MYO exhibited tunneling nanotube (TNT) connections. Time-lapse imaging confirmed TNT formation and unidirectional mitochondrial transfer from M1-MACs to MYO. In co-culture, M1-MACs increased ATP levels in MYO, while metformin pre-treatment blocked mito-transfer and ATP surge. M1 conditioned media had no effect while mitochondria from M1-MACs induced 20α-HSD in MYO. In-vivo, infused MD were detected in the uteri of pregnant mice throughout gestation, while mitochondrial transfer to MYO occurred only during labor.
**Conclusion**: M1-MACs induce P4 withdrawal in MYO through TNT-mediated mitochondrial transfer, triggering labor onset and supplying additional energy.

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## Diverse effects of mitochondrial transfer from wharton's jelly- and bone marrow-mesenchymal stem cells on cellular antioxidant mechanisms in osteoartritic chondrocytes

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**Introduction**: Osteoarthritis (OA), a degenerative disease characterized by progressive cartilage damage, mostly affects elderly people. Elevation in mitochondrial ROS production might accelerate cartilage degeneration and facilitate the development of OA. The alteration of mitochondrial function was associated with the OA pathogenesis.
**Material & Methods**: Inflammatory environmental stress mediated OA chondrocyte (OAC) model was conducted using conditioned medium (CM) of activated M1 polarized macrophages obtained by induction the U937 cells. hSF-MSC-derived chondrocytes were cultured in CM to create OAC. Mitochondria were obtained from Wharton's-Jelly and bone-marrow derived mesenchymal stem cells (mt-WJ-MSC and mt-BM-MSC, respectively) and transferred naked. After 48 hours, ROS, MDA, ATP levels and HMOX-1 gene expression were analyzed.
**Results**: Following isolation of mitochondria, transfer to OAC was confirmed by TMRM-staining. The

intracellular ROS-level, which was increased after CM treatment, was dropped in mt-WJ-MSC and mt-BM-MSC groups, subsequent to mitochondrial transfer. MDA-levels displayed similar alterations. Compared to Control, HMOX-1 expression increased 2.8-fold in OAC, decreased 1.1-fold in mt-WJ-MSC, and increased 1.3-fold in mt-BM-MSC. ATP-levels significantly elevated in mt-WJ-MSC, whereas no effect was observed in mt-BM-MSC.

**Conclusion**: mt-WJ-MSC and mt-BM-MSC transfers exhibit different effects on the antioxidant defense mechanisms in OAC. Age-related variations in mitochondria might play an important role in mitochondria-based therapy.

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# Liposome-based mitochondrial targeted delivery of hydrogen sulphide for the treatment of neurodegenerative diseases

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Hydrogen sulphide (H2S) donors are emerging candidates for treating mitochondrial dysfunction associated with Parkinson's disease (PD). However, their poor solubility and limited mitochondrial accumulation hinder their therapeutic translation.

We designed mitochondriotropic liposomes encapsulating H2S donor, ADT-OH, prepared by the lipid film hydration technique. Mitochondrial targeting was attested by NMR and liposomes characterised using dynamic light scattering, Zeta Plus, and TEM. Uptake/kinetics of H2S were determined by HPLC. The blood-brain-barrier (BBB) was recapitulated in vitro while neuroprotective effects of H2S were attested using an XFe24 Seahorse Analyser, fluorescence microscopy (mitoSOX), and anti-inflammatory profiles by ELISA in a model of PD established in human astrocytes exposed to 6-hydroxyidopamine (6-OHdop).

NMR demonstrated successful mitochondrial targeting. Size and charge were maintained homogenous within a range of mitochondriotropic polymer content (0-22.5)% in the lipid preparation. Liposomes crossed the BBB, delivered ADT-OH to the mitochondria (97% of total ADT-OH) offering sustained release of H2S. ADT-OH, permeated through the BBB, improved cell viability, restored mitochondrial function, reduced mitochondrial-specific superoxide and cytokine levels in comparison to 6-OHdop-exposed cells.

Mitochondrial-targeted liposomes are a suitable carrier for the sustained and targeted delivery of H2S to the mitochondria that successfully cross the BBB offering protection against 6-OHdop-mediated mitochondrial dysfunction.

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# Mitochondrial transplantation as a therapeutic strategy to mitigate viral-induced lung injury through mavs-dependent antiviral pathways

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**Introduction**: Impaired mitochondrial function frequently arises in the context of viral respiratory infections. Mitochondria (MT) play a crucial role in antiviral immune responses through the MAVS (Mitochondrial Antiviral Signaling) pathway regulating the production of IFN and pro-inflammatory cytokines. Mitochondrial dysfunction impairs MAVS signaling, exacerbating viral-induced lung damage. This work evaluates the therapeutic potential of MT-transplantation in restoring MAVS signaling in damaged lung cells.

**Materials & Methods**: MT and MAVS-enriched MT from MSC (MT-MAVS) were transplanted into A549 cells, previously activated with RIG-I ligand and viral NS-1/PB1 plasmids. We assessed the impact on cell viability, ATP production, membrane potential, OCR, ROS, and MAVS pathway activation. Co-cultures with macrophages or T cells were used to measure immune response, polarization, and cytokine levels.

**Results**: MT-MAVS transplantation enhanced the biological functions of activated-A549 cells with inhibited MAVS signaling, simultaneously restoring MAVS activity. MT-MAVS transplantation promoted the polarization of macrophages and lymphocytes towards M2 and Treg anti-inflammatory phenotypes, respectively. This effect was associated with reduced levels of pro-inflammatory cytokines (IL-1, IL-8) and increased IL-10.

**Conclusion**: These findings suggest that MT-transplantation may restore A549 cell functionality and rewire the impaired MAVS pathway. Future in vivo studies will further evaluate its potential to mitigate virus-induced lung injury.

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15th WMS World Congress on

# Targeting Mitochondria

## Abstracts of Poster Presentations by Alphabetic Order

## Mitoquinol mesylate (mito-mes) prevented diethylnitrosamine (den) induced hepatocellular carcinoma via the mitochondrial apoptotic pathway in male wistar rats

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**Introduction**: Mitoubiquinol mesylate (Mito-MES)-a mitochondrial-targeted ubiquinol derivative is being investigated for its apoptotic effects in diethylnitrosamine (DEN)-induced hepatocellular carcinogenic rats.

**Material & Methods**: One hundred male Wistar rats (120 -150mg/kg) were randomly divided into five groups namely control, DEN, Mito-MES, Mito-MES +DEN, and DEN+Mito-MES. Animals in Mito-MES +DEN group were pretreated with 10 mg/kg Mito-MES for a week followed by co-administration of Mito-MES 10mg/kg b.wt and DEN for 20 weeks while the DEN+Mito-MES group received 10mg/kg DEN for eight weeks, then co-administration of 10mg/kg of DEN and Mito-MES until end of 20th week. Rat livers were excised for molecular assays after 12, 16, and 20 weeks

**Results**: At weeks 12 and 16, cytochrome c released in DEN and DEN+Mito-MES groups were significantly higher than in control ($P<0.0001$). However, at week 20, all groups showed low cytochrome c compared to control. DNA quantity was elevated in Mito-MES group at week 12. In the Mito-MES + DEN group and DEN+Mito-MES group, lower DNA quantification was observed at weeks 12, 16, and 20. In DEN +Mito-MES group, intensity of DNA laddering was lower compared to Mito-MES+DEN and Mito-MES groups.

**Conclusion**: These findings suggest that chemoprotective potential of Mito-MES in early cancer developmental stages involve apoptosis.

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## The potential clinical utility of a non-invasive 13c-breath test to assess mitochondrial capacity in patients with long covid

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**Introduction**: The biological mechanism underlying symptoms of long COVID is poorly understood and recent evidence suggests impaired mitochondrial capacity may be involved.[1] A non-invasive test called 13C-ketoisocaproate breath test (13C-KICA BT) has been shown to evaluate mitochondrial capacity.[2] Thus, the aim of this study is to determine whether there is a relationship between mitochondrial capacity and symptoms of long COVID.

**Material & Methods**: 10 healthy volunteers and 7 patients with long COVID were recruited to undergo the 13C-KICA BT before (visit 1) and after

undergoing a maximal exercise challenge (visit 2). The 13C-KICA BT involved the participant taking an oral dose of 1mg/kg body weight of 13C-KICA and collecting breath samples for 90mins. Breath samples were analysed by mass spectrometry for 13CO2 enrichment. Mitochondrial capacity was expressed as the difference in 13C-KICA BT measurements (% 13CO2 recovered on breath at 70mins) between visits.
**Results**: Mitochondrial capacity was significantly reduced in patients with long COVID compared to healthy volunteers (0.04% ± 0.03% vs 0.23%±0.16%; p = 0.02).
**Conclusion**: This study demonstrated the feasibility of non-invasive 13C-KICA BT to assess mitochondrial capacity in patients with long COVID who exhibit reduced mitochondrial capacity in comparison with heatlhy volunteers.

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# Standardized protocol for measuring nematode respiration via seahorse flux analysis

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**Introduction**: Accurate measurement of mitochondrial respiration is vital for studying cellular metabolism. Seahorse Flux analysis has advanced this field1,2, but standardized methods for nematode respiration are lacking. We developed a reliable SOP for assessing Complex I and II activity in nematodes using Seahorse Flux analysis, providing consistent, reproducible results and deeper insights into their metabolic function3.
**Material & Methods**: We created an optimized SOP for nematode respiration using the Seahorse XF Analyzer. This includes sample preparation, instrument calibration, and reagent application to measure Complex I and II activities. Troubleshooting guidelines address common issues, such as sample variability and reagent degradation, ensuring robust, repeatable data.
**Conclusion**: Our SOP offers a reliable method for measuring Complex I and II respiration in nematodes, minimizing sample variability and instrument errors. This standardized approach enhances understanding of nematode metabolism and mitochondrial function, addressing limitations in current research methods.

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## Efficacy and selectivity of second-generation FAHD1 inhibitors in breast cancer 2D and 3D Models

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**Introduction**: FAH domain-containing protein 1 (FAHD1) acts as mitochondrial oxaloacetate decarboxylase1,2, and its inhibition is a promising cancer therapy target3. We developed second-generation FAHD1 inhibitors that kill breast cancer cells in 2D cultures and tested their efficacy in 3D spheroid models to mimic in vivo conditions.

**Material & Methods**: We tested FAHD1 inhibitors on breast cancer cells using 2D monolayer cultures and 3D spheroid models. Cells were treated with increasing doses of inhibitors, and viability was measured. Selectivity was evaluated by comparing effects on breast cancer cells versus non-cancerous fibroblasts to determine therapeutic potential.

**Conclusion**: Second-generation FAHD1 inhibitors show dose-dependent cytotoxicity in breast cancer cells, with higher sensitivity in 3D models and reduced effects on fibroblasts, offering a promising targeted treatment for breast cancer.

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## Utilizing mitochondrial haplogroups as a potential screening tool for risk of primary graft dysfunction

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Primary graft dysfunction (PGD) is a significant complication following lung transplantation (LTx), affecting around 30% of recipients and contributing to a 30-day mortality rate of 36%. Its pathophysiology is poorly understood, making it challenging to diagnose and treat. Expanding our understanding of PGD's mechanisms is vital for improving screening methods and treatments to enhance LTx outcomes and increase patient survival.

Mitochondria, essential regulators of cellular processes in lung tissue, play critical roles in energy metabolism, oxygen consumption, and immune response. The efficiency of these functions is influenced by mitochondrial haplogroups, yet research on the impact of haplogroups in LTx outcomes is sparse. Studies suggest that mitochondrial haplogroups may affect transplantation success and treatment efficacy.

In collaboration with the Toronto Lung Transplant Program, we have access to over 1,000 donor-recipient biopsy samples and clinical data, enabling a retrospective 1:1 matching case-control study. Our pilot study of 30 donor-recipient pairs will help us assess the relevance of mitochondrial haplogroup matching between donors and recipients to the risk of PGD. We also aim to understand the link between mitochondrial injury markers and early transplantation outcomes, which could lead to novel screening tools and therapies for improved LTx success.

## Funding

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# The role of lipids in human IPSC - derived microglia, a model of neurodegenerative stress

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**Introduction**: Hyperlipidemia is often associated with neuroinflammation and mitochondrial dysfunction. Inflammatory neuronal-glia crosstalk leads to neuronal death. Ferroptosis, unlike apoptosis, is caused by the breakdown of antioxidant defences, which are responsible for detoxifying lipid peroxides. Mitochondria are important for cellular energy, yet are key producers of oxidative stress. Therefore, in this study, we hypothesized that inflammatory and ferroptotic pathways could lead to retinal neurodegeneration.

**Methods**: We have used lipotoxicity in microglial cells as a model to mimic neurodegenerative stress. We employ assays such as phagocytosis, migration, immunofluorescence and metabolic assays.

