INTERNATIONAL MEETING OF THE ITALIAN GROUP OF BIOMEMBRANES AND BIOENERGETICS (GIBB)

June 8-10, 2023

Riva del Garda Centro Congressi

Parco Lido, 1

Riva del Garda, 38066

Trento (TN)

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Program

Thursday, June 8

TIME	SESSIONS AND PRESENTERS
12:00-13:50 ***On-site registration***	
13:50-14:00 ***Welcome address***	
14:00-14:30	**Plenary Lecture 1 – Leonid Sazanov** *(ISTA, Klosteneuburg, Austria)* **The beginning and the end: complexes I and V of respiratory chain** *Chairperson, Paolo Bernardi*
	Session 1 – Redox reactions and dynamics of energy-transducing membranes (I) *Chairpersons: Alessandro Giuffrè and Paolo Bernardi*
14:30-14:45	**P1. Sebastian Pintscher**, *Assistant Professor (Jagiellonian University)* Putative respiratory supercomplexes in *Sulfolobus acidocaldarius*
14:45-15:00	**P2. Katarzyna Lorencik**, *PhD student (Jagiellonian University)* The routes of electron transfer in respiratory alternative complex III of *Flavobacterium johnsoniae*
15:00-15:15	**P3. Daniel Ken Inaoka**, *Associate Professor (Nagasaki University)* Sulfide:quinone oxidoreductase from *Schistosoma mansoni* forms a long-lived charge transfer complex
15:15-15:30	**P4. Gaia Tioli**, *Postdoc (University of Bologna)* Biochemical and computational approaches to dissect the effect of MT-CYB pathogenic mutations on respiratory chain activity and assembly
15:30-15:45	**P5. Jakub Pagacz**, *PhD student (Jagiellonian University)* Cytochrome bc_1 propensity towards ROS generation: involvement of redox potentials of heme cofactors
15:45-16:00	**P6. Silke Morris**, *Postdoc (University of Muenster)* Mitochondrial Inner Membrane Protein Dynamics in the Senescent Heart
16:00-16:15	**P7. Alba Timón-Gómez**, *Postdoc (Oroboros Instruments)* A reference substrate-uncoupler-inhibitor titration protocol to create a mitochondrial pathway and coupling control database
16:15-16:30	**P8. Mizuki Hayashishita**, *Postdoc (Nagasaki University)* The importance of ATP and quinol oxidase in cell death induced by oligomycin A and antimycin A
16:30-17:00 ***Coffee break***	
	Session 2 - Redox reactions and dynamics of energy-transducing membranes (II) *Chairperson: Karin Nowikovsky and Michela Carraro*
17:00-17:15	**P9. Francesca Giordano**, *PhD student (University "La Sapienza" Roma)* Persulfide dioxygenase from *Pseudomonas aeruginosa* at the crossroad between the bioenergetically-relevant gaseous signaling molecules nitric oxide and hydrogen sulfide
17:15-17:30	**P10. Martina R. Nastasi**, *PhD student (University "La Sapienza" Roma)* The cyanide insensitive oxidase sustains sulfide-resistant respiration and confers nitric oxide resistance to *Pseudomonas aeruginosa*

17:30-17:45	**P11. Mauricio Cárdenas-Rodríguez**, *Postdoc (University of Padova)* OPA1 processing regulates mitochondrial outer-inner membranes contacts and the TIM23 protein import complex
17:45-18:00	**P12. Tiago Branco,** *Postdoc (University of Padova)* Deletion of Mitochondrial Fission Protein 1 in vivo leads to mitochondrial dysfunction and premature death
18:00-18:15	**P13. Federica Vinelli**, *PhD student (University of Padova)* RAP1 inhibition restores mitochondria elongation and lysosomal distribution downstream of Opa1 deletion
18:15-18:30	**P14. Margherita Zamberlan**, *Postdoc (University of Padova)* The small GTPase Rap1 links the mitochondria-shaping protein Opa1 to angiogenesis inhibition
18:30-18:45	**P15. Giada Zanini**, *PhD student (University of Modena and Reggio Emilia)* Novel functions of extramitochondrial forms of LonP1
18:45-19:00	**P16. Tancredi Bin**, *PhD student (University of Bologna)* Use of bioenergetically active particles for studying the interactions of green chemicals with native membranes

Friday, June 9

TIME	SESSIONS AND PRESENTERS
09:00-09:30	**Plenary Lecture 2 - Ambre Bertholet** *(UCLA, Los Angeles, USA)* **The mitochondrial patch-clamp to redefine the mechanism of action of chemical uncouplers** *Chairperson, Cesare Indiveri*
	Session 3 - Mitochondrial channels and transporters (I) *Chairpersons: Cesare Indiveri and Vito De Pinto*
9:30-9:45	**P17. Chiara Brunocilla**, *PhD student (University of Calabria)* In silico study of LAT1 interaction with substrates and inhibitors
9:45-10:00	**P18. Veronica Carpanese**, *PhD student (University of Padova)* BioID-based proteomic analysis of the human volume regulated chloride channel (VRAC) interactome
10:00-10:15	**P19. Deborah Giudice**, *PhD student (University of Calabria)* Overproduction, Purification, and Stability of the Functionally Active Human Carnitine Acetyl Transferase (hCAT)
10:15-10:30	**P20. Giuseppe Battiato**, *PhD student (University of Catania)* VDAC1 knock-out in mammalian cells affects mitochondrial respiration forcing the Complex I activity
10:30-10:45	**P21. Sami E. M. Mohammed**, *PhD student (University of Veterinary Medicine Vienna)* Exploring the pH-sensing mechanism of the mammalian mitochondrial Ca^{2+}/H^{+} exchanger TMBIM5/MICS1
10:45-11:00	**P22. Aurora Maracani**, *PhD student (University of Padova)* The aspartate-glutamate carrier is a mitochondrial metabolic sensor orchestrating mitochondrial morphology and ultrastructure
11:00-11:30 ***Coffee break***	
	Session 4 - Mitochondrial channels and transporters (II) *Chairperson: Valentina Giorgio and Nazzareno Capitanio*
11:30-11:45	**P23. Ludovica Tommasin**, *PhD student (University of Padova)* Assessing the relative contribution of ATP synthase and Adenine Nucleotide Translocator in the mitochondrial permeability transition

11:45-12:00	**P24. Clarissa Gissi**, *Postdoc (University of Udine)* A novel pathogenic mutation in the ATP5MC3 gene of ATP synthase is associated with lysosomal alterations
12:00-12:15	**P25. Elena Frigo**, *PhD student (University of Padova)* Effects of downregulation of subunits e and g of *Drosophila melanogaster* ATP synthase *in vivo*
12:15-12:30	**P26. Martina Grandi**, *Research fellow (University of Bologna)* The IF1 protein binds to the OSCP subunit of ATP synthase and protects cancer cells from apoptosis
12:30-12:45	**P27. Gabriele Coluccino**, *PhD student (University of Udine)* Characterisation of a N-terminal cleaved form of Cyclophilin D: a new player in an old game?
12:45-13:00	**P28. Valentina Pia Muraca**, *PhD student (University of Udine)* Identification of a N-terminal-cleaved form of Cyclophilin D in animal and human tissues
13:00-13:15	**P29. Alessia Nisco**, *PhD student (University of Bari)* Adaptive Flavin Adenine Dinucleotide production to metabolic changes in Pancreatic Ductal Adenocarcinoma
13:15-13:30	**P30. Serena Barile**, *PhD student (University of Bari)* Preliminary characterization of a novel peroxisomal transporter in *Arabidopsis thaliana*
13:30-14:30 ***Lunch***	
14:30-15:00	**Plenary Lecture 3 – Erich Gnaiger** *(University of Innsbruck, Austria)* **Nonlinearity of the proton leak and mitochondrial membrane potential - protonmotive pressure as a unifying concept** *Chairperson, Luigi Palmieri*
	Session 5 - Mitochondria and metabolism *Chairperson: Luca Scorrano and Luigi Palmieri*
15:00-15:15	**P31. Adrianna Budzinska**, *PhD student (Adam Mickiewicz University, Poznan)* Zoledronate and alendronate induce aerobic metabolism adaptations in endothelial cells
15:15-15:30	**P32. Maria A. Desbats**, *Postdoc (University of Padova)* Redundant and divergent roles of COQ8A and COQ8B in cell metabolism
15:30-15:45	**P33. Antigoni Diokmetzidou**, *Postdoc (University of Padova)* Unraveling the role of mitochondria-endoplasmic reticulum contacts in breast cancer progression: targeting metabolic plasticity
15:45-16:00	**P34. Denis Komarov**, *Research fellow (University of Padova)* Investigating amino acid metabolism in neurofibromatosis type 1-related tumours
16:00-16:15	**P35. Francesca Scantamburlo**, *PhD student (University of Padova)* Taming the metabolism of tumor associated macrophages to fight NF1-related tumors
16:15-16:30	**P36. Aristide Ferrante**, *PhD student (University of Foggia)* Characterization of the Metabolic Phenotype and Reliance of Human Osteosarcoma-Derived Stem and Differentiated Cancer cells: is combination of metabolic and chemotherapeutic drugs the best choice?
16:30-17:00 ***Coffee break***	
	S ession 6 - Mitochondria in health and disease (I) *Chairpersons: Elena Forte and Giancarlo Solaini*
17:00-17:15	**P37. Silvia Castagnaro**, *Postdoc (University of Padova)* A mitochondrial therapy for muscular dystrophies