**Results**: Microglia undergoes changes in morphology, viability, and its phagocytic functions. Our analysis showed that microglial homeostasis was restored when the PA-exposed microglia were treated with ferroptotic inhibitors.

**Conclusion**: This could lead to a better understanding of the involvement of microglia in neuroinflammation. We could target certain genes involved in oxidative stress, but mainly metabolic reprogramming of microglia. Our data demonstrated that targeting microglial reprogramming could support neuroprotection during neuroinflammatory processes.

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## The protein glycosylation machinery in B cells controls mitochondrial function

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**Introduction**: B lymphocytes are immune cells that, upon activation by pathogens, differentiate into antibody-secreting plasma cells (ASCs) and subsequently mount long-lasting immune responses by secreting glycosylated antibodies. Thus, ASCs must control protein (de)glycosylation and degradation, which requires high energy and functional mitochondria. Dysfunctional mitochondria have recently been associated with congenital-disorders-of-deglycosylation (CDG). Thus, we will investigate whether dysfunctional deglycosylation impacts mitochondria in B cells and ASCs.
**Material & Methods**: We activated naive B cells in vitro while inhibiting deglycosylating enzymes and analyzed their mitochondrial function. Furthermore, we deleted specific deglycosylating enzymes in a plasmacytoma cell line and analyzed the impact of the loss-of-function on their mitochondria.
**Results**: Inhibition of deglycosylating enzymes drastically alters the viability, mitochondrial mass, and mitochondrial membrane potential of in vitro activated B cells. Genetic ablation of deglycosylating enzymes in MPC11 plasmacytoma cells reduces their mitochondrial mass and primarily targets those cells with the highest cytoplasmic antibody concentrations.
**Conclusion**: Disrupting the deglycosylation machinery in B cells and ASCs alters their mitochondrial capacity and viability. These findings may help to further elucidate the mechanisms that drive the differentiation of B cells into ASCs. Additionally, this observation is of particular interest for patients with CDG.

### Funding

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## Oxidative drug combination of arsenic trioxide and D-vitamin C induces dysfunction of mitochondria and cytotoxic ros production

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**Introduction**: KRAS mutations occur in approx.25% of human cancers, leading to aggressive tumours with poor prognosis. Targeting the KRAS mutations is challenging, even with specific inhibitors showing drug resistance. A promising approach combines arsenic trioxide(ATO) and D-vitamin C(D-VC).[1,2,3]
**Material & Methods**: CD1 Mouse was injected with pancreatic adenocarcinoma AK192 cells (DOX-inducible KRAS(G12D) and treated with ATO, D-VC and L-V. AK192 and human HCT116 KRAS (G13D/WT) cancer cell lines were also used. Apoptosis, mitochondrial function, and ROS generation were analyzed FACS, and electron transport chain(ETC) activity was measured.
**Results**: Treatment of KRAs mutant cancer cell lines with ATO and enantiomers of VC showed comparable potency in inducing oxidative stress, but D-VC was less toxic in the CD1 mouse model. ATO/D-VC

induced apoptosis, reduced mitochondrial mass, caused mitochondrial dysfunction, decreased membrane potential, and generated cytotoxic ROS levels. It also altered the activities of Complex II and III.

**Conclusions**: ATO/D-VC shows potent tumor suppressive effects via glucose-dependent oxidative stress with suicidal ROS production by mitochondria (SRPM). These findings underscore the complex impact of ATO/D-VC treatment on KRAS-mutant cancer cells, suggesting its potential as an effective therapeutic strategy. The combination of ATO/D-VC is currently being evaluated in clinical trials in Kazakhstan.

## Funding

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# Investigating the role of mitochondria-containing extracellular vesicles in alzheimer's disease

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**Introduction**: Extracellular vesicles (EVs) have emerged as important mediators of intercellular communication in the brain1. Mitochondria are previously reported as cargos in EVs that is called mitoEVs2. Altered mitochondrial biogenesis is frequently linked to Alzheimer's disease (AD). However, it remains unclear whether mitochondrial-related contents are differentially encapsulated and delivered through EVs at the neural progenitor cell (NPC) stage.

**Material & Methods**: We employed size exclusion chromatography (SEC) to isolate mitoEVs from conditioned medium collected from AD NPCs (AD-NPCs) derived from an AD patient with PSEN1 mutation and isogenic control (ISO-NPCs). Followed by charaterization studies, LC-MS/MS was performed to determine the protein contents of isolated mitoEVs. Seahorse XF assays was ultilized to investigate whether mitochondrial dysfunction occured in AD-NPCs, which was further compared with mitochondrial contents indentified in EVs.

**Conclusion**: We successfully isolated mitoEVs from AD-NPCs and ISO-NPCs, both of which tested positive for EV and mitochondrial markers, while no significant differences were observed between AD- and ISO-EVs regarding protein concentrations, particle sizes, or quantities. Proteomic analysis revealed that ISO-EVs contained a higher overall number of proteins compared to AD-EVs. Notably, several proteins related to EVs, mitochondria, and AD pathology were identified, offering insights into the pathobiological processes of AD.

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# Identification of the best protocol for mitochondria isolation from human renal cells

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**Introduction**: renal ischemia-reperfusion damage (I/R) alters mitochondrial functionality; indeed mitochondria transplantation results in organ rescue. Our aim is to identify the best method for renal cell mitochondria isolation and the best storage conditions, to treat kidney failure.
**Material & Methods**: mitochondria were isolated through 1) sucrose-gradient ultracentrifugation of the cellular lysate (obtained using a tissue homogenator) or 2) after cell disruption through syringe passages and subsequent centrifugations 3) additional use of magnetically labelled beads. Isolated mitochondria were stored in different conditions and then analysed by flow citometry and super resolution microscopy. ATP production was assessed through a luminescence-based assay. Finally, mitochondria uptake by renal cells (ciPTECs) was tested in a model of chemical anoxia, mimicking I/R.
**Results**: magnetic separation allowed the best recovery of intact and functional mitochondria, also after long term storage at -80°C, as shown by flow citometry, super resolution microscopy and ATP production assays. In addition, uptake of labelled mitochondria by ciPTECs was dose dependent and higher in damaged cells compared to control ones.
**Conclusion**: the best protocol for mitochondria isolation from renal cells was identified. Further functional assays will be performed to confirm their therapeutic effect in damaged cells for a future use in regenerative medicine.

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# Mitochondrial alterations in fibroblasts from a kufor-rakeb patient with two novel mutations in ATP13A2

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**Introduction**: Kufor-Rakeb syndrome (KRS) is a rare autosomal recessive form of juvenile-onset Parkinson disease, characterised by the presence of motor symptoms and iron accumulation among others. KRS is caused by mutations in the ATP13A2 gene, which encodes ATP13A2 protein[1]. ATP13A2 has

a lysosomal location thus its main function is autophagy regulation[2]. Recent studies show an interaction between ATP13A2 and mitochondrial function[2]. The aim of this work is to further study the mitochondrial alterations associated with ATP13A2 mutations and KRS.

**Material & Methods**: Fibroblasts from a KRS patient carrying 2 novel loss-of-function mutations were used. Results were compared with a sibling carrying one of the mutations in heterozygosis and healthy subjects.

**Results**: Patient fibroblasts mimicked key KRS features, including increased cell death, iron accumulation, and reduced autophagic flux. They also showed mitochondrial alterations, such as elevated mitochondrial membrane potential and increased ROS production. Additionally, KRS fibroblasts exhibited higher mitochondrial mass, likely due to impaired mitochondrial degradation by autophagy. Interestingly, sibling fibroblasts displayed mitochondrial damage but normal mitochondrial mass, without autophagic flux blockage.

**Conclusion**: Our results validate the use of fibroblasts for the study of KRS and suggest a link between ATP13A2 and mitochondria

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# Amadori product, fructoselysine, alters mitochondrial function and impairs energy metabolism in human beta cell line 1.1B4 shifting to glycolysis

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**Introduction**: Advanced glycated end products, (AGEs) formed by non-enzymatic Maillard reaction or polyol pathway flux, have direct impact on cellular redox states and development of diabetes related complications. This study aimed to examine the effects in mitochondrial energy metabolism mediated by AGEs, Nε-(carboxymethyl)-L-lysine (CML) and Nε-(carboxyethyl)-L-lysine (CEL), and Amadori product fructoselysine (FL) on beta cells.

**Material & Methods**: Beta cell line 1.1B4 was cultured with CML, CEL and FL at doses that maintained 80% cell viability. Cellular metabolites and ATP levels were measured after 48 hrs.

**Results**: Inflammatory signaling and RAGE expression were induced by CML, CEL and FL. Despite continued insulin production, insulin secretion in response to high glucose was impaired and mitochondrial ATP synthesis capacity was reduced by CML, CEL, and FL. Treatment with antimycin A and oligomycin showed that FL cells became primarily dependent on glycolysis for energy requirement. PFKFb3 expression was increased by CML, CEL, and FL, while L-lactate levels were not significantly changed.

**Conclusion**: FL-mediated mitochondrial function changes differ from CML- and CEL-ones, and mitochondrial dysfunction negatively affects cellular function and energy metabolism. These disruptions resulted in a shift in cellular energy metabolism from mitochondrial respiration to glycolysis.

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## Metabolic Reprogramming In Chemically Induced Cardiomyocytes Through Mitochondrial Transfer

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**Introduction**: Heart degenerative disease, a leading cause of global mortality, presents a significant challenge due to the failure to restore functional cardiomyocytes. Recently, direct conversion of fibroblasts into cardiomyocyte-like cells using small molecules (CiCMs) has emerged as a potential source of functional cardiomyocytes. However, low conversion efficiency and fetal-like characteristics of CiCMs limit their application in regenerative medicine. Here, we report a metabolic reprogramming strategy to generate functional cardiomyocytes through mitochondria transfer. After transferring mitochondria into CiCMs, we observed enhanced structural maturation, increased respiratory metabolic capacity, and improved electrophysiological functionality of CiCMs compared to the conventional method. Metabolically reprogrammed CiCMs also exhibited mature cardiomyocyte-like hypoxia sensitivity. We suggest that mitochondrial transfer-based metabolic reprogramming can strengthen CiCM functionality.

**Material & Methods**: Primary mouse embryonic fibroblasts (PMEFs) were isolated from ICR mouse embryos and induced into CiCMs using a small molecule-supplemented reprogramming medium. Mitochondria from mouse tissues were isolated via serial centrifugation and transferred into CiCMs. CiCM maturation was assessed using immunostaining and hypoxia sensitivity analysis, while functionality was evaluated through calcium imaging.

**Conclusion**: In this study, we confirmed that CiCMs with mitochondrial transfer exhibited enhanced myocardium structure and improved cellular respiration metabolism, indicating that mitochondrial transfer effectively aided CiCM maturation.

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## Mitochondria functionality and its interaction with smooth endoplasmic reticulum in D-aspartate-treated rat testis

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**Introduction**: D-Aspartate (D-Asp), a D-amino acid endogenously present in testis, affects steroidogenesis/spermatogenesis by up-regulating testosterone byosinthesis. Mitochondria and their interactions with the Endoplasmic Reticulum (ER) through the Mitochondria-Associated ER membranes (MAMs) play a key role in the steroidogenic process [1,2]. Furthermore, mitochondria support the metabolic shift that occurs during meiosis in spermatocytes.

**Material & Methods**: To understand the action of D-Asp on mitochondrial funcitionality/dynamics and MAM stability, in vivo and in vitro experiments were performed. Male Wistar rats were treated with 20 mm D-Asp for 15 days (oral administration); germinal GC2-

spd (spermatocytes) and steroidogenic somatic TM3 cell lines (Leydig cells) were treated with 200 μM D-Asp.
**Results & Conclusion**: In TM3 Leydig cells upregulation of mitochondrial biogenesis, fusion, and mitochondrial mass, whereas a decrease in fission, were observed. Furthermore, D-Asp increased MAM stabilization and reduced ER stress, indicating its potential benefits in steroidogenesis efficiency. D-Asp enhanced also spermatogenesis by improving mitochondrial functionality, critical for the metabolic changes occurring during meiosis. Specifically, in GC-2 spermatocytes D-Asp increases mitochondrial biogenesis and fusion, whereas reduces fission. Furthermore, in GC2-cells D-Asp increased expression of OXPHOS complexes necessary for ATP production, improving mitochondrial function. These actions of D-Asp in steroidogenesis/spermatogenesis were supported by in vivo experiment.

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# Proteomic analysis of mitochondrial cardioprotective responses to reduced oxygen consumption: the role of dichloroacetate

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**Introduction**: The myocardium under partial oxygen deficiency faces increased energy demands, activating cardioprotective signaling pathways. Cardiac mitochondria are essential in these processes [1]. This study aimed to elucidate key signaling pathways related to respiratory chain regulation, mPTP opening, and ROS signaling in an acute streptozotocin (STZ)-induced diabetes mellitus model treated with the metabolic modulator dichloroacetate (DCA).
**Material & Methods**: Male Wistar rats (n=24), aged 12-15 weeks, were used. An acute diabetes was induced with a single STZ dose (65 mg/kg i.p.). DCA was administered (150 mg/kg or 75 mg/kg i.p.) 60 and 15 minutes before heart excision. Isolated rat heart mitochondria underwent quantitative LC-MS/MS proteomic analysis.
**Results**: Proteomic analysis revealed significant stimulation of proteins in respiratory chain complexes I and II (both $p < 0.0001$) in diabetic rats compared to controls. In DCA-treated diabetic rats, expressions of complex II ($p < 0.001$) and IV ($p < 0.05$) proteins decreased. AOFA, was upregulated in diabetic mitochondria. DCA downregulated AOFA and stimulated PRDX3. The diabetic condition enhanced mitochondrial resistance to calcium overload via mPTP regulation. DCA reduced ROS levels, downregulating VDAC1 and VDAC3 ($p < 0.05$).
**Conclusion**: DCA effectively modulates mitochondrial signaling, enhancing myocardial function and ROS regulation under increased energy demands.

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## Adaptive cellular mechanisms in cells with mitochondrial dna mutations induced by defective dna polymerase γ exposed to cigarette smoke extract

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**Introduction**: Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide and cigarette smoke (CS) exposure is its main risk factor 1,2. Mitochondrial bioenergetic failure and increased oxidative stress are induced by CS and are hallmarks of COPD2-4. However, the role of the mitochondrial DNA (mtDNA) mutations on the cellular response to CS, development and progression of COPD remain unclear. We hypothesized that mtDNA mutations could negatively affect the cellular response to CS inducing premature cell death or oncogenic initiation.

**Material & Methods**: Lung fibroblasts (LFs) were isolated from WT and homozygous mice carrying a proofreading-deficient polymerase γ (D257A) which increases mtDNA mutation rate5. Cells were exposed to cigarette smoke extract (CSE) and tested for: cell viability, respiration and glycolysis, markers of cell death, oxidative stress and integrated stress response (ISR).

**Results**: PolGD257A LFs showed a higher viability and de novo DNA synthesis compared to WT exposed to sublethal dose of CSE, whereas they were less sensitive to CSE-induced apoptosis via cleaved caspase-3. PolGD257A LFs expressed a pro-survival ISR profile, antioxidants and glycolytic enzymes, with inhibited respiration.

**Conclusion**: Mutations of mtDNA induced by PolGD257A cause an adaptive metabolic rewiring that sustains the growth of LFs under CSE-induced stress.

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## The role of oxphos during early B cell development

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**Introduction**: The hallmark of early B cell development is antibody (μ heavy chain, μHC) gene rearrangement, leading to pre-B cell receptor (pre-BCR) expression. The pre-BCR elicits pre-B cell

expansion. Early B cells oscillate between cycling and resting states and through niches with different oxygen tensions. This suggests that OxPhos may be regulated in the different stages. We have previously found that pro- and pre-B cells show different extents of glycolysis and OxPhos. The function of OxPhos during early B cell development has been unclear.
**Material & Methods**: Genetic depletion of mtDNA in developing B cells (ΔmtB).
**Results**: Early B cell development in ΔmtB mice shows a developmental block between the pro- and pre-B cell stage. In vitro cultivation of pro-B cells from ΔmtB mice with IL-7 reproduced the in vivo data. Strikingly, μHC expression in pro-B cells from ΔmtB mice was significantly reduced and transgenic μHC expression rescued B cell development in ΔmtB mice.
**Conclusion**: The fact that pre-arranged μHC genes rescue B cell development in ΔmtB mice suggests that pre-B cells can develop despite defective OxPhos, provided that sufficient pre-BCR signals are delivered. Hence, OxPhos is a prerequisite for pre-BCR function, but not for its downstream metabolic pathways

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# Modulating immunometabolism in murine primary macrophages by mitochondria-targeting nanocapsule, mito-porter

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**Introduction**: Macrophages play a critical role in chronic inflammatory conditions. Modulation of immune cell metabolism has been recently receiving attention as a novel treatment strategy to counteract such conditions. In this study, we evaluated the potential of direct mitochondrial delivery of coenzyme Q10 (CoQ10) by using unique mitochondria-targeting lipid nanoparticles, MITO-Porter in primary murine bone marrow-derived macrophages (BMDMs), as a novel approach to modulate immunometabolism.
**Material & Methods**: MITO-Porter encapsulating CoQ10 (CoQ10-MITO-Porter) and empty MITO-Porter were prepared using a microfluidic device as previously described. Primary BMDMs treated with different MITO-Porters (nanoparticle composition and content) or PBS (vehicle) were assessed for cell import (by flow cytometry), mitochondrial transfer (by confocal laser scanning microscopy), and mitochondrial function (mitochondrial reactive oxygen species (ROS) levels and mitochondrial respiration).
**Results**: We successfully transferred MITO-Porters into BMDMs without affecting cell viability, as confirmed by flow cytometry and confocal laser scanning microscopy. CoQ10-MITO-Porter treatment significantly reduced mitochondrial ROS levels and significantly increased the mitochondrial oxygen consumption rate compared to empty MITO-Porter in BMDMs.
**Conclusion**: We have demonstrated for the first time that CoQ10-MITO-Porter treatment successfully alters mitochondrial function in primary murine immune cells, BMDMs, potentiating its immunomodulatory effects to modulate chronic inflammation.

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## The study of the impact of hypoxia and epigenetic modulation on mechanisms involved in resistance of testicular tumors

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**Introduction**: Hypoxic regions in solid tumors prompt cellular adaptation through the HIF transcription factor, influencing expression of various genes. In testicular germ cell tumors (TGCTs), upregulated miR-218 in cisplatin-resistant cells may inhibit selected phosphatases, contributing to resistance. Our hypothesis was that HIF/miR-218/PPP2R2A-PPP2R5A regulatory axis plays a critical role in TGCT resistance.
**Material & Methods**: Transfection with siRNA inhibitors (anti-miR-218-5p and anti-HIF) was performed to confirm regulation of key markers, followed by experiments to assess mRNA, protein expression, and cell viability. Techniques such as qPCR, Western blot, pyrosequencing, MTT assay, and immunocytochemistry were employed to study responses to cisplatin and hypoxia
**Results**: miR-218-5p expression was significantly elevated in resistant TGCT cell lines and correlated with increased SLIT2/3 gene expression. Since SLIT2/3 promoters contain HRE elements, HIF factor expressions were analysed. We found uniformly elevated HIF-1α expression in resistant cell lines, even under normoxia, PPP2R5A expression was low in resistant cells, except in 1411HP, where PPP2R2A expression was low, both increased after miR-218-5p inhibition. Further experiments confirmed the role of HIF/miR-218/phosphatase axis in TGCT resistance.
**Conclusion**: Our findings show that miR-218-5p downregulates phosphatases in resistant TGCTs, influenced by hypoxia and HIF-1α. This may also affect mitochondrial metabolism and suggests new biomarkers and targets for overcoming resistance.

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## Prospective monitoring of changes in mitochondrial respiration in patients with parkinson´s disease

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**Introduction**: Idiopathic Parkinson's disease (iPD) is primary or secondary mitochondrial disorder, hallmarked with mitochondrial dysfunction and deteriorated mitochondrial homeostasis (MiH).[1] Currently, in clinical settings are not available any reliable markers sufficiently specific for iPD. Here we have examined parameters contributing to mitochondrial fitness (MiF) in leukocytes of iPD patients and sex- and age-matched control probands.

**Material & Methods**: We have assembled two study groups: newly diagnosed iPD patients, N = 30 and patients with advanced stages of iPD, N = 30; control group to group 1, N = 30; and control group to group 2, N = 30. Mitochondrial respiration of isolated leukocytes was analyzed with High Resolution Respirometry (HRR, Oroboros, AT)-SUIT003ce-cpeO2. Furthermore, metabolites linked to energy metabolism and profiles of bile acids were examined

in blood plasma with magnetic resonance spectroscopy and targeted MS analyses. Acquired parameters were ranked by significance and analyzed with Machine Learning (Random Forest algorithm).
**Results**: We have established cumulative markers consisting of HRR parameters, bile acids, and components of energy metabolism that can reliably (more than 95% precision) distinguish between iPD patients and corresponding healthy controls.
**Conclusion**: MiF-related cumullative markers may contribute to precise and expedited diagnostics of iPD in clinical settings.

## Funding

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# Genetic changes in mitochondria: a potential marker of testicular tumor chemoresistance?

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**Introduction**: Metabolic dysfunction is one of the hallmarks of malignant cells. Mutations in mitochondrial genome contribute to metabolic deficit and their accumulation is related to disease progression and development of chemoresistance.
**Material & Methods**: The mutation profile of the mitochondrial genome we determined by using the TWIST panel. Mitochondrial respiration was measured with high-resolution respirometry method using O2k-respirometer and to analyze the metabolic pathway dependence we used the Glycolysis/OXPHOS Assay Kit.
**Results**: We identified new mutations in respiratory chain genes. Functional analysis of respiratory chain revealed differences between TGCT cell lines. Resistant TGCT cell line 1777NRpmet showed the highest O2 consumption, while resistant metastatic 1411HP TGCT cell line showed the opposite. Resistant 2102EP Cis cell line showed only minor differences in O2 consumption compared to their sensitive counterparts. In regard of typical hallmark of cancer cells, switching metabolism from OXPHOS to glycolysis, we observed 2102EP and H12.1 ODM TGCT cell lines preferentially utilizing OXPHOS while 1411HP and H12.1 preferring glycolysis. In case of metastatic 1777NRpmet, it was not clear which energy pathway was preferential.
**Conclusion**: Our results point on significant heterogeneity of TGCT cell lines. This novel data provide new potential biomarkers for precise patients stratification and new targets for their personalized treatment approach.

## Funding

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## Impact of chromosome 18 numerical imbalances on mitochondrial BCL-2 protein expression In HPV-positive cervical squamous intraepithelial lesions

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**Introduction**: High Risk Human Papilloma Virus (HR-HPV) subtypes are implicated in a variety of gains/losses in specific regions (loci) of chromosomes 1, 2, 4, 6, 8, 17 predominantly in intraepithelial squamous lesions (SILs). Concerning their influence in other chromosomes (ie chromosome 18), there are limited data. B-cell lymphoma 2 (Bcl-2) (gene locus: 18q21.33) -located on the outer mitochondrial membrane- is an anti-apoptotic factor suppressing/blocking the signal transduction. Our aim was to co-analyze Bcl-2 expression levels/chromosome 18 status in a series of SILs (LSIL-HSIL).

Materials and Methods: Using a liquid based cytology assay, thirty (n = 30) HPV-positive vaginocervical cell specimens were analyzed by implementing immunocytochemistry (ICC) and fluorescence in situ hybridization (FISH) assays. Digital image analysis was also performed.

**Results**: Bcl-2 high expression was observed in 16/30 (53.5%) cases, whereas the rest (14/30-46.5%) demonstrated moderate/low expression. Among HSIL cases, 12/16(75%) were overexpressed ($p = 0.022$). Concerning chromosome 18, polysomy was detected in one HSIL case correlated with high Bcl-2 protein expression.

**Conclusions**: Bcl-2 over activation is involved in a progressive LSIL to HSIL epithelia transformation. Chromosome 18 numerical imbalances (polysomy) seems to be a rare mechanism leading to Bcl-2 overexpression, whereas 18q21.33 loci amplification/loss of heterozygosity (LOH)-mutations are alternative mechanisms in HR-HPV infected cervical epithelia.

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## Exploring cellular metabolism through novel light-based method targeting mitochondrial activity in live endothelial cells

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**Introduction**: Mitochondria play a vital role in maintaining cellular function and are key players in numerous metabolic processes, i.e. vascular inflammation and oxidative stress1. However, examining the molecular mechanisms behind these issues is challenging due to limited techniques that offer spatial and molecular insights at the subcellular level. Raman spectroscopy (RS), a laser-based technique, provides detailed molecular information of specific biomolecules that undergo biochemical reactions in live cells. We create a technology that facilitates labeled-RS to monitor biochemical alterations at the cellular level. Through

mitochondria-specific probes, RS enables the visualization of mitochondria and monitoring their activity2.
**Material & Methods**: Two mitochondrial targeting probes MitoBADY and the novel RAR-BR3, designed for visualization and detecting mitochondria activity, were utilized. Human aortic endothelial cells (HAECs) were imaged with RS to assess probe activity and localization.
**Results**:RS imaging confirmed the probe activity as being related to mitochondrial membrane potential (MPP) changes and demonstrated their effectiveness in visualizing the localization of these organelles. The signals from MitoBADY and RAR-BR colocalized with cytochrome c, a known label-free spectral marker of mitochondrial activity.
**Conclusion**: We believe that RS combined with mitochondrial-oriented probes has the prospect of being used to track mitochondrial activity in the future.