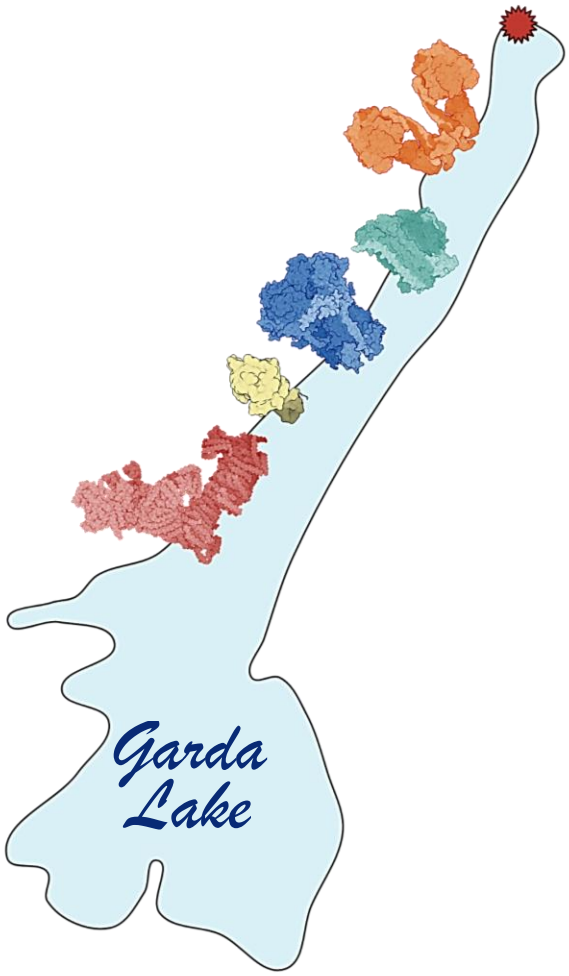
17:15-17:30	**P38. Cristina Calderan**, *Postdoc (University of Padova and Istituto di Ricerca Pediatrica Città della Speranza)* Development of a yeast model to characterize OPA1 mutations associated with different neuromuscular disorders
17:30-17:45	**P39. Andre Djalalvandi**, *Postdoc (University of Padova)* Antagonizing microRNAs that target the mitochondria shaping protein Opa1 ameliorates denervation-induced muscle atrophy
17:45-18:00	**P40. Ana Paula Mendonça**, *Postdoc (University of Padova)* Microparticle sustained delivery of the calcineurin inhibitor FK506 to curtail autophagy and restore vision in an ADOA mouse model
18:00-18:15	**P41. Alice Lacombe**, *Postdoc (University of Padova)* Establishment of a new cell model to identify drugs for Autosomal Dominant Optic Atrophy (ADOA)
18:15-18:30	**P42. Mirko Tamma**, *PhD student (University of Foggia)* iNPC-derived dopaminergic neurons attained from PARK2 mutated patient fibroblasts unveil an impaired interplay between mitochondrial functions and circadian clockwork
18:30-18:45	**P43. Krzysztof Wojcicki**, *PhD student (Adam Mickiewicz University, Poznan)* The effects of statins on the bioenergetic activity of mitochondria isolated from the rat's brain
18:45-19:00	**P44. Maria Laura Matrella**, *PhD student (University of Bari)* Biochemical characterization of functional differentiated SHSY5Y cells as an "in vitro" cellular model in neuroscience research
19:00-19:30 ***Assemblea soci GIBB***	
20:30 Social dinner	

Saturday, June 10

TIME	SESSIONS AND PRESENTERS
09:00-09:30	**Plenary Lecture 4 – Diego De Stefani** *(University of Padova, Italy)* **Molecular control of mitochondrial calcium fluxes** *Chairperson, Annamaria Tonazzi*
	Session 7 - Mitochondria in health and disease (II) *Chairperson: Annamaria Tonazzi and Marcello Pinti*
9:30-9:45	**P45. Martina Semenzato**, *Postdoc (University of Padova)* OPA1 ablation in the heart causes mitochondrial dysfunction and cardiac hypertrophy through inactivation of autophagic process
9:45-10:00	**P46. Hualin Fan**, *PhD student (University of Padova)* The landscape of tether-spacers in mitochondria-endoplasmic reticulum contacts in isoproterenol-induced heart failure
10:00-10:15	**P47. Jonathan Lambert**, *Postdoc (University of Padova)* Performing discovery-based proteomics using a novel Opa1-TurboID stable cell line to determine the metabolically dependent Opa1 protein interactome
10:15-10:30	**P48. Anna Pellattiero**, *Postdoc (University of Padova)* Specific OPA1 inhibitors that enhance apoptotic release of cytochrome c and cell death
10:30-10:45	**P49. Francesca Landini**, *PhD student (University of Foggia)* Bioenergetic Profile and Redox Control during in vitro Osteogenesis of Dental Pulp Stem Cells

10:45-11:00	**P50. Silvia Grillini**, *PhD student (University of Bologna)* The pro-oncogenic protein IF1 does not inhibit ATP synthase physiological activity and confers a proliferative advantage on tumor cells exposed to stress conditions
11:00-11:30 ***Coffee break***	
	Session 8 - Mitochondria in health and disease (III) *Chairperson: Angela Messina and Francesco Francia*
11:30-11:45	**P51. Carlotta Paoli**, *PhD student (University of Padova)* NME4 elevation promotes OPA1 activity to accelerate pancreatic carcinogenesis
11:45-12:00	**P52. Erwan Rivière**, *PhD student (University of Padova)* A genome-wide screening identifies conserved regulators of mitochondrial fission
12:00-12:15	**P53. Stefano Miglietta**, *Postdoc (University of Bologna)* MCJ/DNAJC15 mitochondrial chaperonine increases vulnerability to ferroptosis of chemoresistant ovarian cancer cells
12:15-12:30	**P54. Ludovica B. Zambello**, *PhD student (University of Padova)* A genome wide screening to identify mediators of cellular senescence induced by loss of the mitochondrial fission protein
12:30-12:45	**P55. Keisuke Takeda**, *Postdoc (University of Padova)* Defining a process of mitochondrial quality maintenance based on lateral separation of aggregated proteins
12:45-13:00	**P56. Michela Rosiello**, *PhD student (University of Foggia)* Mitochondria: a time to sleep, a time to wake up
13:00-13:30 ***Awards, closing and remarks (Lunchbox)***	

Abstract Book



Speakers of the Plenary Lectures

Plenary Lecture 1

Leonid Sazanov *(ISTA, Klosteneuburg, Austria)*
The beginning and the end: complexes I and V of respiratory chain

Plenary Lecture 2

Ambre Bertholet *(UCLA, Los Angeles, USA)*
The mitochondrial patch-clamp to redefine the mechanism of action of chemical uncouplers

Plenary Lecture 3

Erich Gnaiger *(University of Innsbruck, Austria)*
Nonlinearity of the proton leak and mitochondrial membrane potential - protonmotive pressure as a unifying concept

Plenary Lecture 4

Diego De Stefani *(University of Padova, Italy)*
Molecular control of mitochondrial calcium fluxes

P1. Putative respiratory supercomplexes in *Sulfolobus acidocaldarius*

Joanna Florek, Sebastian Pintscher

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Sulfolobaceae, a remarkable family within the Archaea domain, inhabit some of the most extreme environments on Earth: the solfataric lakes in volcanically active regions [1]. This study focuses on the unique energy conversion enzymes found in *Sulfolobus acidocaldarius* (DSM 639), which demonstrate both similarities and differences when compared to their eubacterial and mitochondrial counterparts. Three alternate terminal oxidases (SoxB, SoxM, and DoxB) and three cytochrome-ISP complexes ($SoxCL_2$, SoxNL, and SoxGF) were identified in *S. acidocaldarius* [1,2]. Building on recent discoveries of type III_2-IV_2 supercomplex formation in other respiratory systems [3,4], we hypothesize that the *S. acidocaldarius* complexes are organized similarly, resulting in two large dimeric assemblies: $[SoxABCDL_2]_2$ and $[SoxEFGHIM]_2$. In order to elucidate the molecular structure of these enzymatic machineries, we propose employing state-of-the-art biochemical techniques for the isolation of respiratory supercomplexes from native sources, as well as cutting-edge structural biology tools such as AlphaFold and cryo-electron microscopy.

[1] G. Schäfer, M. Engelhard, V. Müller, Bioenergetics of the Archaea, Microbiology and Molecular Biology Reviews. 63 (1999) 570–620.

[2] L.F. Bischof, M.F. Haurat, L. Hoffmann, A. Albersmeier, J. Wolf, A. Neu, T.K. Pham, S.P. Albaum, T. Jakobi, S. Schouten, M. Neumann-Schaal, P.C. Wright, J. Kalinowski, B. Siebers, S.V. Albers, Early response of Sulfolobus acidocaldarius to nutrient limitation, Frontiers in Microbiology. 10 (2019) 1–17.

[3] W.-C. Kao, C. Ortmann de Percin Northumberland, T.C. Cheng, J. Ortiz, A. Durand, O. von Loeffelholz, O. Schilling, M.L. Biniossek, B.P. Klaholz, C. Hunte, Structural basis for safe and efficient energy conversion in a respiratory supercomplex, Nat Commun. 13 (2022) 545.

[4] B. Wiseman, R.G. Nitharwal, O. Fedotovskaya, J. Schäfer, H. Guo, Q. Kuang, S. Benlekbir, D. Sjöstrand, P. Ädelroth, J.L. Rubinstein, P. Brzezinski, M. Högbom, Structure of a functional obligate complex III2IV2 respiratory supercomplex from Mycobacterium smegmatis, Nature Structural and Molecular Biology. 25 (2018) 1128–1136.

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P2. The routes of electron transfer in respiratory alternative complex III of *Flavobacterium johnsoniae*

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Alternative complex III (ACIII) is a membrane complex that catalyses quinol oxidation, the function of enzymes from cytochrome *bc* family. Interestingly, it is evolutionarily unrelated and structurally different from them. The molecular structure of ACIII, resolved by cryo-EM, revealed a supercomplex with aa_3 oxidase and highly intriguing architecture of cofactors suggesting different routes for electrons.

Until now, the lack of genetic system, that allows for ACIII modification, severely limited further mechanistic studies. Our recent work described the first genetic manipulations within ACIII of *Flavobacterium johnsoniae*, opening new possibilities for research on this enzyme. We used this system to delete the heme-containing subunits, ActA and ActE, as the ones involved in the catalytic mechanism.

The enzymatic activity of generated mutants was measured by tracking oxygen consumption in isolated native membranes. The experiment showed lack of cytochrome aa_3 activity only in ActA deletion mutant, but not in ActE mutant. This indicates that ActE is not required for electron transfer between ACIII and cytochrome aa_3.

Another candidate that may act as a linker between ACIII and cytochrome aa_3 is a monoheme mobile domain of ActA (mdA). To verify the proposed role of mdA, we obtained mutant that lacks mdA (ΔmdA) and examined whether and how the absence of this domain alters the activity of the supercomplex. The results showed that in ΔmdA mutant the electron transfer from ACIII to the cytochrome aa_3 does not occur, and the consumption of oxygen by this strain is residual. This confirmed that mdA heme is the sole donor of electrons to aa_3 oxidase.