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# The effect of mitochondrial CA2+ abundance on mitochondrial function and morphology in B cells

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**Introduction**: B cells are essential for effective immune responses against pathogens. During B cell development in the bone marrow, mitochondrial function changes in different developmental stages. Mitochondrial Ca2+ is a crucial regulatory cell-intrinsic signal. EF-hand domain-containing protein 1 (EFhd1) is a mitochondrial Ca2+-binding protein that is abundant in pro-B cells but not in later developmental stages. EFhd1 interacts with the Mitochondrial Ca2+ Uniporter Channel (MCUC) to control mitochondrial Ca2+ abundance. We hypothesize that EFhd1 controls the early B cell development via the regulation of mitochondrial Ca2+.
**Material & Methods**: Bone marrow single cell suspensions and in vitro cultures of pro-B cells derived from wildtype, EFhd1-KO and EFhd1 transgenic mice are used to measure mitochondrial Ca2+ and to identify the effect of EFhd1 on proliferation and differentiation of early B cell populations.
**Results**: We reveal that prolonged expression of EFhd1 disfavours B cell development beyond the pro-B cell stage in vivo. Moreover, IL-7 mediated in vitro cultivation of pro-B cells reveals a blocking effect of EFhd1 on both cell proliferation and differentiation, which is further enhanced by hypoxia.
**Conclusion**: EFhd1 expression controls the early B cell development. Ongoing analyses aim at characterizing mitochondrial function and Ca2+ abundance in B cell precursors.

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## The effect of mitochondrial transplantation on the covid-19 associated acute respiratory distress syndrome

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**Introduction**: The two most common lung conditions in COVID-19 patients are lung failure and acute respiratory distress syndrome (ARDS). The defining features of ARDS include oxidative and inflammatory alveolar injuries, which lead to damage to nuclear DNA and mitochondria as well as epithelial cells. As a possible treatment for ARDS, mitochondrial transplantation (MT) may be able to reverse the mitochondrial dysfunction in pulmonary cells.
**Material & Methods**: Experimental groups are control, ARDS and ARDS+Mito. A dose of 2 mg/kg of lipopolysaccharide was administered intratracheally to male rats. Thereafter, isolated mitochondria from the biceps femoris muscle were transplanted intranasally. The function and mass of isolated mitochondria were determined by Mitotracker Red CMXROS and MitoSpy Green FM. Lung damage and MT efficacy were evaluated histopathologically. IL-10, IL-1β, catalase, SOD analyses were also performed for inflammation and oxidative stress.
**Results**: The isolated mitochondria had sufficient function and mass. MT reduced fibrotic tissue formation and ameliorated lung injury. Moreover, MT improved inflammation and anti-inflammation balance. MT increased antioxidant levels through the reduction of oxidative stress.
**Conclusion**: Our findings highlight the effects of MT in the treatment of COVID-19 associated-ARDS.

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## Effects of a highly hydrophilic mitochondria-targeted iron chelator

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**Introduction**: Mitochondrial iron is a potential cancer therapy target due to its role in energy generation for high-demand cancer cells. The widely used mitochondria-targeting triphenylphosphonium (TPP+) moiety ensures membrane potential-dependent accumulation. However, highly lipophilic TPP+ compounds can inhibit the electron transport chain1,2. Therefore, we designed a hydrophilic derivative of the iron chelator deferiprone (mitoDFP) to selectively deplete mitochondrial iron while diminishing the inhibitory effects of TPP+ moiety.
**Material & Methods**: Isolated rat liver mitochondria were treated with mitoDFP and +/- FCCP, and the accumulated compound was quantified using HPLC. The

iron-complexing ability was investigated via competition studies with calcein. MTS assay was used to asses cell viability. Cellular respiration was measured using Seahorse XFe24 Analyzer. Changes in protein levels were analysed using SDS-PAGE and Western blot.
**Results**: Accumulation of mitoDFP in isolated rat liver mitochondria is membrane potential-dependent. The iron-complexing ability of deferiprone is not impaired upon derivatization. Incubation of PANC-1 cells at an IC10 concentration of mitoDFP decreases maximal respiration and depletes cellular iron, as evidenced by an increase in transferrin receptor 1 and a decrease in cytosolic ferritin protein levels.
**Conclusion**: New mitochondria-targeted deferiprone derivative accumulates in mitochondria and influences both energetic and iron metabolism in PANC-1 cells.

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# PCSK9 regulates mitochondrial cholesterol levels by acting on the degradation of the mitochondrial translocator protein TSPO in the heart

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**Introduction**: Cholesterol is critical for mitochondrial membrane structure and function. We have shown that cardiac Pcsk9 expression is required for preserving mitochondrial and cardiac function by maintaining mitochondrial cholesterol homeostasis. Here, we sought to elucidate how PCSK9 regulates mitochondrial cholesterol in the heart.
**Materials & Methods**: We found that cardiac Pcsk9 deletion in mice (CM-Pcsk9-/-) upregulates TSPO, a mitochondrial protein involved in cholesterol transport. High TSPO expression in human heart is associated with a strong downregulation of mitochondrial and cardiovascular functions, and TSPO protein levels were higher in failing human hearts compared to non-failing hearts. Mechanistically, PCSK9 degrades TSPO through a proteasomal mechanism and this axis modulates mitochondrial cholesterol content and function in cardiac cells. Additionally, gene therapy-induced TSPO downregulation prevented mitochondrial cholesterol accumulation and improved cardiac function in CM-Pcsk9-/- mice
**Conclusion**: Thus, PCSK9/TSPO axis regulates mitochondrial cholesterol homeostasis and targeting TSPO and mitochondrial cholesterol may represent a promising therapeutic approach for heart failure.

# Protective effects of plant extracts on mitochondrial dysfunction in alzheimer's disease

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**Introduction**: Mitochondrial dysfunction is one of the major pathological hallmarks of Alzheimer's disease, mainly induced by amyloid β protein (Aβ). Aβ disrupts the activity of mitochondrial respiratory chain complexes I and IV. Mechanistically, the post-transcriptional modification N1-methyladenosine

(m1A) at a single site on mRNA of the ND5 subunit of complex I appears to repress its translation, leading to complex I dysfunction and alterations in mitochondrial function, such as decreased ATP levels, reduced mitochondrial membrane potential (MMP), and mitochondrial morphology changes. Therefore, we aim to investigate whether plant extracts interfere with this newly identified mechanism [1–3].

**Material & Methods**: A screening was performed by assessing mitochondrial key parameters after extract treatments in SH-SY5Y cells (ATP levels, MMP, lactate production). Confocal microscopy was conducted to detect mitochondrial morphology changes. The Agilent Seahorse analyzer enabled us to measure key parameters of mitochondrial respiration.

**Results**: Our group identified 9 out of 48 most promising plant extracts with protective effects on mitochondrial function.

**Conclusion**: To elucidate the molecular mechanism of these plant extracts, we will examine alterations in protein expression after extract treatments, followed by Illumina Sequencing. Our findings could highlight a new potential therapeutic approach for Alzheimer's disease treatment, specifically targeting mitochondrial health.

## Funding

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# Oxidative phosphorylation as a predictive biomarker of oxaliplatin response in colorectal cancer

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**Introduction**: Oxaliplatin is effectively used in advanced colorectal cancer to eradicate micrometastases1. However, its benefits in early stages of colorectal cancer remain controversial, as approximately 30% of patients experience unexpected relapses2. In this study, we evaluate the efficacy of oxidative phosphorylation as a predictive biomarker of oxaliplatin response in colorectal cancer.

**Materials & Methods**: A database consisting of individuals treated with oxaliplatin, were analyzed by categorizing them based on their response to the drug and performing a Gene Set Enrichment Analysis (GSEA). Through the analysis of multiple oxaliplatin-resistant and -sensitive cell lines, we identified oxidative phosphorylation KEGG pathways as a valuable predictive biomarker of oxaliplatin response, with a high area under the curve (AUC). Additionally, some oxidative phosphorylation-related biomarkers were validated in tumorspheres derived from primary and metastatic tumors through western blotting, confirming the in silico results. The clinical significance of low expression of these biomarkers was assessed by analyzing relapse-free survival curves.

**Conclusion**: These results underscore the value of oxidative phosphorylation as a reliable marker for predicting the response to oxaliplatin treatment in colorectal cancer. This study proposes using oxidative phosphorylation-related protein expression levels as a predictor of response to oxaliplatin-based treatments to prevent relapse.

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# Pancreatic cancer cells exchange functional ribosomes through tunneling nanotubes


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**Introduction**: Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers. PDAC cells form intercellular tunneling nanotubes (TNTs), which transmit signals and cellular components between distant cells. Cytotoxic medications induce the formation of TNTs in PDAC, which may contribute to chemoresistance and cancer spread.

**Material & Methods**: In cultured PANC-1 cell line and patient biopsy-derived cells we used a range of microscopy methods (immunocytochemistry, FISH, proximity ligation assay) to detect components of the translational machinery. Transfection with HaloTag labelled ribosomal protein S9 enabled us to detect a directed intercellular transfer of this component and of whole labelled ribosomes.

**Results**: In PANC-1 cells, we observed that TNT formation is stimulated by gemcitabine in a dose-dependent manner. In both PANC-1 cells and patient biopsy-derived cells we detected a number of ribosomal and translational machinery components (RPS6, RPS9, RPL24, poly mRNA, 5.8S rRNA) in TNTs between cancer cells, and observed their transport within TNTs. We also show that HaloTag labelled ribosomes are transferred into unlabelled PDAC cells.

**Conclusion**: PDAC cells can exchange functional ribosomes via TNTs, which may potentially enhance the survival of cancer cells and contribute to treatment resistance.



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# Mitochondrial transplantation: latest patent analysis


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**Introduction**: Mitochondrial therapeutics encompass methods for isolation, processing, storage, and transplantation. This study aims to establish a strategy for securing new patents and directing R&D efforts by identifying technological gaps through patent analysis.

**Material & Methods**: We conducted patent search in the Wontelips database on October 10, 2023.

**Results**: 470 were identified as relevant technologies focused on mitochondrial transplantation. The distribution of patent filings by country indicated that China (38%) leads, followed by the United States (19%), international applications (17%), South Korea and Japan (9% each), and Europe (8%). Notably, domestic applicants dominate in China and South Korea, while

foreign applicants are more prevalent in Japan and Europe; the U.S. shows a balanced representation of both.

The analysis positioned the overall patent landscape in a growth phase, with MINOVIA THERAPEUTICS (Israel) being the leading applicant. Second-ranked PAIAN TECHNOLOGY (South Korea) concentrates on injection/delivery and therapeutic improvement technologies, while CHILDREN'S MEDICAL CENTER (U.S.) follows in miscellaneous devices and therapeutic technologies. Within the field, patents are primarily categorized as miscellaneous (42%), therapeutic agents (31%), isolation/extraction (11%), and injection/delivery (10%).

**Conclusion**: The increasing trend of patent filings in mitochondrial transplantation highlights that these technologies are gaining prominence in major countries.

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# The effect of high glucose concentration on the expression of UPRMT genes LONP1, OMA1, YME1L, HSPD1 aND HSPA9 in myocardial samples of patients with heart failure

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**Introduction**: Mitochondrial dysfunction and hyperglycemia are linked to cardiac injury and heart failure (HF).[1] Mitochondrial unfolded protein response (UPRmt) can protect heart from injury.[2] Here we investigated the association between UPRmt and hyperglycemia in human HF.

**Material & Methods**: Myocardial samples were collected from heart transplant patients and were divided into HF with hyperglycemia (HFHG, HbA1c >6.5%, n = 31) and HF with normoglycemia (HFNG, HbA1c <6.5%, n = 34) groups. Six healthy heart samples were obtained from the National Disease Research Interchange organisation. Gene expression of HSPA9,HSPD1, LONP1, OMA1 and YME1L was analyzed using RT-qPCR with TaqMan probes.

**Results**: Significant differences were found in the expression of UPRmt genes LONP1, HSPD1, and YME1L, which were higher in HFHG than in HFNG group. LONP1 and YME1L are mitochondrial proteases and HSP60 is a chaperone (encoded by HSPD1). In healthy hearts, OMA1, HSPD1, and HSPA9 were downregulated compared to both HFHG and HFNG.

**Conclusion**: Our results indicate that hyperglycemia induces LONP1, HSPD1, and YME1L in myocardium of HF patients and that HF upregulates OMA1, HSPD1, and HSPA9,suggesting that both hyperglycemia and HF induce UPRmt, acting as likely adaptive response to stress.[3]

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## The role of COX4 in glioblastoma: exploring its therapeutic potential and impact on mitochondrial function — a preliminary study

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**Introduction**: Glioblastoma (GBM) is a fast-growing, invasive brain tumor with a poor prognosis and high recurrence rate. Mitochondrial dysfunction, including disrupted cellular energetics, is a hallmark of cancer. Cytochrome c oxidase subunit 4 (COX4), a mitochondrial enzyme involved in oxidative phosphorylation, may influence GBM, but its role remains insufficiently explored.

**Material & Methods**: This study investigated COX4's role in LN-18 GBM cells by assessing the expression of COX4 isoforms (COX4-1 and COX4-2) using RT-qPCR and downregulating COX4 with siRNA. Following silencing, experiments evaluated cell proliferation, migration, mtDNA content, and ATP levels to understand COX4's effects on mitochondrial function.

**Results**: COX4-1 was significantly overexpressed in LN-18 GBM cells compared to normal cells, while COX4-2 showed lower expression. Silencing COX4-1 with siRNA reduced its expression by 85.07%, significantly inhibiting cell proliferation. Additionally, COX4-1 knockdown enhanced cell migration in wound-healing assays. The knockdown also led to a marked reduction in mtDNA copy number and ATP levels, indicating compromised mitochondrial function.

**Conclusion**: Preliminary findings suggest that COX4 may be a promising therapeutic target for GBM due to its crucial role in mtDNA maintenance and cell migration. Ongoing experiments aim to further elucidate COX4's impact on mitochondrial function in GBM.

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## M.10010T>C mitochondrial disease: a case report with hypocalcemia

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**Introduction**: Recent advancements in genetic analysis have enhanced the discovery of rare mitochondrial DNA (mDNA) variants. However, the phenotypic characteristics and heteroplasmy levels of these variants are often not well documented. Here we present a case of a female manifesting myalgia for 19 years and

unexplained persistent hypocalcemia heteroplasmic for m.10010T>C mDNA.

**Material & Methods**: This case report includes a detailed clinical evaluation and heteroplasmy measurements across multiple tissues.

**Results**: The patient initially presented with myalgia and dyspnea, yet pulmonary and cardiac evaluations were unremarkable. The patient exhibited hypocalcemia with an inappropriate parathyroid hormone response, potentially contributing to the observed myalgia. After 19 years with progressive myalgia electron microscopy of muscle biopsies revealed abnormal mitochondrial morphology, and subsequent genetic analyses revealed the m.10010T>C variant in skeletal muscle. Further analysis of DNA isolated from other tissues revealed buccal mucosa (4%), and urine (20%), however absent in blood. The variant was accordingly absent in tissues from the mother. Notably, previous cases with m.10010T>C have not reported hypocalcemia.

**Conclusion**: Muscle fatigue and myalgia are common symptoms that may serve as the sole indicators of mitochondrial disease. In the present case, persistent hypocalcemia represented a rare and unexplained finding, potentially excabating myalgia.

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# AD-TAU pathology induces mitochondrial dysfunction in a rat model of sporadic alzheimer's disease

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**Introduction**: Tau pathology in Alzheimers disease (AD) is accompanied by mitochondrial dysfunction [1]. However, most studies investigating mitochondrial dysfunction have employed animal models expressing either wild-type or familial mutant forms of tau protein, making them translationally less effecient. To address this gap, we aimed to investigate potential defects in various mitochondrial parameters in the presence of tau pathology by utilizing a rat model of sporadic AD [2].

**Material & Methods**: Following confirmation of tau pathology by immunohistochemistry, the isolated mitochondria from brainstem (BS) and cortex (CTX) were characterized via flow cytometry (n=24). A comparative mitochondrial proteomic analysis was performed using advanced mass spectrometry technique.

**Results**: In BS, 120 proteins were upregulated and 33 were downregulated while in CTX 32 were upregulated and 18 were downregulated. In BS, the dysregulated proteins were primarily associated with metabolism, ATP synthase, respiratory complexes I,III,V, transport, autophagy, dynamics, and calcium channels. In contrast, the dysregulated proteins in CTX were related to only metabolism, respiratory complexes I,III, calcium channels, and outer membrane.

**Conclusion**: Both truncated tau expression and tau pathology contribute to mitochondrial dysfunction, with tau tangles exacerebating mitochondrial dysfunction. These findings offer new insights into potential pathways through which tau pathology contributes to AD progression.

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# Cardiovascular autonomic neuropathy in M.3243A>G mitochondrial disease


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**Introduction**: Carriers of m.3243A>G have an increased risk of cardiomyopathy and cardiac death.[1] Cardiac autonomic neuropathy (CAN), an impaired autonomic nerve regulation of the heart rate may contribute to increased mortality[2], and we investigated if m.3243A>G carriers have decreased parasympathetic tone and heart adaptability.

**Material & Methods**: Cross-sectional case-control study including 28 carriers of m.3243A>G and 28 healthy controls matched on sex and age, and with a body mass index of 20-25kg/m2. Cardiac measures included CAN-score from cardiovascular autonomic reflex test (CART), 24-hour heart rate variability (HRV), and cardiac vagal tone (CVT).[3]

**Results**: CART revealed 54% of m.3243A>G carriers have established or borderline CAN vs. 21% of controls ($p = 0.014$). Evaluating HRV-measures they had a lower mean [95% confidence interval (CI)] heart adaptability using the standard deviation of normal to normal (127ms [113;142] vs. 157 [140;175], $p = 0.010$) and of parasympathetic tone including mean [95% CI] root square of successive RR interval differences (29.2ms [24.1;34.3] vs. 38.8 [32.5;45.2], $p = 0.020$) and lower median (interquartile range) CVT (3.4 (1.8;5.9) vs. 7.1 (5.4;12.1), $p<0.001$).

**Conclusion**: The 3243A>G mitochondrial disease is associated with dysfunctional autonomic cardiac regulation. CAN may potentially be a marker for early cardiac disease.


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## Mitochondria transfer, a potential mechanism of CD4+T cell exhaustion in oral cancer

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**Introduction**: Oral squamous cell carcinoma (OSCC) is the main type of oral-cancer. We have previously identified the presence of mitochondrial proteins in the secretomes of human OSCC biopsies, which also have the capacity to induce exhaustion markers on CD4+T-cells1. Several cells have the capacity to transfer mitochondria, including cancer cells 2,3. However, it has not been evaluated whether mitochondria transfer from oral-cancer cells into CD4+T-cells induces an exhausted phenotype; thus, we analyse the effect of artificial transfer of malignant mitochondria on T-cell exhaustion.

**Material & Methods**: Isolated MitoTracker Green(MTG)-labelled mitochondria from oral-cancer cells were artificially transferred into CD4+T-cells. Then, surface molecule expression, proliferation, cytokine secretion and metabolic pathways mediated by tumour mitochondrial transfer were analysed by flow cytometry, metabolomics and proteomics.

**Results**: Artificial mitochondria transfer from oral-cancer cells into activated CD4+T-cells impaired cell proliferation, Th-cytokine secretion and promoted an exhausted phenotype. Proteomics-derived pathways such as hypoxia, reactive oxygen species production and glycolysis were significantly upregulated in CD4 +T-cells that acquired malignant mitochondria, whereas metabolomics revealed that thiamine was reduced. Thiamine is a cofactor of enzymes from the Krebs's cycle, suggesting that the uptake of malignant mitochondria affect cellular respiration in CD4 +T-cells.

**Conclusion**: The acquisition of mitochondria from oral-cancer cells induces cell-exhaustion in CD4 +T-cells.

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## Gestational physical activity is associated with the altered expression of mitochondrial markers in term placenta and differs based on tissue location

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Physical activity (PA) benefits the gestational parent (gesP) and fetus during pregnancy. These benefits are hypothesized to occur via the placenta, potentially because of placental mitochondrial adaptations. Furthermore, the effect of different tissue locations of the placenta on mitochondria has yet to be studied in detail.

Term placental tissue was collected from healthy women and characterized as objectively active (n=5) or inactive (n=5). Mitochondrial complexes (CI-CV),

SOD1, SOD2, OPA1, and DRP1 were measured by western blot. mtDNA copy number and network formations via mitochondrial network analysis (miNA) were also assessed.

PA significantly influenced the protein expression of CII (p=0.024) and CIV (p=0.024). We also found significantly greater expression of SOD1 and CI-CV in active individuals and CI-CII and CV in inactive participants in peripheral placental tissue. mtDNA and miNA analysis yielded no significant results.

While PA had a smaller influence than anticipated, we noted differences in essential mitochondrial complexes. Peripheral tissue, more so in active individuals, shows greater expression of mitochondrial markers, indicating potential differences in function by tissue location. Overall, we provide mechanistic insight into the benefits of PA during pregnancy and the possible role of tissue location.

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# Brown adipose tissue mitochondria is a target for the rapid effects of ketone bodies

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**Introduction**: Brown adipose tissue (BAT) mitochondria play a thermogenic role due to its ability to dissipate energy obtained by substrate oxidation as heat through uncoupling protein-1 (UCP-1) mediated uncoupling. We evaluated whether BAT mitochondria could be a target of the rapid effects of ketone bodies.

**Material & Methods**: 1,3-butanediol, a precursor of β-hydroxybutyrate (β -OHB), was in vivo administered to rats, and its effects were evaluated after 3-hours from the injection. A large-scale quantitative proteomic approach (LC-MS/MS) associated with respirometry was performed on mitochondria isolated from BAT. Western blot of respiratory chain complexes subunits, UCP-1 and total lysine β-hydroxybutyrylation were also performed.

**Results**: BD administration induced a 5-fold increase in β-OHB plasma levels. This was associated with an enhancement in mitochondrial respiration rate due to increases in respiratory chain activity and UCP-1 mediated uncoupling. Proteomic analysis indicated the ability of BD to induce rapid remodeling of respiratory chain and to increase mitochondrial UCP-1 levels. BD administration rapidly induced β- hydroxybutyrylation post-translational modification of mitochondrial proteins.

**Conclusion**: Our data indicate that β-OHB could be a signaling molecule able to rapidly affects mitochondrial BAT functionality.

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## Impaired mitophagy in huntinton´s disease striatal astrocytes

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Huntington's disease (HD) is an inherited dominant neurodegenerative disorder that gradually impairs patient's motor, cognitive and affective functions1. This disorder is originated by a CAG repeat expansion in the Huntingtin gene that mostly affects the Medium Spiny Neurons within the Striatum2. Among the alterations defined in neurons, dysfunction in mitochondrial quality control mechanisms (MQCM) have been described3. Although HD pathology have mainly been studied in neurons, mHtt also disrupts astrocytes metabolism potentially affecting neurons4. Our group has previously described mitochondrial dysfunctions in HD striatal astrocytes. In this study we hypothesize that MQCM could be also altered. Therefore, we have focused on mitophagy, the specific degradation of mitochondria, in the R6/1 mouse model of HD. Mitophagy is carried out mainly by the PINK1-Parkin pathway5. Our results show that after treatment with Bafilomycin A1, p62 protein levels are significantly increased in R6/1 astrocyte primary cultures but not LC3II levels. Also, higher colocalization of p62 with TOMM20 was observed in the R6/1 model either after treatment with DMSO or Bafilomycin A1. Besides, p62 protein levels were increased in ACSA2 + astrocytes from 12 and 20-week-old mice.

Altogether, we suggest that the mitophagy pathway is stuck at p62 accumulation in mitochondria in HD striatal astrocytes.

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## Novel intracellular fluorimetric probes for organelles with photoinduced bioactivity

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Bioimaging by fluorescent mitochondria-probing small molecules significantly contributed to understanding biological functions but few probes can fluorometrically distinguish between DNA and protein/membrane and upon outer stimuli damage cellular organelles. Triarylborane chromophores were considered unstable in water, until we recently discovered cationic bis-triarylboranes (TAB), with outstanding intracellular bioimaging properties.

Marder group synthesized novel TABs and Piantanida group studied interactions with biomacromolecules by UV/Vis, fluorimetric, and circular

dichroism techniques. The confocal microscopy in living human cells demonstrated cell uptake and intracellular accumulation and distribution and photoinduced bioactivity.

We showed that novel TABs bind exceptionally strongly (with 10-100 nM affinity) to DNA, RNA and proteins. Some dyes can be applied as a dual luminescence probe, efficiently distinguishing between DNA/RNA and protein (BSA) by fluorescence emission separated by $\Delta\lambda$= 100 nm, supporting the accumulation of dye in a protein-like target of mitochondria in human cells.1 Also, novel generations of TABs showed that by simple modification of a central core, fluorescence colour can be finely tuned, as well as intracellular selectivity toward organelles.2 Further studies demonstrated that some non-toxic TABs could be photoinduced by visible light to exceptionally strong bioactivity, attributed to singlet oxygen production,3 thus offers numerous applications in theranostic studies.