The results of this work help us to define the electron transfer paths connecting ACIII with aa_3, and provide first insight into the functional organisation of the supercomplex.

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P3. Sulfide: quinone oxidoreductase from *Schistosoma mansoni* forms a long-lived charge transfer complex

Augustin Tshibaka Kabongo[1,2], Talaam Keith Kiplangat[3,4], Yuichi Matsuo[1,5], Acharjee Rajib[3,4], Tetsuo Yamashita[6], Euki Yazaki[6], Endah Dwi Hartuti[1,2], Tetsuro Matsunaga[8], Tomoaki Ida[8], Tomoyoshi Nozaki[9], Takaaki Akaike[8], Shinjiro Hamano[1,2], Kiyoshi Kita[1,9,10] and Daniel Ken Inaoka[1,2,9]

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Intestinal parasites like *Schistosoma mansoni* have evolved to survive in environments with high sulfide levels, but little is known about their sulfide metabolism. In this study, the putative sulfide:quinone oxidoreductase (SmSQOR) of *S. mansoni* was investigated. Purified recombinant SmSQOR could not reduce quinones in the presence of sulfide alone, but required sulfite as a sulfur acceptor. Unlike human SQOR, SmSQOR was not active with glutathione or coenzyme A. SmSQOR also generated sulfane-sulfur, which could be detected using a specific probe (SSP4, Dojindo), only in the presence of both sulfide and quinone. Furthermore, SmSQOR was also active with rhodoquinone, indicating its potential role in hypoxic environments. Purified rSmSQOR was green in color and showed a long-lived charge transfer complex between FAD and sulfane-sulfur, which was stable for months. UV-vis spectra of rSmSQOR showed a stable and long-lived CT complex with a peak at 650 nm, which disappeared after adding SDS and was replaced by a classical FAD spectrum (peak at 450 nm). Moreover, an unusual FAD fluorescence at Ex/Em of 590/664 nm was observed, which was titratable with ubiquinone. We found similar FAD fluorescence in apoptosis-inducing factor, but not to mitochondrial dihydroorotate dehydrogenase. The distinct biochemical features of rSmSQOR suggest that sulfide metabolism in intestinal parasites follows a different pathway to that of mammals, and could be exploited to identify specific inhibitors as potential drug targets.

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P4. Biochemical and computational approaches to dissect the effect of MT-CYB pathogenic mutations on respiratory chain activity and assembly

Gaia Tioli[1], Francesco Musiani[1], Luisa Iommarini[1], Anna Maria Porcelli[1] and Anna Maria Ghelli[1]

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Complex III ($CIII_2$) is a multisubunit enzyme and in its native form is a symmetrical homodimer. $CIII_2$ is central for mitochondrial respiratory chain and is associated with different stoichiometry with Complex I and Complex IV to form supramolecular assemblies, called supercomplexes (SCs). Defects in $CIII_2$ are rare and mostly associated with mutations in MT-CYB gene that encodes for one of the catalytic core subunits, cytochrome b (cyt b). It has been suggested that pathogenic mutations in MT-CYB are mitigated when $CIII_2$ is assembled in SCs [1]. Therefore, we applied biochemical approaches in human cellular models carrying pathogenic point mutations in cyt b to analyse the structural stability and enzymatic activity of $CIII_2$ in its isolated form or assembled in SCs. The results support and strengthen the idea that the deleterious effect of cyt b pathogenic mutations could be mitigated when $CIII_2$ is organized in SCs. Moreover, we applied the Protein Stability Prediction with a Gaussian Network Model (PSP-GNM) approach [2] to evaluate global changes in the unfolding Gibbs free energy change and study the effects of single amino acid mutations on cyt b stability on the available $CIII_2$ structures both in its isolated and bound form. Preliminary results indicate that some pathogenic mutations may affect the unfolding free energy of $CIII_2$, stiffening the structure of the enzyme, in agreement with the reduction of $CIII_2$ activity. This dual experimental and biocomputational approach may be very useful to better understand the effect of these rare pathogenic mutations to design new strategies for possible therapeutic options.

1. M. Rugolo, C. Zanna, A.M. Ghelli, Organization of the Respiratory Supercomplexes in Cells with Defective Complex III: Structural Features and Metabolic Consequences, Life (Basel) 2021 Apr 17;11(4):351

2. S.K. Mishra, PSP-GNM: Predicting Protein Stability Changes upon Point Mutations with a Gaussian Network Model, Int J Mol Sci. 2022 Sep 14;23(18):10711

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P5. Cytochrome bc1 propensity towards ROS generation: involvement of redox potentials of heme cofactors

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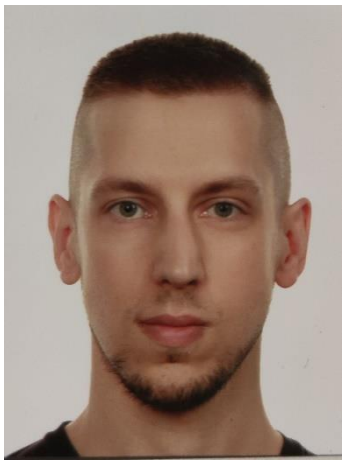
[2]*Department of Plant Biotechnology, Jagiellonian University, Krakow (Poland)*

Cytochrome bc_1 (mitochondrial complex III, CIII) is a key enzymatic complex of the electron transport chain (ETC) linking electron transfer with membranous proton transport in most bacteria and mitochondria. It catalyzes the reduction of cytochrome *c* by ubiquinol which consists of a set of sub reactions operating according to the P. Mitchell's Q-cycle. This reaction engages three active sites: quinol oxidation site (the Q_o site), quinone reduction site (the Q_i site) and cytochrome *c* reduction site (C_c site). Besides, it involves a series of electron transfer reactions occurring within two distinct cofactor chains: the high-potential c-chain (Rieske cluster and heme c_1) and the low-potential b-chain (heme b_L and heme b_H) connecting Q_o – C_c and Q_i – C_c sites, respectively. This reaction when uncompleted can lead to the propagation of side reactions within the system and subsequent generation of reactive oxygen species (ROS). In the present study, we explored electron transfer sequences that were postulated to drive ROS production benefiting from ROS detection method based on a reconstituted hybrid system of bacterial or mitochondrial cytochrome bc_1 coupled to mitochondrial cytochrome *c* oxidase (complex IV, CIV). Thus enabling measurements under non-inhibition. Towards this goal we analyze *R. capsulatus* mutant strains of hemes *b* (with changed redox potential or a knockout) and heme c_1 (mimicking redox potential of its mitochondrial equivalent). With this approach we examine how changes in electron distribution along the cofactor chains impact on ROS production under various conditions. Comparative analysis of ROS generation of different mutational cytochrome bc_1 variants helps us understand the mechanism of ROS formation in CIII, contributing to future developments regarding regulatory functions of ETC complexes and mitochondrial bioenergetic efficiency.



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P6. Mitochondrial Inner Membrane Protein Dynamics in the Senescent Heart

Silke Morris[1], Isidora Molina[2], Gonzalo Barrientos[2], Leonhard Breitsprecher[3], Katherina Psathaki[3], Verónica Eisner[2], Karin Busch[1]

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2: Department of Cellular and Molecular Biology, Pontificia Universidad Católica de Chile, Santiago de Chile (Chile)

3: Center of Cellular Nanoanalytics, University of Osnabrueck, Osnabrueck (Germany)

Heart disease is the leading cause of death in the elderly population and the heart requires large amounts of energy. Energy in the form of ATP is mainly produced in a process called oxidative phosphorylation (OXPHOS) within the inner mitochondrial membrane (IMM). Proteins involved in OXPHOS are highly dynamic. Complexes associated to oligomers or super-complexes may have different functions and are more restricted in their mobility because of their larger size. Another influence on the spatio-temporal dynamics is the IMM ultrastructure. Diffusion within the IMM is restricted by the characteristic cristae structure and complexes may be located in sub-compartments. We asked the question whether IMM protein dynamics change in senescent cardiomyocytes and how this influences mitochondrial function.

As a model system we chose human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) in which we induced senescence by doxorubicin treatment. We could show that senescent cells had functionally compromised mitochondria and saw changes in the IMM ultrastructure, manifested through reduced cristae density and morphological alterations. Fusion and fission were unaffected, although we did see increased IMM fusion velocity. We carried out Tracking and Localization Microscopy (TALM) to study the dynamics of the ATP Synthase. We could show in the past that this enzyme changes its spatio-temporal dynamics depending on its function (ATP synthesis/hydrolysis, structural function as dimers) [1]. In our model we observed a decrease of immobile dimers, which would be involved in cristae stabilization and we saw decreased mobility of the mobile population, which may contribute to mitochondrial dysfunction.

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P7. A reference substrate-uncoupler-inhibitor titration protocol to create a mitochondrial pathway and coupling control database

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Comparative OXPHOS analysis is essential for understanding the physiological implications of tissue- and species-specific respiratory coupling and pathway control patterns. Their analysis is an essential component of the diagnosis of mitochondrial diseases. To address these challenges, we developed a substrate-uncoupler-inhibitor titration reference protocol (SUIT-RP) for measuring metabolic fluxes by high-resolution respirometry. The scope of investigations into mitochondrial respiratory function is limited when using living cells, due to the impermeability of the plasma membrane to certain compounds and mitochondrial preparations are required for OXPHOS analysis.

The SUIT-RP protocol consists of two complementary protocols, RP1 and RP2, that evaluate 20 respiratory states with multiple electron entries feeding the Q-junction and with cross-linked states for harmonization. Defined coupling and pathway states are established in different mitochondrial preparations (isolated mitochondria, tissue homogenate, permeabilized cells). RP1 focuses on NADH-linked linear coupling control (LEAK-OXPHOS-ET), separating coupling control and substrate control. RP2 evaluates the effect of fatty acid oxidation (FAO) in the OXPHOS state. We demonstrated the applicability of SUIT-RP in different tissues (heart, brain, blood), and organisms (human and mouse models).

Importantly, the SUIT-RP protocol was designed to avoid classical artifacts in measuring FAO pathway and Complex IV activity, which have previously confounded research in the field. The SUIT-RP protocol represents a valuable baseline for developing a mitochondrial pathway and coupling control database, which can contribute to a better understanding of the diversity of mitochondrial function and to comparative studies for functional diagnosis in health and disease.