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# Macrophage polarization in wound healing impacts on adipose-tissue derived stem cell metabolism and mitochondrial function

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**Introduction**: Chronic non-healing wounds pose a significant health challenge, often leading to persistent inflammation and premature scar tissue formation. Regenerative cells from neighboring fat tissue contribute to healing[1], but their fate is closely tied to the macrophage-dominated inflammatory microenvironment. This study examined the presence of adipose-derived stem cells (ASC) in human granulation tissue and explored the effects of macrophage polarization on ASC metabolism and mitochondrial function.

**Material & Methods**: ASC presence was assessed by immunofluorescence and flow cytometry. Macrophage-ASC crosstalk was modeled using conditioned medium (CM) from M1(IFNG/LPS) or M2 (IL4/IL13) macrophages. Mitochondrial activity was evaluated using seahorse technology, flow cytometry, and fluorescence microscopy.

**Results**: Approximately 1.5% ASC (CD45-/CD31-/CD34+) were present in single-cell suspension of patient granulation tissue[2]. CM from M1 macrophages induced a pro-fibrotic response in ASC, increasing stress fiber formation, attachment, and integrin expression, while reducing mitochondrial function, ATP production. A metabolic shift from OXPHOS-driven to glycolysis-dominated metabolism and changes in mitochondrial structure were detected, while protein levels of OPA1 or DRP1 remained unchanged.

**Conclusion**: M1 macrophages influence ASC behavior by promoting a pro-fibrotic response, mitochondrial reorganization and function. These findings provide insights into chronic wound dynamics and highlight the critical role of microenvironmental signaling in regulating regenerative cell functions.

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# Mutant P53 induces mitochondrial fragmentation in pancreatic ductal adenocarcinoma (PDAC)

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**Introduction**: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer marked by frequent mutations in the TP53 tumor suppressor gene1. TP53 mutations have severe impacts on tumour cell metabolism, enhancing glucose metabolism even under aerobic conditions promoting tumour aggressiveness2. Conversely, the role of mutant p53 in mitochondrial respiration of cancer cells is less understood. Our goal is to investigate the impact of mutp53 on the mitochondrial metabolism of PDAC cells, as it may represent a promising target for cancer therapy.

**Material & Methods**: Using CRISPR/Cas-9 technology, we generated TP53 KO PANC-1 cells and we compared them with parental PANC-1 cells (mutp53R273H). Different analysis were performed: seahorse mito stress test to determine OXPHOS metabolism, mass spectrometry study on puridied mitochondria and transmission electron microscopy and confocal microscopy to analyze mitochondria morphology.

**Results**: TP53 KO PANC-1 cells are less metabolic than PANC-1 cells. This is associated with a significantly higher expression of MYH14 in PANC-1 cells, protein that is involved in mitochondrial fragmentation. Microscopy analysis revealed more fragmented mitochondria. 3D mitochondrial network study showed a more fractionated network in PANC-1 cells.

**Conclusion**: Mutant p53 is responsible, through a mecahnism that involves MYH14, of the enhanced mitochondria fragmentation in PDAC.

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## Mitochondrial genes expression profile in extracellular vesicles of bowel lavage fluid in patients with colorectal cancer and colonic lesions

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Colorectal cancer (CRC) is the third most commonly diagnosed cancer. Polyps are colonic lesions that can trigger CRC in the medium/long term1. Extracellular vesicles (EVs) from bowel lavage fluid (BLF) could be an important source of cancer biology knowledge2. The objective was to investigate the enrichment of mitochondrial gene sets in EVs from BLF of patients with colonic lesions (including CRC), and the possible role of mitochondria in CRC.

The BLF was obtained during colonoscopy. EVs were isolated by ultracentrifugation, and characterized through NTA, TEM, AFM, and tetraspanin expression analyses. RNA was obtained using Qiagen's miRNeasy mini kit. mRNA array was performed using Clariom S Human mRNA Array, and bioinformatic analysis was performed with TAC software (Applied Biosystems) and GSEA Software3.

Bioinformatic analysis revealed many mitochondrial-related mRNAs upregulated in patients with colonic lesions, 11 of them with a Fold Change > 4. Enrichment analysis of Gene Ontology, Pathways, Reactome, and Hallmarks unveiled that mitochondrial-related pathways were also upregulated.

These results confirm the presence of mitochondrial-specific mRNAs in the EVs of patients with colonic lesions. These findings suggest a role of mitochondria in CRC development and progression, and possibly in the communication between cancer and surrounding cells through the EVs.

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## Megamitochondria formation in primary human hepatocytes: a pathway to therapeutics for alcohol-related liver disease

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**Introduction**: Megamitochondria, a histological hallmark of alcohol-related liver disease (ALD), have been associated with improved patient survival [1], suggesting that promoting their formation could be beneficial in ALD. However, the mechanism underlying their formation and impact remains unclear due to limitations

in current animal models [3]. This study aimed to test whether primary human hepatocytes (PHH) cultured in 3D collagen scaffolds develop megamitochondria in response to alcohol, enabling further functional studies on their role in ALD.

**Material & Methods**: PHH from 3 donors were seeded in collagen sponges and treated with 100mM ethanol for 6/12 days. Immunofluorescent staining for TOM20, CYP2E1, ADH1B was performed on paraffin sections. Mitochondrial morphology was assessed using super-resolution microscopy, followed by automated segmentation and morphometric analysis via NIS Elements software.

**Results**: Mitochondrial size was variable among donors (up to 16,000mitochondria/condition/donor analysed). In 2 donors, alcohol treatment increased mitochondrial length, width, and surface area and the proportion of megamitochondria (defined as >1 μm length, width) compared to untreated control.

**Conclusion**: These results demonstrate suitability of PHH grown in collagen scaffolds for studying megamitochondria in response to alcohol. This system captures patient-specific mitochondrial differences and offers a platform for investigating megamitochondria and developing potential therapies for ALD.

## Funding

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# Measuring mitochondrial morphology and network abberations in a cellular model of P301S tauopathy

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The role of mitochondrial dysfunction in tauopathies is of growing interest and the pharmacological modulation of mitochondrial maintenance mechanisms (mitophagy, mitochondrial biogenesis, and dynamics) may offer novel therapeutic strategies. To explore this, this research investigates changes in mitochondrial morphology and dynamics in a MAPT P301S neuroblastoma model of tauopathy1. The P301S mutation gives rise to frontotemporal dementia and animal models present with marked mitochondrial dysfunction2.

This study has utilised SH-SY5Y cells possessing a tetracycline inducible P301S mutation. Cells were stained with MitoTracker Red CMXRos, and Z-stacks were acquired using confocal microscopy. The 'Mitochondria Analyzer' pipeline plugin for ImageJ3 was then used to acquire metrics of morphology and dynamics: volume, surface area, sphericity of the network, and branch features.

This research is in a preliminary stage; however, pilot data suggests that volume and surface area are reduced after induction both across the cell and for individual mitochondria. Increased sphericity and reductions in branches and branch junctions may also indicate fragmentation of the mitochondrial network.

This preliminary data is promising and offers means for measuring mitochondrial health in vitro. The next phase of this research will be to see how the induction of mitophagy and mitochondrial biogenesis might ameliorate the observed dysfunction.

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## SORL1 deficiency in alzheimer´s patients: impact on mitochondria and autophagy

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**Introduction**: Alzheimer's disease (AD) is the most common neurodegenerative disorder. SORL1 mutations have been associated with both early-onset AD and sporadic late-onset AD. Moreover, reduced levels of Sorl1 protein have been found in lymphoblasts and in post-mortem AD brains. Sorl1 is a sorting receptor implicated in amyloid-precursor protein (APP) processing and Aβ peptide clearance in lysosomes. Using animal models of AD, brain transcriptome studies have shown that SORL1 deficiency could affect mitochondrial function. Additionally, studies on cellular models have linked SORL1 deficiency to defective autophagy. Here we aimed to determine the role of Sorl1 protein in mitochondrial function and the interplay between SORL1 deficiency and autophagy.
**Material & Methods**: We used fibroblasts from AD patients with SORL1 mutations and a stable SORL1 knockdown cellular model.
**Results**: We demonstrate that SORL1 deficiency negatively affect the mitochondrial function. Furthermore, SORL1 deficient cells showed increased mitochondrial mass, which could be related with alterations in the mitochondrial dynamic processes. In adition, we found that SORL1 mutations could also affect autophagy and the mitochondrial recycling process, known as mitophagy.
**Conclusion**: This research could lead to a better understanding of how Sorl1 deficiency impacts mitochondrial function and the pathophysiology of AD, eventually suggesting therapeutic interventions.

### Funding

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## A putative involvement of telomerase reverse transcriptase in mitochondrial turnover

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**Introduction**: Telomerase Reverse Transcriptase (TERT), the catalytic subunit of telomerase, aside from the role in telomere lengthening, is recognized to have some extra-telomeric roles [1], including the localisation inside mitochondria [2]. Previously, we showed that TERT has a protective function against oxidative stress and a role in preserving mitochondrial functionality [3]. The aim of this work is to understand the TERT mechanism of action inside mitochondria.
**Material & Methods**: U2OS osteosarcoma cells, which naturally lack TERT, were transduced with TERT protein. mtDNA immunoprecipitation was used to detect TERT binding to mitochondrial DNA, and immunofluorescence, immunoblotting (anti-BrdU), and RT-qPCR were performed to analyse mitochondrial

replication and gene expression. MitoTracker measured mitochondrial mass, and transmission electron microscopy (TEM) confirmed TERT localization in mitochondria.

**Results**: in TERT-overexpressing cells, TERT was detected inside mitochondria, bound to mtDNA sequences, leading to an increase of mtDNA replication and transcription. Mitochondrial mass decreased, but mitochondria were smaller and more numerous. Autophagy appeared to increase as well.

**Conclusion**: we suggest an involvement of TERT in mitochondrial turnover, to mediate the clearance of damaged mitochondria. Further studies are required to confirm if TERT influences mitophagy, with future research aimed at identifying potential mitochondrial proteins that interact with TERT.

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# Investigating the role of FAHD1 expression in cellular respiration in U2OS cells

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FAHD1 is an oxaloacetate decarboxylase involved in regulating mitochondrial respiration, specifically impacting Complex I and II activities. We explored the effects of FAHD1 expression on cellular respiration using U2OS cells. This study aims to assess how wild-type FAHD1, as well as FAHD1 mutants (T192S and K123A), influence mitochondrial function, particularly focusing on temperature-dependent changes in Complex I and II activities. Respiration rates were measured in U2OS cells using high-resolution respirometry (Oxygraph O2k) to evaluate Complex I and II activity in wild-type cells, FAHD1-overexpressing cells, and those expressing FAHD1-T192S and FAHD1-K123A mutants. Measurements were taken at 25°C and 37°C to assess temperature-dependent changes in mitochondrial function.

Our findings reveal that all tested U2OS cell lines exhibited higher Complex I activity at 25°C compared to 37°C. In contrast, Complex II activity was largely comparable across temperatures, except in FAHD1-K123A mutant cells, where a notable increase in Complex II activity was observed at 37°C.

These results highlight the temperature sensitivity of Complex I activity in U2OS cells and demonstrate that the FAHD1-K123A mutant uniquely increases Complex II activity at higher temperatures. This suggests that specific FAHD1 mutations may differentially regulate mitochondrial respiration, providing further insight into FAHD1's role in cellular metabolism.

# Mitochondrial function and other important parameters of ageing are improved by caloric restriction in humans

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**Introduction**: The concept of caloric restriction (CR) has become an integral part of gerontological research due to its ability to delay the ageing process. Positive effects have been demonstrated in many model organisms. In contrast, studies in humans are scarcer and more diverse but suggest a similar beneficial effect. To

further elucidate the potential effects of CR in humans, we performed several ex vivo studies. Our studies aimed to analyse peripheral blood mononuclear cells (PBMCs) from individuals who underwent F.X. Mayr therapy – a specific form of CR – with regard to various cellular parameters.

**Material & Methods**: PBMC isolation and sample analysis were performed immediately after blood collection (day 0, day 5 or 7, day 11 of therapy). Autophagy was analysed by antibody labelling and flow cytometry and mitochondrial function by XF Analyzer. In addition, mRNA levels and mtDNA copy number were determined by real-time PCR.

**Results**: Autophagy is rebalanced by CR, depending on the initial autophagy levels. All mitochondrial parameters measured by XF Analyzer show a significant increase, while mtDNA copy number remained unchanged.

**Conclusion**: Our results indicate a positive influence of CR on key cellular parameters, particularly mitochondrial function and autophagy.

## Funding

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# Variation in mitochondrial functions across vital organs and brain sub-regions in a swine model: a novel reference targets for TBI and polytrauma

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**Introduction**: In potential future combat situations, US forces may encounter systemic injuries affecting multiple organs, often resulting from a combination of mechanical trauma, thermal injury, and/or exposure to chemical, biological, radiological, and nuclear elements. It is crucial to comprehend the post-injury mechanisms and identify treatments for delayed secondary injuries. Our study focused on understanding post-injury mechanisms following TBI by examining organ-specific mitochondrial differences in swine. The aim is to establish reference values for assessing TBI and polytrauma and for evaluating future therapies in combat and field care environments. The swine model has recently come to the forefront due to its large brain mass and mammalian-specific architecture, which are key features in replicating the TBI and polytrauma pathophysiology in humans.

**Material & Methods**: This study used Yorkshire Swine to create initial baseline data for potential future TBI/polytrauma injury in a swine model. It examined mitochondrial function in various organs (heart, kidneys, liver, lungs) and brain regions of uninjured control Swine, including real-time respirometry, $Ca^{2+}$ buffering capacity, and redox profile. Statistical comparisons were conducted using ANOVA ($N = 6$, $^{*}p < 0.05$)

**Results**: In comparison to other organs, the brain and heart exhibited the highest levels of bioenergetics and $Ca^{2+}$ buffering capacity. Additionally, both organs demonstrated increased expression of membrane proteins such as Cytochrome C and VDAC. The expression of antioxidants also varied across different organs.

**Conclusion**: The vital organs are equipped with unique profiles to combat pathological insults. Establishing organ-specific reference values may offer valuable insights for the development of mitochondria-targeted therapeutic interventions for TBI and polytrauma.

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## Disclaimers

Research was conducted under an IACUC-approved animal use protocol in an AAALAC International - accredited facility with a Public Health Services Animal Welfare Assurance and in compliance with the Animal Welfare Act and other federal statutes and regulations relating to laboratory animals.

## Dietary l-carnitine and intravenous lipopolysaccharides (LPS) modify the mitochondrial functionality of bovine peripheral blood mononuclear cells (PBMC)

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**Introduction**: L-carnitine is essential for the beta-oxidation of free fatty acids in mitochondria[1] and may therefore influence the mitochondrial functionality of bovine PBMC during an energy-demanding immune challenge.
**Material & Methods**: Heparinized blood was collected -14d, +24h and +14d relative to an LPS injection (0.5µg/kg body weight) from pluriparous German Holstein cows which were assigned to a control group and an L-carnitine supplemented group (25g per cow and day). PBMC were isolated by density gradient separation and stimulated ex vivo with LPS or concanavalin A (ConA). Oxygen consumption rate was measured using the Seahorse XFe96 Analyzer (Agilent, Santa Clara, USA), and mitochondrial functionality was assessed using the mito stress test (Agilent). Statistical analyses were performed using the MIXED procedure of SAS (9.4), including feeding group, time relative to LPS injection, stimulus, and their interactions as fixed factors.
**Results**: Mitochondrial functional parameters were not significantly affected by L-carnitine supplementation. However, non-mitochondrial respiration was significantly influenced by L-carnitine supplementation over time (pGroup*Time=0.038). Maximal respiration was significantly lower in PBMC stimulated ex vivo with LPS than in unstimulated or ConA-stimulated PBMC.
**Conclusion**: These findings suggest that the combination of immune challenge and L-carnitine supplementation leads to increased non-mitochondrial enzymatic activity.

### Funding

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## Decreasing heparan sulfate biosynthesis suppresses neurodegeneration, promotes mitochondrial function, autophagy flux and regulates lipid metabolism

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**Introduction**: Heparan sulfate modified proteins (HSPGs) are co-receptors for many growth factor signaling pathways. HSPGs are poised to regulate cellular and metabolic processes, including autophagy. Reducing HS biosynthesis suppresses cell loss in Drosophila models of Parkinson's (parkin mutants) and Alzheimer's disease (presenilin knockdown). Commensurate with rescue of these degenerative phenotypes, reducing HS increases mitochondrial function, promotes autophagy to lysosome trafficking, and reduces intracellular lipid stores. To better understand the effects of reducing HS-mediated signaling on metabolic pathways, we conducted RNAseq-based gene expression analysis of human Hep3B cells bearing a knockout mutation in EXT1, an essential HS co-polymerase.
**Material & Methods**: RNA isolated from Hep3B +/+ and EXT1 -/- cells in quadruplicate, processed as described.

**Results**: Significant expression changes in genes encoding components of glycolysis, fatty acid oxidation, as well as fatty acid and cholesterol biosynthesis were apparent. More than 50% of mitochondrial localized protein encoding genes (MitoCarta) showed significant changes in EXT1-dependent gene expression. Overall, the gene expression profiles are indicative of increased glycolysis, elevated fatty acid catabolism, decreased fatty acid and cholesterol biosynthesis, increased autophagy flux and mitochondrial biogenesis.
**Conclusion**: Metabolic changes mediated by inhibiting HS biosynthesis, could counter metabolic changes found in neurodegenerative diseases.

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# Penetrance of leber's hereditary optic neuropathy in h and j1 mitochondrial haplogroups

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**Introduction**: Leber's hereditary optic neuropathy is a common cause of blindness resulting from mutations in the mitochondrial DNA that encode the subunits of complex I. Penetrance is low, but greater with mitochondria from the J1-haplogroup compared to the parent H-haplogroup.
**Materia l& Methods**: OCR, membrane potential (ΔΨ), pH gradient (ΔpH) and proton motive force (ΔP) were measured with a prototype of the Iberius (CellSpex Ltd) in 143B cybrid cells lines.
**Results**: ΔP was very similar in all the cells lines, as was the OCR in the H1 and J1c wildtype cell lines but decreased in the mutants. The G11778A mutation decreased ΔΨ from ≈130 to ≈110mV and increased ΔpH from ≈25 to ≈45mV at near constant ΔP in the H1-haplogroup compared to wildtype. Surprisingly, the wildtype J1c cells already had low ΔΨ (≈110mV) and high ΔpH (≈45mV), and the T14484C mutation had minimal effect on components of ΔP, G3460A decreased ΔΨ and increased ΔpH by ≈15mV, and G11778A changed them by ≈30mV, correlating with the severity of disease.
**Conclusion**: The mechanism of disease is associated with the decrease in ΔΨ and increase in ΔpH, and that penetrance is greater in individuals with J1-mitochondria because they already have decreased ΔΨ and increased ΔpH.

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## Mitochondrial quality control in sjögren's disease

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**Introduction**: Sjogren's disease (SjD) is a chronic autoimmune disease characterized by salivary and lachrymal gland dysfunction, clinically presented as sicca symptoms. Increasing evidence indicates a possible role of mitochondrial dysfunction in this disease. A proteomic study of salivary gland epithelial cells (SGEC) from SjD patients showed decreased expression of proteins involved in mitochondrial quality control (MQC), such as mitochondrial transcription factor A and mitochondrial fission factor. In our study, we aim to evaluate the dysregulation of MQC pathways in the salivary glands of SjD patients and to evaluate if this dysregulation is reflected in mitochondrial mass and function.
**Material & Methods**: Based on proteome analysis (via mass spectrometry) of minor salivary glands (MSG) of 18 patients with SjD and 6 sicca controls, we will evaluate the dysregulation of MQC pathways. In addition, we will measure mitochondrial mass and mitochondrial membrane potential with flow cytometry analysis of SGEC from SjD patients and sicca controls.
**Results**: We expect to find dysregulation of MQC pathways in the MSG of SjD patients, accompanied by decreased mitochondrial mass and altered mitochondrial membrane potential in SGEC.
**Conclusion**: Our study will provide new insights into the role of MQC dysregulation, mitochondrial mass and function alterations in SjD.

### Funding

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## The foundation of decay: modeling aging promoted retinal degeneration with mitochondrial dna mutation burden

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**Introduction**: This study characterizes the retinal degenerative phenotype presented in the PolgD257A (D257A) mouse that accumulates rapid systemic mitochondrial DNA (mtDNA) mutations with age.[1] Previous studies of this model have indicated that both the retina and retinal pigment epithelium (RPE) are among the tissues with the most significant mtDNA mutation burden.[2]
**Material & Methods**: The age-related retinal degenerative phenotype presented in the D257A mouse was characterized morphologically, physiologically and biocheically.
**Results**: D257A displayed a significant loss of cone photoreceptor thickness and function starting at 6-months of age. D257A RPE showed an increased abundance of autofluorescent granule formation and accumulation. In addition, the RPE showed disordered pigment, disorganized microvilli and basal infoldings. D257A RPE mitochondria were significantly smaller and appear to have dilated cristae.

Interestingly, there was no significant difference in several measures of oxidative stress. D257A RPE showed a significant increase in receptor mediated mitophagy markers. Electron transport complex proteins I and IV were most significantly depleted in this model. Our observations were not associated with increased oxidative stress and parkin-mediated mitophagy in the ages analyzed.

**Conclusion**: This study suggests that the accumulation of mtDNA mutations promotes accelerated aging, resulting in retinal degeneration.

## Funding

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# 3D cell culture models to study mitochondrial function in airway diseases

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**Introduction**: Mitochondrial dysfunction in the airway epithelium is suggested to contribute to the development of Chronic Obstructive Pulmonary Disease (COPD)1–3. Development of in vitro models are therefore needed to study mitochondrial function in the airway epithelium. Here we aim to develop a physiologically-relevant 3D epithelial cell culture model used to measure cellular bioenergetics.

**Material & Methods**: Primary bronchial epithelial cells from healthy and COPD donors were used to generate apical-out 3D organoids consisting of differentiated epithelial cells. The cellular bioenergetics of the organoid and monolayer cultures was analysed using a Seahorse XF analyser.

**Results**: Seahorse assays showed that the organoid cultures generated 80% of their ATP through mitochondrial respiration while this was only 50% for epithelial monolayers from the same donors ($p<0.001$). The COPD cells had similar sources of ATP production compared to healthy cells in both models. There were no differences in oxygen consumption between COPD and healthy monolayer cultures, however, COPD organoid cultures had lower oxygen consumption rates than healthy organoids ($p=0.0495$).

**Conclusion**: These results emphasise the importance of using differentiated, 3D cell culture models to study mitochondrial function in vitro. Further studies are needed to fully characterise mitochondrial function in airway epithelial cells from COPD patients.

## Funding

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## CXCR4 and mitochondrial biogenesis

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Our previous study has shown that CXCR4 cardiac-specific knockout (CXCR4-cKO) mice develop a progressive cardiomyopathy. To better characterize cardiac structural abnormalities, we used conventional H&E and Masson's trichrome staing. Our histological assessment reveals that that by 6-months of age, CXCR4 cKO hearts have significant cardiac myocyte hypertrophy, shows the presence of cellular infiltrates, and progressive interstitial/peri-vascular fibrosis. Transition electron microscopy was performed to further assess the subcellular abnormalities in the cardiomyocytes of CXCR4 cKO mice hearts at 6-months of age.

Quantitative analyses of mitochondrial morphology revealed that CXCR4 cKOs have a significantly higher numbers of mitochondria, however, mitochondria were much smaller in the CXCR4 cKO group as compared to controls implicating an important role for CXCR4 in regulating mitochondrial fission/fusion mechanism. We also observed significant changes in mitochondrial gene expression profile in our model of chronic exposure to isoproterenol-induced hypertrophy. A significant reduction in mRNA expression of cytochrome c oxidase subunit IV (COX4), NADH: ubiquinone oxidoreductase complex 1 & 9 (Ndufv1 and Ndufa9) were detected in CXCR4-KO LacZ-treated mice; while their expression levels were returned to normal in animal going under AAV9.CXCR4 gene therapy suggesting a great reduction in mitochondrial function in the absence of cardiac CXCR4.

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## Linking myrcene to tricyclic hydroquinone derivates improves the antiplatelet potential by regulating mitochondrial function

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Myrcene and tricyclic hydroquinone are known for their anti-inflammatory and antioxidant properties, the latter also affecting the mitochondria. These properties can contribute to the prevention of thrombosis, critical in many cardiovascular conditions. This study aims to determine the effects of compounds that link myrcene to different hydroquinone derivates on platelets in vitro.

Platelets from healthy donors were treated with compounds (up to 100 μM) and tested for cytotoxicity, aggregation, activation, and mitochondrial and platelet function. Microplate aggregation, flow cytometry and microscopy were used to measure these effects.

While none of the compounds evaluated exhibited cytotoxic effects, MVqEMF1 showed the most promise. It did not affect platelet viability or apoptosis, even at high concentrations, while also significantly inhibiting aggregation and activation markers. Further tests revealed that MVqEMF1 reduced mitochondrial membrane potential and calcium and ATP levels and increased the production of reactive oxygen species (ROS). Finally, during spreading, the size of platelets incubated with MV1EMF1 was smaller, while the number of mitochondria per platelet was reduced.