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P8. The importance of ATP and quinol oxidase in cell death induced by oligomycin A and antimycin A

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Trypanosomatids are parasitic protozoa that cause neglected tropical diseases. The blood stream form of *Trypanosoma brucei*, which causes Sleeping sickness, uses trypanosome alternative oxidase (TAO) as the sole terminal oxidase and ATP is synthesized through the acetate:succinate CoA transferase/succinyl-CoA synthase (ASCT/SCS) cycle [1]. Mitochondrial diseases arise from mutations in the components of mitochondrial oxidative phosphorylation (OXPHOS), with unclear mechanisms and no available cure. We engineered trypanosome alternative energy metabolism pathways in HeLa cells, and analyzed the impact of specific inhibitors on cellular respiration and ATP synthesis. TAO or ASCT expressing cells became resistant to Antimycin A or Oligomycin A, respectively, and restored oxygen consumption rate or ATP levels, while control cells died within 6 days. Our findings demonstrate the importance of upstream metabolic pathways in Antimycin A-induced cell death and mitochondrial-ATP homeostasis in Oligomycin A-induced cell death. These results provide a proof-of-concept for the use of non-canonical trypanosomal alternative energy metabolism pathways in HeLa cells as molecular tools to study mitochondrial diseases in detail.

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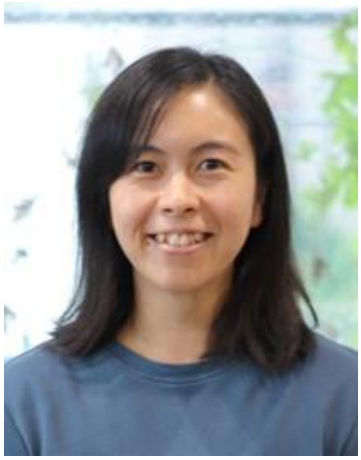

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P9. Persulfide dioxygenase from *Pseudomonas aeruginosa* at the crossroad between the bioenergetically-relevant gaseous signaling molecules nitric oxide and hydrogen sulfide

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Hydrogen sulfide (H_2S), as nitric oxide (NO) and carbon monoxide (CO), is a physiologically relevant, yet potentially toxic, endogenous gaseous modulator of energy metabolism and several other cellular pathways. The role of H_2S in cell physiology, while thoroughly investigated in mammals, is yet to be assessed in bacteria. Unlike Eukaryotes, bacteria are endowed with a H_2S-insensitive respiratory oxidase (cytochrome *bd*) enabling O_2 respiration in H_2S-rich microenvironments [1] and reportedly use endogenous H_2S to defend themselves against antibiotics and oxidative stress [2]. Targeting H_2S metabolism therefore represents a promising antibacterial strategy [3]. In bacteria H_2S is detoxified to sulfite and (thio)sulfate by a multienzymatic unit (located in mitochondria in Eukaryotes), which comprises persulfide dioxygenase (PDO), a non-heme Fe containing enzyme that metabolizes glutathione persulfide and O_2 to reduced glutathione and sulfite. Here, the PDO from the multidrug-resistant pathogen *Pseudomonas aeruginosa* was structurally and functionally characterized by X-ray crystallography and high-resolution respirometry combined with NO amperometry and was unexpectedly found to be potently and reversibly inhibited by NO, suggesting a novel mechanism of crosstalk between H_2S and NO seemingly conserved in the human mitochondrial PDO, named ETHE1.

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P10. The cyanide insensitive oxidase sustains sulfide-resistant respiration and confers nitric oxide resistance to *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa antibiotic resistance represents an increasing threat to global public health as it prevents the effective treatment of chronic infections caused by this opportunistic pathogen. Remarkably, among the bacterial strategies to fight antibiotics-induced stress is the endogenous production of hydrogen sulphide (H_2S), recently recognized as a general protective molecule, which renders multiple prokaryotic species, including *P. aeruginosa*, highly resistant to oxidative stress, host immune responses and antibiotics [1]. Like nitric oxide (NO), H_2S is a potent inhibitor of bacterial O_2 respiration [2]. The highly branched respiratory chain of *P. aeruginosa* has three cytochrome *c* oxidases, caa_3, cbb_3-1 and cbb_3-2, and two quinol oxidases, cytochrome bo_3 and the cyanide-insensitive oxidase (CIO). CIO belongs to the *bd*-type oxidases, enzymes responsible for bacterial adaptation to different stress conditions and thus identified as potential drug target [2]. Here, using *P. aeruginosa* wild-type and its isogenic mutants in one or more terminal oxidases, we found that CIO supports bacterial growth under condition of increased H_2S levels and allows respiration in the presence of high H_2S concentrations. Interestingly, after NO inhibition, CIO fully recovers its O_2 consumption activity more quickly than the other oxidases. The insensitivity of CIO respiration and bacterial growth to H_2S as well as the resistance to NO-induced stress suggest a role of this oxidase in both oxidative and nitrosative stress response in *P. aeruginosa*.

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P11. OPA1 processing regulates mitochondrial outer-inner membranes contacts and the TIM23 protein import complex

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Contacts between the mitochondrial inner (IM) and outer (OM) membranes participate in biogenesis of nascent proteins imported from the cytosol as well as in transduction of signals that need to reach the inner membrane from the extramitochondrial space. Our knowledge of the mediators of IM-OM contacts is scant, besides the roles for the interaction between TOM40 and TIM23 complexes and of the mitochondrial intermembrane space bridging (MIB) complex formed between the SAM and MICOS complexes. Here we show that processing of the mitochondria-shaping protein OPA1 controls the extent of contacts between OM and IM. Reintroduction of a mutant of OPA1 that is not processed into its short (S) form in an Opa1-/- cell line resulted in decreased OM-IM juxtaposition but not in cristae tightness, suggesting that OPA1 processing is required for IM-OM contacts. These ultrastructural changes were paralleled by defects in MICOS, MIB and the TIM23 import complex. This import pathway accounts for the import of most matrix proteins and is essential for mitochondrial biogenesis and respiration. Indeed, growth and respiration of cells lacking S-OPA1 were impaired. Our data indicate a role for OPA1 processing in the stability of the mitochondrial protein import complex TIM23, linking the core mitochondrial dynamics machinery to mitochondrial biogenesis.

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P12. Deletion of Mitochondrial Fission Protein 1 in vivo leads to mitochondrial dysfunction and premature death

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Mitochondrial fission is fundamental for a plethora of cellular processes. Fission protein 1 (Fis1) was identified in yeasts as the first receptor of Drp1, the master regulator of mitochondrial fission. Fis1 was later shown to be dispensable for the division of mitochondria in mammals, where its role remains highly controversial and its relevance in mitochondrial physiology unexplored. We set out to elucidate the physiological roles of Fis1 by generating a hypomorphic mouse model, characterised by a constitutive Fis1 downregulation, and a Fis1-floxed, FIS1flfl, mouse line. Fis1 hypomorphism resulted incompatible with life. Fis1 hypomorphic (FIS1hh) pups die within just two weeks of life, and present mitochondrial ultrastructure and mitochondrial respiration severely compromised. These Fis1hh defunct mitochondria elicit not only a mitochondrial integrative-stress response (ISRmt), with upregulation of serum Fgf21, but also the cGAS-STING pathway. Likewise, depletion of Fis1 from adult life - tamoxifen-induced KO of Fis1 in Fis1flfl mice - leads to weight loss and mitochondrial respiration abnormalities. In vitro, deletion of Fis1 does not compromise mitochondrial division and Drp1 recruitment, revealing that Fis1 is dispensable for mammalian Drp1-mediated fission. We have shown for the first time Fis1 is an essential mammalian protein and that its loss is incompatible with life.

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P13. RAP1 inhibition restores mitochondria elongation and lysosomal distribution downstream of Opa1 deletion

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Changes in core components of the mitochondria-shaping machinery trigger retrograde signaling pathways that remodel gene expression and influence cellular signaling cascades, like angiogenesis that is transcriptionally controlled by Optic Atrophy 1 (Opa1) [1]. However, the nature and the cellular effects of these retrograde signals is largely unknown. Here we show that in endothelial cells the Ras-associated protein-1 (Rap1) signaling cascade activated when Opa1 is deleted regulates mitochondrial morphology. Unbiased RNA sequencing of ECs where we genetically ablated Opa1 revealed activation of the Rap1 small GTPase pathway. Indeed, Rap1 localized in proximity of mitochondria. Surprisingly, Rap1 downregulation in ECs lacking Opa1 restored mitochondrial morphology without affecting the core fission mediator Drp1. Conversely, Rap1 inhibition restored the normal distribution of lysosomes that is instead skewed towards the perinuclear region in cells lacking Opa1. Thus, by exploiting Opa1 deletion as a tool to investigate mitochondria-nucleus retrograde signals we unveiled a hitherto unappreciated role for Rap1 in mitochondrial dynamics, perhaps by modulating mitochondria-lysosome contact sites and lysosomal priming of mitochondrial fission.

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P14. The small GTPase Rap1 links the mitochondria-shaping protein Opa1 to angiogenesis inhibition

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Changes in mitochondrial shape impact cell division, migration, activation, and differentiation across different tissues by inducing changes in gene expression profiles that appear cell type and context dependent. Signals linking mitochondrial morphology to gene expression rewiring are unclear. Here we identify that loss of OPA1 inhibits angiogenesis by engaging the Ras-like GTPase RAP1. RNA sequencing of endothelial cells where OPA1 was downregulated uncovers a Ras-like GTPase RAP1, and its cyclic AMP (cAMP)-activated nucleotide exchange factor EPAC1 signature. EPAC1 and RAP1 localize proximal to mitochondria and upon OPA1 silencing, perimitochondrial cAMP levels increase, EPAC1 is recruited on mitochondria and activated, leading to RAP1 activation that impinges on NFκB to blunt angiogenesis. Genetic or pharmacological EPAC1 and RAP1 inhibition curtails NFκB activation and restores angiogenesis in endothelial cells lacking OPA1. Our results nominate EPAC and RAP1 as rheostats of OPA1 loss in angiogenesis.

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P15. Novel functions of extramitochondrial forms of LonP1

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LonP1 is a mitochondrial protease that degrades oxidized and damaged proteins, assists protein folding, and contributes to the maintenance of mtDNA. LonP1 was believed to be exclusively located in the mitochondria, but we recently detected LonP1 also in the nucleus. Nuclear localization is detectable under all conditions, but the amount is dependent on a response to heat shock (HS). Lonp1 in the nucleus interacts with heat shock factor 1 (HSF1) and modulates the HS response. Silencing of LonP1 enhances HS response, suggesting that LonP1reduces HS response in physiological conditions. Besides the full-length isoform (ISO1), we also identified two other isoforms of LonP1, resulting from alternative splicing: Isoform-2 (ISO2) lacking aa 42-105 and isoform-3 (ISO3) lacking aa 1-196. Public database TSVdb inspection revealed that that ISO1 was upregulated in lung, bladder, prostate, and breast cancer, ISO2 in all the cancer types, and ISO3 did not show significant changes. We found that ISO1 is exclusively mitochondrial, ISO2 is present in the organelle and in the cytoplasm, and ISO3 is exclusively cytoplasmatic. The overexpression of ISO1 and, at a letter extent, of ISO2 enhanced basal, ATP-linked, and maximal respiration without altering the mitochondria number or network, mtDNA amount or mitochondrial dynamics. A higher ECAR was observed in ISO1 and ISO2, overexpressing cells, suggesting an increase in glycolysis. Cells overexpressing the different isoforms did not show a difference in the proliferation rate but showed a great increase in anchorage-independent growth. ISO1 and ISO2, but not ISO3, determined an upregulation of EMT-related proteins, which appeared unrelated to higher mitochondrial ROS production, nor due to the activation of the MEK ERK pathway, but rather to global metabolic reprogramming of cells. Collectively, these findings reveal novel extramitochondrial functions for LonP1 in response to stress and in cancer progression.

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P16. Use of bioenergetically active particles for studying the interactions of *green* chemicals with native membranes

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Ionic liquids (ILs) are salts composed of a combination of both organic and inorganic ions. Their low melting point is due to structural elements that shield the ion charged center and impede the formation of crystals, causing them to be liquid below 100°C. This feature, together with their high ionic conductivity, negligible vapour pressure and low flammability, makes them suitable for replacing canonical volatile organic compounds, with a reduction of industrial activities impact on the environment. Yet, ILs sustainability is still debated. While the overall toxicity against model organisms of a large amount of ILs has been tested, data on their specific effects on biological membranes are almost completely missing. Moreover, the few available data derive from studies performed on liposomes, which give limited information about native biological membranes containing protein complexes. We have chosen to investigate the effect of some ILs on native membranes using chromatophores, photosynthetic vesicles that can be easily isolated from *Rhodobacter capsulatus*. Here, carotenoids associated with the light-harvesting complex II act as endogenous spectral probes of the membrane electrical potential ($\Delta\Psi$). In fact, when a membrane electric field is generated by photoexcitation of the photosynthetic reaction center (RC), the visible spectrum of the carotenoids undergoes an electrochromic shift that responds linearly to the $\Delta\Psi$ amplitude. By measuring the time evolution of the carotenoid band shift induced by a single RC photoexcitation, information on the $\Delta\Psi$ dissipation due to ionic currents across the membrane can be obtained.

We found that some of these compounds cause a rather fast dissipation of the membrane $\Delta\Psi$ at low concentrations, and that this behavior is dose-dependent.

In order to see if such effects occur on a different model of bioenergetically active membranes, experiments on submitochondrial particles are being carried on. Preliminary data will be discussed.

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P17. *In silico* study of LAT1 interaction with substrates and inhibitors

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LAT1 is a membrane transporter crucial for homeostasis because of its role in providing cells with essential amino acids. It is virtually over-expressed in all tumors representing a target for anticancer drug discovery. So far, interaction studies of LAT1 with drugs did not consider that the transporter undergoes at least four different conformations to complete the transport cycle: outward-open, outward-occluded, inward-occluded, and inward-open. The Cryo-EM inward-open and outward-occluded structures were already deposited [1,2], whereas the outward-open and the inward-occluded conformations were built by homology modeling in our study. The models were optimized, the binding site were characterized and docking analysis were performed. The substrates and the inhibitor JPH203 [3] of LAT1 showed a higher affinity for the occluded conformations. Therefore, the two occluded conformations are the major determinants of the substrate affinity. The outward open structure revealed two binding sites, suggesting that the substrate first takes contact to a more external site and then to a central one. These data suggest that the substrate translocation is not due to the movement of the substrate itself but to the conformational changes of the transporter. This highlight that when performing in silico drug design, the conformational issue cannot be neglected.

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P18. BioID-based proteomic analysis of the human volume regulated chloride channel (VRAC) interactome

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The Volume-Regulated Anion Channel (VRAC) is ubiquitously expressed in vertebrate cells. Although its biophysical properties have been well characterized, its molecular identity remained a mystery for three decades. To date, it is known that VRAC comprises the essential LRRC8A subunit, co-assembled with at least one other family member. The role played by VRAC in the cellular context is equally uncertain. In addition to the canonical function that involves it in cell volume regulation mechanisms, new roles have been proposed. Particularly interesting is the putative involvement of VRAC in mediating processes typically associated with carcinogenesis, such as cell proliferation and migration, invasiveness, and resistance to apoptosis. However, studies related to the role of VRAC in cancer are limited and rather debated. To understand the role of VRAC in cellular processes, we studied the interactome LRRC8A through the BioID approach and characterize the phenotype of HCT116 KO cells for LRRC8A, obtained using CRISPR/Cas9 technology, to assess the role of VRAC in cell proliferation, migration, and invasiveness, in the context of colorectal cancer.

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P19. Overproduction, Purification, and Stability of the Functionally Active Human Carnitine Acetyl Transferase (hCAT)

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Carnitine Acetyl Transferase (CAT) catalyzes the reversible transfer of the acetyl-moiety from acetyl-CoA to carnitine, modulating the acetyl-CoA/CoA ratio in mitochondria. Derangement of this ratio leads to metabolic alterations in Diabetes and Fibrosis [1] in which the level of acetyl-CoA increases, affecting glucose disposal, fat metabolism, and hence energy homeostasis. Thus, CAT stimulation might contribute to restoring the correct acetyl-CoA/CoA ratio. Studies on CAT can be performed with the sole commercially available pigeon enzyme. However, the human enzyme differs from the pigeon one, which is not a proper model. Thus, we dedicated our efforts to pointing out a strategy to produce the active recombinant human CAT. The cDNA of hCAT cloned into the pH6EX3 vector was used in combination with the *E. coli* Rosetta strain to produce hCAT. The overexpression of the enzyme was achieved by lowering the IPTG concentration and the growth temperature with respect to conventional expression conditions. The recombinant protein was then purified by Ni-affinity chromatography. A spectrophotometric assay was used to measure the activity of the purified enzyme. hCAT showed comparable activity to that of the commercially available pigeon CAT. Moreover, the protein was stable, retaining its activity for at least two months if stored at -20°C with glycerol. A natural compound, curcumin, identified as a pigeon CAT activator [2], was tested on the hCAT. Curcumin stimulated the activity of the hCAT by more than 45%. The hCAT production is the basis for in vitro screening for identifying new and more potent modulators.

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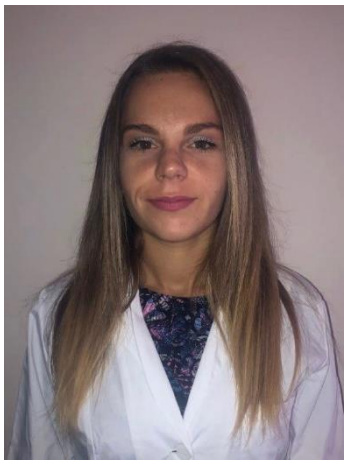

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P20. VDAC1 knock-out in mammalian cells affects mitochondrial respiration forcing the Complex I activity

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Voltage-Dependent Anion-selective Channels isoform 1 (VDAC1) is the most abundant transmembrane β-barrel protein of the outer mitochondrial membrane (OMM) in eukaryotes. VDAC1 is the principal gate for ions, Krebs's cycle intermediates, ATP/ADP, NAD^+/NADH to and from the organelle, and participates in the regulation of apoptosis, calcium and ROS homeostasis [1]. Although the protein is not directly involved in mitochondrial respiration, in yeast *S. cerevisiae* its deletion triggers the inactivation of mitochondrial functions, prompting the cells towards a complete metabolic rewiring [2]. Here, we investigated the impact of VDAC1 knockout on mitochondria by high-resolution respirometry (Oroboros) in the near-haploid human cell line HAP1. Despite the presence of other two VDAC isoforms, knockout of VDAC1 correlated with a drastic impairment of the oxygen consumption in the main respiratory states, and with a re-organization of the relative contributions of the electron transport chain enzymes [3]. Precisely, we observed that complex I-linked respiration (N-pathway) was increased in VDAC1 knockout cells by drawing resources from respiratory reserves [3]. Overall, our data set up VDAC1 as a key regulator of mitochondrial functionality.

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P21. Exploring the pH-sensing mechanism of the mammalian mitochondrial Ca^{2+}/H^{+} exchanger TMBIM5/MICS1

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As mitochondrial Ca^{2+} is crucial for mitochondrial metabolism and life/cell decision, regulation of its homeostasis is an important factor. Mitochondrial Ca^{2+} homeostasis is controlled by uptake and release transporters: mitochondrial Ca^{2+} uniporter complex (MCUC), $Na^{+}/Li^{+}/Ca^{2+}$ exchanger (NCLX), Na^{+}-independent Ca^{2+}/H^{+} exchanger (CHE), which we molecularly identified as Transmembrane BAX inhibitor-1 motif-containing protein 5 (TMBIM5/MICS1). In addition, the mitochondrial permeability transition pore is a critical Ca^{2+} determinant through transient opening.

TMBIM5 belongs to the TMBIM family. Members of this family are conserved regulators of cell death and intracellular Ca^{2+} homeostasis, with TMBIM5 as the only mitochondrial member. The crystal structure of the family founder, BsYetJ suggests a pH-sensitive regulation of Ca^{2+} transport, which is likely mediated by highly conserved amino acid residues.