In conclusion, MVqEMF1 effectively inhibits platelet function without causing cell damage while affecting the mitochondrial function, suggesting a possible mechanism through mitophagy. However, its potential as an antiplatelet drug require further experimentation.

## Funding

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# Sulfur metabolism in patients with mitochondrial diseases

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**Introduction**: Catabolism of sulfur compounds generates reduced equivalents used by mitochondrial oxidative phosphorylation (OXPHOS) complexes to generate ATP. H2S oxidation by sulfide:quinone oxidoreductase (SQOR) produces reduced coenzyme Q (CoQ10), and sulfite oxidation generates reduced cytochrome-c. Primary mitochondrial disorders (MD) may thus elicit secondary disturbances in sulfur catabolism.

**Material & Methods**: We analyzed sulfur compounds (including bioavailable sulfide, H2S) in plasma and urine from 52 patients with different MDs and 63 controls. The cohort was divided into subgroups based on their disease: complex I deficit (Leber hereditary optic neuropathy, m.3460G>A – group LHON ND1, m.11778G>A – group LHON ND4), complex IV deficit, combined OXPHOS complex deficiency (m.3243A>G, MELAS), short-chain (SCADD) and long-chain acyl-CoA dehydrogenase deficiency (LCHADD).

**Results**: We found markedly increased plasma levels of bioavailable sulfide in LHON ND4, hypotaurine in the plasma and urine of all MDs except LHON ND1, and dimethylglycine in the plasma and urine of LCHADD and SCADD. MDs, including beta-oxidation of fatty acids deficits, disturb CoQ10, the NAD+/NADH, resp. FAD/FADH2 pools in cells, thus hampering their use by other enzymes (SQOR, FMO1, or DMGDH).

**Conclusion**: The study demonstrates, for the first time, marked disturbances in sulfur metabolism observed in the plasma and/or urine of MD patients.

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## Exercise-induced changes in mitochondrial unfolded protein response in rat skeletal muscle

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**Introduction**: Exercise is a potent stimulus for mitochondrial adaptations. In addition to their unique role in energy metabolism, mitochondria are equipped with the mitochondrial unfolded protein response (UPRmt) to adapt to mitochondrial proteotoxic stress. The aim of this study was to examine the changes in the UPRmt to acute endurance exercise in rat skeletal muscle.

**Material & Methods**: Male rats were subjected to a single session of endurance exercise on a treadmill at a speed of 25 m/min for 60 min. Oxidative stress analyses as well as measurements of gene and protein expression of UPRmt markers and mitochondrial activity were carried out in plantaris muscle collected at sedentary (SED) and 0, 1, 3, 6, 12, and 24h post-exercise.

**Results**: Oxidative stress levels showed an increase at 0, 1, 3, and 6h post-exercise. We identified a different pattern of gene and protein expression of UPRmt markers in response to acute exercise, with mRNA expression responding early (0-6h) compared to protein expression (6-24h) following acute exercise. Histological staining of skeletal muscle showed an increase in mitochondrial activity (6-24h) following acute exercise.

**Conclusion**: Our findings revealed that acute exercise increased UPRmt and improved skeletal muscle oxidative capacity.

### Funding

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## Gallbladder mitochondria function as a prognostic factor for liver transplantation outcomes: a pilot study

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**Introduction**: Biliary complications are the most frequent cause of post-operative morbidity and mortality following liver transplantation (LT) [1]. Assuming that the mucosa of gallblader reflect changes in bile

ducts, we investigated whether gallbladder mitochondrial functions are associated with short and long-term outcomes after LT.

**Material & Methods**: Data from 16 LT patients was compared with 14 controls. Cholecystectomy was performed following implantation and arterial blood supply restoration of the donor liver (LT group) and during pancreaticoduodenectomy (Control). Gallbladder mitochondria were isolated by differential centrifugation.Oxygen consumption rates were measured in freshly isolated mitochondria with an Oroboros oxygraph. Biliary and systemic complications were classified according to the time of onset into early outcomes (up to 2 months after surgery) and late outcomes (later than 2 months after surgery).

**Results**: We have found that mitochondria of LT group present markedly diminished phosphorylating respiration rates compared to the control group. LT group was classified into sub-groups according to the respiratory rates in various metabolic states into strong and mild mitochondrial injury. Logistic regression analysis showed that ADP-stimulated respiration with glutamate and malate is significantly associated with early poor outcomes in LT patients.

**Conclusion**: Gallbladder mitochondrial function is a potential prognostic factor for early outcomes after liver transplantation.

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# Cardiac hypertrophy in growth associated protein 43 null cardiomyocytes is associated with altered mitochondrial proteomic profile

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**Introduction**: Growth associated protein 43 (GAP-43), is a Calmodulin (CaM) binding protein expressed in cardiac muscle close to dyads. In mouse model, the GAP-43 deletion lead to cardiac hypertrophy (CF)1. Mitochondrial dysfunction has been indicated as important player in the development of CF2,3. The aim of this study is to investigate the role of GAP-43 in mitochondria dynamics.

**Material & Methods**: Newborn C57BL/6 mouse GAP-43 knockout (GAP43-/-) and Wild type, was used for isolation of primary cardiomyocytes. We analize protein expression via Western blot (WB), shotgun proteomic and cellular metabolism through Seahorse after 7 days of colture.

**Results**: WB analysis revealed increased expression levels of GATA4 and Nkx2.5, myosin heavy chain and α actinin in GAP43-/- confirming in vitro the hypertrophic phenotype. Proteomic analysis highlighted alteration in mitochondria at different level, including reduction in subunits forming mitochondrial Complex I, III, IV while Fe-S cluster assembly proteins are absent in GAP-43-/-. Seahorse analysis confirmed a reduction in mitochondrial efficiency in ATP production in GAP-43-/-.

**Conclusion**: The lack of GAP-43 leads to alterations in cardiac remodelling and in cellular bioenergetics, demonstrating that it is an important mediator in the mitochondrial functions in cardiomyocytes.

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## A new view on the role of mito-ribosomal fidelity on human disease: structural analysis of deafness-related mtdna variants mapping to mitochondrial rrna genes

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We have reviewed the issue of mito-ribosomal fidelity during mitochondrial protein synthesis and placed it in the context of the emerging high-resolution structures of the mito-ribosome. All available evidence shows that altered mito-ribosome function, even at very low levels, as is often the case with fidelity mutations, may become highly pathogenic. This shifts the theoretical framework for the evaluation of the pathogenic potential of mitochondrial (mt-) rRNA variants. Under this new light, essentially, any base change capable of inducing a fidelity phenotype may be considered non-silent. We have used this new framework to study the potential pathogenicity of 93 mt-rRNA variants, reported by others as presumably associated to deafness. After inspection of these variants in the mito-ribosomal structure with the highest resolution currently available (2.2Å), 49 such variants were deemed as potentially non-silent.

These results drastically update our view on the implication of the primary sequence of mt-rRNA in the etiology of deafness and mitochondrial disease in general. In particular, our data shed much needed light on how mt-rRNA variants, specially those located at non-conserved positions, may lead to mitochondrial disease. Our data also provides clues on the predicted effect of haplotype context in the manifestation of some mt-rRNA variants.

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## Targeting mitochondrial dysfunction to improve skin aging through bioactive compounds

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**Introduction**: Mitochondria are the primary organelles affected during both chronological and ultraviolet (UV)-induced skin aging. Numerous bioactive compounds have been identified to enhance mitochondrial function, proving effective in mitigating aged skin. Here, we screened and identified effective compounds targeting mitochondrial function, achieving skin anti-aging benefits.

**Material & Methods**: Human dermal fibroblasts (HDFs) were used to establish an in vitro UVA irradiation model. Potential active compounds improving mitochondrial function were screened by analyzing cellular respiration using the Seahorse XF Analyzer. Multidimensional biological validation, including mitochondrial morphology, ATP and NAD+ levels, mitophagy, membrane potential, and collagen I levels, was performed. The identified active compounds were then incorporated into cosmetic formulations and evaluated for their skin anti-aging effects through a multicenter clinical trial.

**Results**: We identified seven active compounds capable of enhancing cellular respiration. They improved

mitochondrial morphology, intracellular ATP and NAD+ levels, mitophagy, membrane potential, and collagen I in UVA-irradiated HDFs. The clinical trial demonstrated that cosmetics containing seven active compounds improved wrinkles, tightness, and dermal density after 56 days of use.

**Conclusion**: We identified seven active compounds that restore mitochondrial function and delay skin aging. This study promotes the broader implications of mitochondrial function in age-related conditions, including skin aging.

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# Effects of supervised exercise training on platelet mitochondrial bioenergetics in hemodialysis patients with end-stage renal disease

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**Introduction**: Platelet dysfunction contributes to thrombotic/hemostatic disturbances in hemodialysis patients with end-stage renal disease (ESRD-HD). Mitochondrial functionalities in platelets involve cellular bioenergetics and thrombogenesis. Whether exercise training influences the risk of thrombosis in ESRD-HD patients remains unclear. This study aimed to elucidate the effect of supervised exercise training on platelet mitochondrial bioenergetics in ESRD-HD patients.

**Material & Methods**: Twenty patients with ESRD-HD received supervised exercise training (i.e., cycling exercise at ventilation threshold for 30 minutes/day, 3 days/week) for 24 weeks in the rehabilitation center, and then home base-medical care for 12 weeks. Systemic aerobic capacity (i.e., VO2peak) and platelet mitochondrial bioenergetic efficiency that included oxidative phosphorylation (OXPHOS) and electron transport system (ETS) were evaluated at pre-training status, training 12- and 24-weeks, and post-training 12-week follow-up.

**Results**: Supervised exercise training for 24 weeks significantly (i) elevated work rate and VO2peak, (ii) increased the capacities of OXPHOS and ETS by facilitating the Complex I-/II-mediated O2 consumption rates, and (iii) heightened the bioenergetic health index in platelet mitochondria. However, post-training12-week follow-up reversed these training effects on systemic aerobic capacity and platelet mitochondrial bioenergetics.

**Conclusion**: Supervised exercise training effectively increases systemic aerobic capacity and enhances mitochondrial bioenergetics efficiency of platelets in patients with ESRD-HD.

## Funding

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## Analysis of phenotypic changes in drug tolerant cells - the role of mitochondria and stat3 signaling

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**Introduction**: Understanding the phenotypic characteristics of drug-tolerant persister (DTP) cells is essential for improving the efficacy of cancer therapies. These traits are not static; they dynamically change over time, often independent of genetic mutations, leading to diminished therapeutic responses. This variability is particularly notable in STAT3-dependent (signal transducer and activator of transcription) cancers - acting downstream of many pro-oncogenic signals, where fluctuations in its level and activity could contribute to fractional killing. Mitochondria seem to play a critical role in this phenomenon, with variations in their function, structure, and activity significantly influencing STAT3 signaling and drug response.

**Material & Methods**: To characterize the phenotype of the chemotherapy-resistant cancer cell population, a research model was employed in which H1975 cancer cells were treated with the Janus kinase inhibitor: Ruxolitinib. Mitochondrial function, structure, and STAT3 levels were analyzed at distinct timepoints (pre-treatment, post-treatment, drug-free recovery and re-treatment) using immunocytochemistry, fluorescent probes, and high-content microscopy analysis.

**Results**: It was confirmed that the population of H1975 cancer cells resistant to Ruxolitinib displays a distinct mitochondrial function phenotype when compared to untreated cells.

**Conclusion**: Mitochondrial alterations are likely to play a crucial role in survival and persistence of cancer cells under drug treatment.

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## Does BK channel affect senescence - mitochondrial connection?

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**Introduction**: The BKCa channel plays a pivotal role in protecting cardiac and brain cells. Studies on Drosophila melanogaster show that overexpression of this channel correlates with increased lifespan, while its absence leads to enhanced reactive oxygen species (ROS) synthesis and cellular senescence.[3] This study investigates how the loss of BKCa channel function affects superoxide dismutase 2 (SOD2) expression and synthesis during senescence in vascular smooth muscle cells (VSMCs)[1] and U87-MG cells.[2]

**Materials & Methods**: Senescence was induced in VSMCs and U87-MG cells, both with and without functional BKCa channels, using H2O2 and visualized by β-galactosidase activity (SA-β-gal staining). Gene expression was analyzed via q-PCR and RNA

sequencing, while protein levels were examined through Western blotting.

**Results**: In senescent VSMCs, a reduction in BKCa protein was observed, with no concurrent changes at the gene expression level. Additionally, SOD2 expression and protein synthesis were significantly elevated. These findings were compared with results from a deletion line lacking functional BKCa channels.

**Conclusion**: Understanding the impact of BKCa channel loss on ROS production and SOD2 levels could provide valuable insights for the treatment of aging-related diseases.

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