Having previously demonstrated in intact and permeabilised cells, as well as in vitro assays, that TMBIM5 mediates mitochondrial Ca^{2+} and H^{+} fluxes, we are now exploring whether, similarly to the ancestral bacterial homolog BsYetJ model, it may regulate Ca^{2+} fluxes by a pH-sensitive switch between an open and a closed conformation. The amino acid sequence alignment between BsYetJ and TMBIM5 points to highly conserved residues proposed to regulate the conformational changes. We present a simulation model of TMBIM5 with the proposed residues that likely control Ca^{2+} efflux through protonation and deprotonation. We compare the changes in protein structure and Ca^{2+} passage. We generated TMBIM5 with mutated residues. Using a Ca^{2+} fluorophore and permeabilised cells, in which knockout of TMBIM5 (TMBIM5KO) abolishes CHE and induces PTP opening, we functionally explore the CHE activity and PTP sensitivity in TMBIM5KO that re-express or not TMBIM5WT or the pH-sensitive TMBIM5 mutants.

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P22. The aspartate-glutamate carrier is a mitochondrial metabolic sensor orchestrating mitochondrial morphology and ultrastructure

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Mitochondria respond to cellular changes by undergoing fusion and fission events that control their shape, size, and number inside the cell [1]. However, the molecular mechanisms that link cellular metabolism and mitochondrial dynamics are not clear [2]. Here we show that the mitochondrial aspartate-glutamate carrier (Agc1), part of the malate-aspartate shuttle (MAS) that delivers glycolysis-derived NADH to mitochondria, regulates mitochondrial shape changes in response to different carbon sources. Through a mass spec-based screening of dynamic changes in mitochondrial high molecular weight complexes, we identified Agc1 as a potential interactor of Optic atrophy 1 (Opa1), a mitochondria-shaping protein essential for mitochondrial fusion and cristae biogenesis [3,4]. During starvation, Agc1 dimerization was reduced, whereas Opa1 oligomerization increased and mitochondria were elongated. Agc1 deletion similarly resulted in mitochondrial elongation, but it also caused an increase in cristae width, consistent with the impaired Opa1 oligomerization observed in Agc1$^{-/-}$ cells. Our data suggests that Agc1 might be part of a relay system signaling changes in carbon sources availability to the machinery controlling mitochondrial dynamics and cristae morphology.

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P23. Assessing the relative contribution of ATP synthase and Adenine Nucleotide Translocator in the mitochondrial permeability transition

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The Permeability Transition Pore (PTP) is a Ca^{2+}-activated channel that causes matrix swelling. Among its regulators cyclophilin D and thiol reagents (*e.g.* phenylarsine oxide, PAO) are the most potent activators, while inhibitors are acidic pH and adenine nucleotides. To date, the ATP synthase and the adenine nucleotide translocator (ANT) are the main candidates for PTP formation [1]. To assess their relative contribution we tested the effect of the ANT specific ligands, bongkrekate (BKA) and atractylate (ATR), which are reported to inhibit and activate the PT, respectively [2]. Ca^{2+}-dependent swelling of deenergized mouse liver mitochondria in KSCN at pH 7.4 could not be prevented by BKA, indicating that the ANT does not play a major role in permeabilization. Incubation at pH 6.5 prevented PTP opening by Ca^{2+} [3], yet swelling could be activated by ATR and fully prevented by BKA, suggesting that under these conditions the ANT can form a channel. We suspect that ANT and ATP synthase physically interact to regulate formation of the PTP, as supported by co-migration and co-immunoprecipitation of ANT with specific subunits of ATP synthase.

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P24. A novel pathogenic mutation in the *ATP5MC3* gene of ATP synthase is associated with lysosomal alterations.

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ATP synthase consists of the membranous F_O sector and the extrinsic catalytic F_1 part connected by a central and a peripheral stalk. It is considered a 'genetic hybrid' and in humans consists of 17 subunits encoded by nuclear genes and 2 by mitochondrial genes. Recently, in a 13-year-old patient affected by dystonia, a rare missense mutation was identified in the nuclear gene ATP5MC3 that encodes for the c3 subunit (one of the three paralogues that form the c-ring within F_O). This point mutation generates the substitution N106K in the mature c3 subunit that reduces ATPase activity and basal mitochondrial respiration [1].
In this study, we found that in mitochondria from patient fibroblasts the ATP synthase assembly is altered resulting in an accumulation of subunit c, despite a reduction of the subunit c mRNA levels. Moreover, these mitochondria show a reduced propensity to form the permeability transition pore (PTP) that would generate within the c-ring [2]. Electron microscopy analyses of patient fibroblasts revealed the presence of damaged mitochondria, but also accumulation of aberrant autophagy-lysosome structures. Consistently, the levels of autophagic markers (p62, LC3II, LAMP 1-2) were increased. Since the autophagic flux was not blocked in patient fibroblasts, subunit c accumulation appears associated to a lysosomal dysfunction. Taken together, our results suggest that N106K substitution in subunit c3, in addition to compromise the ATP synthase functions, including its transition to the PTP, affects the degradation of subunit c via lysosomal pathways, thus opening new perspectives for identifying new pharmacological targets to reverse the pathologic phenotype.

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P25. Effects of downregulation of subunits e and g of *Drosophila melanogaster* ATP synthase *in vivo*

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We generated *D. melanogaster* knock-down (KD) lines of mitochondrial ATP synthase subunit e (*ATPsynE*) and g (*ATPsynG*), which are essential for dimerization and oligomerization of the enzyme. Ubiquitous downregulation of either subunit leads to an arrest in development at larval stage, impairs the dimerization and oligomerization states of ATP synthase and decreases mitochondrial respiration, yet the total amount of ATP is unaltered. Strikingly, the sensitivity of the Permeability Transition Pore (PTP) to Ca^{2+} is decreased in mitochondria of both KD lines, indicating a key role of these two subunits in the formation of the *Drosophila* PTP. The PTP is a Ca^{2+}-activated, unselective channel that induces an increase in the permeability of the inner mitochondrial membrane. It has been suggested that PTP formation is mediated by ATP synthase [1]. In fruit fly the "PTP" seems to operate as a selective Ca^{2+}-induced Ca^{2+}-release channel [2]. Consistently, *Drosophila* ATP synthase generates Ca^{2+}-activated channels with a conductance of 53 pS, indicating that the channel is smaller than the mammalian PTP [3]. Our results suggest that the phenotype of subunit e and g KD flies is not entirely due to bioenergetic defects, but may also arise from dysregulated Ca^{2+} homeostasis linked to defective activity of the mitochondrial Ca^{2+}-induced Ca^{2+} release channel.

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P26. The IF_1 protein binds to the OSCP subunit of ATP synthase and protects cancer cells from apoptosis

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The mitochondrial protein IF_1 is the natural inhibitor of the F_1F_o ATPase which binds the catalytic sector F_1 inhibiting the ATP hydrolysis under ischemic conditions. IF_1 is overexpressed in many tumors where it plays a relevant role in promoting cell proliferation and cancer growth under anoxia and normoxia, although the latter mechanism(s) is still debated.

We recently showed that the presence of IF_1 promotes tumor mass development *in vivo* through a zebrafish xenograft model.

To study the role of IF_1 in tumors we performed our experiments in a control HeLa cell line, which naturally expresses high IF_1 levels, and in its IF_1 KO counterpart. Proximity ligation assay and immunoprecipitation experiments showed that IF_1 binds to the ATP synthase OSCP subunit in HeLa cells under oxidative phosphorylation condition, suggesting a new IF_1 binding site.

In this condition, the ablation of *ATPIF1* gene did not affect cell proliferation or oligomycin-sensitive mitochondrial respiration, but sensitized cells to the permeability transition pore (PTP) opening and to the related PTP-dependent cell death.

To elucidate which one of the two IF_1 binding sites, the canonical one in the F_1 sector or the new one on the OSCP subunit, is involved in protecting cells from PTP-dependent apoptosis, we overexpressed both an IF_1 Y33R mutant and the wild-type form of the inhibitor protein in mouse embryonic fibroblasts (MEFs). The overexpression of both IF_1 forms induced a tumor phenotype even in non-tumoral MEFs, through desensitization of PTP opening.

Indeed, the overexpression of the IF_1 Y33R mutant, which does not bind into the F_1 sector, allowed us to figure out the role of the new binding site on the OSCP subunit which desensitizes PTP opening.

In conclusion, our study indicates that IF_1 interacts with a new site on ATP synthase in HeLa cells under oxidative phosphorylation and prevents PTP opening and apoptosis.

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P27. Characterisation of a N-terminal cleaved form of Cyclophilin D: a new player in an old game?

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Cyclophilin D (CyPD), the only mitochondrial Cyclophilin (CyP), is a master regulator of the mitochondrial permeability transition pore (mPTP) able to sensitize the Ca2+-dependent pore opening. Different post-translational modifications of CyPD have been found. However, the role of the irreversible protein cleavage has never been explored.

Biochemical evidence in our lab indicated the presence in various tissues of two forms of CyPD, one ~1 kDa shorter than the other, suggesting the possibility that CyPD could be cleaved at the N-term by a mitochondrial protease.

We decided to produce and characterize the full-length CyPD (FL-CyPD) and a truncated form of CyPD (ΔN14-CyPD), lacking the first 14 amino acids but retaining the catalytic domain. The effect of the two CyPD forms on the activity of ATP synthase, the most promising candidate as mPTP component, was studied. FL-CyPD and ΔN14-CyPD exerted a similar inhibition of the ATPase activity in a classical sucrose-based medium but dramatically different effects in a KCl-based one, which better resembles the physiological environment. Co-immunoprecipitation assay showed a different association of the two CyPD forms to the OSCP subunit of F-ATP Synthase in the two buffers.

The structural characterization of FL-CyPD and ΔN14-CyPD was done using NMR spectroscopy. It indicated that the N-terminus does not affect the overall structure of the two forms of CyPD, which showed the same secondary structure, hydrogen bonding pattern, flexibility, and Cyclosporin A binding mode. We used NMR to assess the effect of KCl and found that both the FL-CyPD and ΔN14-CyPD were affected at the gatekeeper region of the active site, but the effect on FL-CyPD involved a larger patch of residues.

Overall, our data suggest the possible presence of a N -terminal-cleaved form of CyPD that behaves differently than full-length one, which opens new insights on the mPTP regulation.

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P28. Identification of a N-terminal-cleaved form of Cyclophilin D in animal and human tissues

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Cyclophilin D (CyPD) is the unique mitochondrial isoform of the CyP family, a ubiquitous protein family that catalyses the reaction of peptidyl-prolyl cis-trans isomerase (PPIase) on their ligand proteins. CyPD is encoded by the genomic Ppif gene and contains a mitochondrial targeting sequence which is cleaved upon entering the mitochondrial matrix, reducing its size from 22 to 19 kDa. Although the physiological role of CypD remains elusive, CypD has been shown to be a master sensitizer of the permeability transition pore (PTP), a Ca^{2+}-dependent, high-conductance channel whose opening can lead to cell death [1].

Here we report that two forms of the mature CyPD can be identified in various animal and human tissues. Mass spectrometry analysis showed that one form is ~1 kDa shorter at the N-terminus, suggesting that the mature CyPD can be cleaved by a mitochondrial protease and that such modification might affect its ability to regulate the PTP.

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P29. Adaptive Flavin Adenine Dinucleotide production to metabolic changes in Pancreatic Ductal Adenocarcinoma

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Riboflavin (Rf) is an essential dietary component and the precursor of FMN and FAD, the redox enzymatic cofactors required for mitochondrial terminal metabolism, nuclear epigenetics and other cellular processes [1]. Alterations in flavin homeostasis are associated with several pathological conditions, among which cancer [1]. We have recently demonstrated that Rf transporters (RFVTs) expression is profoundly altered in colorectal cancer, and we proposed that cancer cells are greedy for Rf [2]. We investigated here flavin homeostasis in two pancreatic ductal adenocarcinoma cell lines and their derived cancer stem cells (CSCs) with different p53 mutations, PANC-1 carrying the R273H mutation in the oncosuppressor p53 [3], and MiaPaca2 carrying the R248W mutation in p53 [3]. As a control, HPDE cells expressing wt-p53 were used. We found that FAD synthase (EC 2.7.7.2) and RFVT3 expression/activities increased with malignancy and even more with stemness. These increments are presumably demanded by the increase in the levels of both flavoprotein subunit of complex II of the mitochondrial respiratory chain, namely SDHA and the nuclear FAD-dependent lysine demethylase 1. With the aim of proposing FADS as a novel target for cancer therapy, the inhibitory effect of Chicago Sky Blue on FADS enzymatic activity was tested on the recombinant 6His-hFADS2 and on PANC-1 derived CSCs' lysate in the context of a selective reduction of cell proliferation.

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P30. Preliminary characterization of a novel peroxisomal transporter in *Arabidopsis thaliana*

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The genome of *Arabidopsis thaliana* encodes 58 members of the mitochondrial carrier family (MCF) most of which have been functionally characterized in our laboratory. Here, the gene At1g74240 encoding for a protein 364 amino acid length with the characteristic sequence features of MCF, has been preliminary characterized upon overexpression in bacteria and subsequent reconstitution into liposomes. Its transport properties and kinetics parameters indicate that this protein transports arginine and to a lesser extent ornithine, but no other amino acid or metabolite. Transport catalyzed by At1g74240 is saturable and inhibited by common mitochondrial carrier inhibitors. Interestingly the protein catalyzes both uniport and antiport of arginine. Although plant contains two other proteins belonging to the MCF able to transport basic amino acid, named BAC1 and 2, localized in the inner mitochondrial membrane, the product of the At1g74240 gene is localized to peroxisomes and its expression is induced by abiotic stress such as heat and high light. We hypothesize that At1g74240 may play a role in the uptake of arginine into peroxisomes where, in stress conditions, it becomes substrate for a NOS-like enzyme. The NO produced can mediate post-translation modifications (nitration and S-nitrosation) and also act as a signaling molecule inside and outside the peroxisomes increasing stress tolerance caused by abiotic agents.

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P31. Zoledronate and alendronate induce aerobic metabolism adaptations in endothelial cells

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Zoledronate and alendronate are used in anti-resorptive treatment of osteoporosis. Their mechanism of action is to block the mevalonate pathway and inhibit osteoclastmediated bone resorption. One of the products of this pathway is coenzyme Q, an important antioxidant and mitochondrial respiratory chain electron carrier. During intravenous treatment endothelial cells are the first to be exposed to these drugs. We studied a direct effect of two nitrogen-containing bisphosphonates, alendronate and zoledronate on respiratory function and membrane potential of mitochondria isolated from human umbilical vein endothelial cells (EA.hy926 cell line). We proved that both did not adversely affect mitochondrial function (phosphorylating and nonphosphorylating respiration, ADP/O ratio, respiratory control ratio) in vitro at lower concentrations. The addition of zoledronate or alendronate to isolated endothelial mitochondria resulted in disturbances in calcium ion uptake and release, indicating inhibition of the calcium uniporter. Furthermore, the aim of our study was to elucidate the effects of the chronic (6day) exposure of cultured EA.hy926 cells to zoledronate and alendronate on aerobic metabolism at the cellular and mitochondrial levels, thus the effects resulting from inhibition of the mevalonate pathway. Our results indicate that in endothelial cells, 6 days of exposure to bisphosphonates induced a significant lowering of coenzyme Q10 level as well as lowering cell viability in higher concentrations.

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P32. Redundant and divergent roles of COQ8A and COQ8B in cell metabolism

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Coenzyme Q (CoQ) is a key redox and antioxidant molecule widely present in biological membranes, yet its synthesis and regulation are still unclear. In addition, the current knowledge about the specific function of many mitochondrial proteins related to CoQ biosynthesis is limited. COQ8 is a mitochondrial member of the UbiB kinase-like family of proteins with a putative ATPase activity involved in CoQ homeostasis. Coq8 in yeast is required for CoQ biosynthesis supporting the stability of COQ synthesis proteins through an unknown mechanism. COQ8A and COQ8B are two mammalian orthologs of yeast Coq8 that share 50% sequence identity. Intriguingly, mutations to human COQ8A cause cerebellar ataxia while mutations to human COQ8B cause steroid-resistant nephrotic syndrome. In this work, we aim to study the mitochondrial role of COQ8A and COQ8B in human cells. To achieve this, we have generated three different HEK293 knockout cell lines: *COQ8A*$^{-/-}$, *COQ8B*$^{-/-}$ and *COQ8A/B*$^{-/-}$ by CRISPR-Cas9. While *COQ8A*$^{-/-}$ and *COQ8B*$^{-/-}$ cells only presented a mild respiration defect, *COQ8A/B*$^{-/-}$ double knockout cells showed a reduction in CoQ levels and a strong impairment in respiration, Complex III activity and supercomplexes organization. At the transcriptomic level, only COQ8A/B$^{-/-}$ cells showed noticeable alterations in mRNAs profile, such us higher expression of respiratory chain subunits and lower expression of TCA cycle, urea cycle and fatty acids synthesis and degradation related genes. Finally, by metabolomic analysis COQ8A/B$^{-/-}$ cells showed a general metabolic shutdown, but strikingly, COQ8A$^{-/-}$ and COQ8B$^{-/-}$ cells presented some interesting differences related to TCA cycle, Proline cycle and Urea cycle metabolites. Overall, these findings indicate some overlapping and specific roles of COQ8A and COQ8B in human cell metabolism. If these changes may be linked to variable tissue involvement in patients harboring COQ8A and COQ8B mutations will be further explored.

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P33. Unraveling the role of mitochondria-endoplasmic reticulum contacts in breast cancer progression: targeting metabolic plasticity

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Despite considerable therapeutic advances, triple negative breast cancer for which targeted therapies are not suitable remains the deadliest cancer among European women, calling for a deeper understanding of the mechanism by which this breast cancer subtype evades chemotherapy and metastasizes. The specialized subdomain where the ER membranes physically associate to mitochondria, known as mitochondria-endoplasmic reticulum contacts (MERCs), modulates lipid and Ca^{2+} homeostasis, mitochondrial morphology and apoptosis. In breast cancer, expression and localization of several MERCs resident proteins is altered, pointing to the potential role of MERCs in the disease pathogenesis and progression. We therefore performed a high content screening of MERCs using a unique FRET based indicator of ER–mitochondria proximity (FEMP) coupled to the Operetta High Content Imaging System and automated image analysis to quantify MERCs dynamically in a panel of different breast cancer cells and therefore correlate their presence to their cancer biology properties. We found that MERCs are upregulated in triple negative breast cancer cells and the invasive cells of the isogenic MCF10 model of breast cancer progression. These tighter MERCs correlated with a higher oxidative metabolism as determined by Seahorse respirometry, indicating a crosstalk between MERCs and breast cancer metabolism. We therefore studied if MERCs were influenced by the available metabolic substrate. Notably, when we force mitochondrial oxidative metabolism with different substrates in triple negative breast cancer cells, we can reduce MERCs. The plastic nature of MERCs in the different breast cancer cells and their responsiveness to metabolic changes suggests that MERCs can participate in breast cancer progression and that they might participate in the definition of intrinsic properties of the different breast cancer subtypes.

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P34. Investigating amino acid metabolism in neurofibromatosis type 1-related tumours

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Loss of neurofibromin in Schwann cells of individuals with Neurofibromatosis type 1 (NF1) results in the constitutive activation of Ras signaling and onset of neurofibromas along peripheral nerves. Neurofibromas are benign tumors, however they infiltrate into adjacent tissues and can progress to malignant peripheral nerve sheath tumors (MPNSTs), highly invasive cancers for which no effective treatment exists. Tumor progression involves interactions of cancer cells with the surrounding microenvironment and the dynamic production and degradation of components of the extracellular matrix (ECM), such as collagen molecules. A growing number of evidence suggests the existence of a link between the metabolic state of cancer cells and the ECM production and arrangement, and the consequent acquisition of invasive properties. Cancer cells undergo a metabolic rewiring to optimize nutrient usage and promote cell survival, growth and proliferation. We have previously demonstrated that the mitochondrial chaperone TRAP1 is a master regulator of the bioenergetic circuits of NF1 cancer cells. Here, we investigate whether changes in amino acid metabolism sustain MPNST cell invasiveness by tuning ECM deposition, and the role played by TRAP1 in this process. We have observed that i) MPNST cells rapidly decrease glutamine (Gln) levels; ii) Gln availability directly correlates with collagen biosynthesis and invasiveness of MPNST cells; iii) and secretion. TRAP1 interacts with enzymes involved in amino acid pathways upstream collagen biosynthesis; collagen levels are decreased in the absence of TRAP1. Nonetheless, the molecular processes behind TRAP1-dependent regulation of collagen production is yet to be defined. A thorough comprehension of how the amino acid metabolism is regulated, and how it does regulate MPNST cell invasiveness, is required to unveil new targetable liabilities of cancer cells and hinder MPNST progression.

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P35. Taming the metabolism of tumor associated macrophages to fight NF1-related tumors

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Neurofibromatosis type 1 (NF1) is a genetic syndrome caused by germline loss-of-function mutations in Neurofibromin gene. Almost 15% of patients develop malignant peripheral nerve sheath tumors (MPNSTs), which are unresponsive to conventional treatments and rely on the mitochondrial chaperone TRAP1 [1]. MPNSTs are significantly infiltrated by tumor associated macrophages (TAMs), but the molecular crosstalk between these two cell types is lacking [2]. The aim of the project is to investigate the MPNST-TAM communication to identify macrophage-specific metabolic actors sustaining MPNST growth. We use WB and qPCR assays to study MPNSTs ability to drive specific macrophage phenotype acquisition; Boyden chamber and Matrigel assays to investigate the effect of TAM metabolism on MPNSTs migration and 3D growth. We show that MPNSTs induce a significant transition of macrophage toward a metabolic state characterized by ARG1 (Arginase 1), CD206 (Mannose Receptor), GLUL (glutamine synthetase), VEGF-A (Vascular Endothelial Growth Factor) and HIF1α (Hypoxia inducible factor 1α) upregulation. These TAMs sustain *in vitro* MPNST 3D growth and migration, and endothelial cell angiogenesis. Macrophage TRAP1 ablation impairs the pro-tumoral functions of TAMs. Our findings suggest a new crosstalk between MPNSTs and TAMs, in which macrophages exposed to MPNST conditioned media acquire a M2-like metabolic state. This inter-cellular signaling may be crucial in facilitating tumor maintenance and invasion. Inhibition of TRAP1 could be used as a therapeutic strategy to reverse this macrophage mis-education, opening the venue for novel therapeutic opportunities against MPNSTs.

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P36. Characterization of the Metabolic Phenotype and Reliance of Human Osteosarcoma-Derived Stem and Differentiated Cancer cells: is combination of metabolic and chemotherapeutic drugs the best choice?

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Understanding the metabolic profile of cancer stem cells and their differentiated counterparts could be a crucial step toward the development of new and more effective therapies. We characterized the metabolic phenotype of human osteosarcoma-derived 3AB-OS cancer stem cells and the parental MG-63 cells by analyzing their metabolic fluxes through the Seahorse technology under a variety of conditions. We also carried out the real time monitoring of cell growth by impedentiometric measurement and confocal imaging to verify the cellular redox state. The results of our analysis under selective respiratory carbon-source deprivation or pathway inhibition clearly show the reliance of 3AB-OS on glycolysis and of MG-63 on glutamine oxidation. Treatment of both cell lines with cisplatin resulted in an additional inhibition of MG-63 cells growth already deprived of glutamine, while it caused a paradoxical pro-survival effect in 3AB-OS cells starved of glucose, promoting a cell-cycle arrest in S phase and antioxidant outcome therof. This study highlights that the efficacy of specific metabolite starvation combined with chemotherapeutic drugs has not always the desired outcome and depends on cancer phenotype, therefore suggests cautions in using this combined approach as a generalizable therapeutic strategy [1].

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P37. A mitochondrial therapy for muscular dystrophies

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Mitochondrial dysfunction due to inappropriate opening of the permeability transition pore (PTP) plays a key role in the pathogenesis of muscular dystrophies of various origin, as for Duchenne Muscular Dystrophy (DMD) and collagen VI-related myopathies (COL6-RM) [1]. This project focuses on the application of high-affinity, triazole-based small molecule PTP inhibitors (TR001, TR002 and their relative prodrugs) for therapeutical purposes. Efficacy will be tested *in vivo* in two disease models: the *mdx*^{5Cv} mouse lacking dystrophin, a severe DMD model; and the *Col6a1*$^{-/-}$ mouse lacking collagen VI, a well-studied model of COL6-RM. Potential off-target effects will be assessed through a panel of established *in vitro* assays. The triazoles under study are extremely effective in zebrafish models of both DMD [2] and COL6-RM [3] and restore defective respiration in zebrafish DMD model and in myoblasts and myotubes from dystrophic patients [2]. This program aims to provide the proof of principle for a broad applicability of a mitochondria-targeted treatment for muscular dystrophies linked to aberrant PTP activation. Our treatment could also be used for combinatorial approaches with drugs acting on different steps in disease onset and progression.

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P38. Development of a yeast model to characterize *OPA1* mutations associated with different neuromuscular disorders

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Mutations in the *OPA1* gene, encoding a mitochondrial GTPase responsible for mitochondrial fusion and overall functionality, have been associated to autosomal dominant optic atrophy (ADOA) and other severe neuromuscular disorders. Here we report the development of a yeast functional complementation model to characterize O*PA1* mutations and to establish a correlation between *OPA1* genotypes and clinically related phenotypes. Since the human gene couldn't complement the absence of the yeast homolog *MGM1*, we generated a Mgm1-OPA1 chimera in which the OPA1 transmembrane region was replaced by the Mgm1 corresponding region. This construct rescued the growth of a *Δmgm1* yeast on respiratory medium, suggesting that lack of complementation with the human gene is due to a different processing between Mgm1 and OPA1. Hence, we performed the same assay on four ADOA/ADOA+-associated *OPA1* mutations, affecting residues in the GTPase domain (NP_056375.2 p.Gly300Glu, p.Lys301Ala, p.Arg445His) or in the GTPase effector domain (p.Val.910Asp). All mutants failed to rescue the respiratory phenotype; moreover, the GTPase domain mutations exerted a dominant negative effect on the wild type isoform. We also examined four recessive *OPA1* mutations associated with complex neurodegenerative disorders. Even in this case, the effect of each mutation on yeast growth correlated with the phenotype of the patients. The p.Ile382Met mutation, observed in patients in *trans* with a null allele, caused only a mild defect in yeast growth; the p.Ala394Thr mutation, reported in patients in the homozygous state, caused instead a more pronounced respiratory defect. Two novel recessive alleles, p.Arg46His and p.Thr330Pro, found in a child with ataxia and neuropathy, caused disruption of OPA1 function. Lastly, we tested four variants whose pathogenicity has been disputed: p.Gln15Lys, p.Arg38_Ser43del, p.Tyr80Cys, and p.Tyr102Cys. None of them impacted yeast growth, suggesting they are neutral polymorphisms.

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P39. Antagonizing microRNAs that target the mitochondria shaping protein Opa1 ameliorates denervation-induced muscle atrophy

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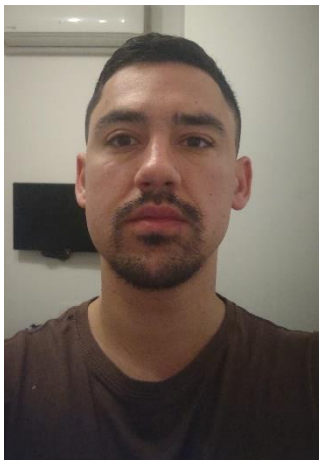
Distorted mitochondrial cristae shape is a pathological hallmark of mitochondrial and disuse myopathies. Genetic overexpression of the master cristae shape regulator Optic atrophy 1 (OPA1) in models of mitochondrial and disuse myopathies ameliorates mitochondrial and muscle function and prolongs lifespan but translating this proof of principle approach into a pharmacological therapy to modulate OPA1 levels remains a challenge. Here we report that antagonising microRNAs (miRNAs) that regulate OPA1 mRNA levels ameliorates a model of disuse myopathy. By bioinformatic approaches we identified that mouse and human *OPA1* is regulated by miRNAs of the 148/152-3p family and by miR-128-3p. By using luciferase sensors, we show that these miRNAs specifically target and regulate mammalian OPA1 levels. Moreover, these OPA1-specific miRNAs are increased upon mitochondrial dysfunction, in complex IV deficient cell lines and in mice undergoing muscle atrophy induced by sciatic nerve denervation. Mechanistically, levels of these miRNAs appear under the control of endoplasmic reticulum stress pathways that are engaged upon mitochondrial dysfunction. Delivery of specific microRNA antagonizers (antagomiRs) increased OPA1 levels and curtailed muscular atrophy induced by denervation in vivo. Our results nominate OPA1-regulating miRNAs as therapeutic targets in mitochondrial myopathies.



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P40. Microparticle sustained delivery of the calcineurin inhibitor FK506 to curtail autophagy and restore vision in an ADOA mouse model

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Autosomal dominant optic atrophy (ADOA) is the most common hereditary neuropathy that affects 1:35.000 people worldwide and for which treatment is not available. Mutations in OPA1 in ADOA patients cause Retinal Ganglion Cells (RGCs) dysfunction leading to blindness. By combining genetics, imaging and in vivo analyses we unveiled a key role for autophagosome and mitochondria accumulation in the soma of RGCs in a mouse model of ADOA that develops visual defects at 4 months of age. Genetic reduction in autophagy corrected mitochondrial distribution and vision in the ADOA mice. Because calcineurin regulates autophagy in RGCs and its inhibition corrects mitochondrial distribution, we decided to harness the potential of FK506, a clinically approved calcineurin inhibitor as a pharmacological therapy to correct ADOA. We therefore efficiently developed PLGA microparticles (diameter=18.40µm) loaded with FK506 (27% w/w) for vitreal injection in the ADOA mouse model. FK506 loaded particles are not toxic, release FK506 over time in vitreal fluid and will be used to investigate whether sustained FK506 exposure curtails excess autophagy and restores mitochondrial distribution and vision in the ADOA mouse.

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P41. Establishment of a new cell model to identify drugs for Autosomal Dominant Optic Atrophy (ADOA)

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Autosomal dominant optic atrophy (ADOA) is a hereditary optic neuropathy characterized by the progressive bilateral loss of vision for which no treatment currently exists. Mutations in the nuclear encoded mitochondrial protein Optic Atrophy 1 (Opa1) are associated with ADOA which affect primarily Retinal Ganglion Cells (RGCs). Upon RGC death, the optic nerve composed of RGC axons degenerates resulting in blindness. The Scorrano lab has demonstrated that RGCs carrying mutated Opa1 display excess autophagy, accumulation of autophagosomes in axonal hillocks and mitochondrial depletion along axons, all associated with loss of vision in an ADOA mouse model. Remarkably, genetic inhibition of autophagy restored both axonal mitochondria distribution and vision in ADOA mice. We hence reasoned that pharmacological inhibition of pathways connecting ADOA mitochondria to autophagy hyperactivation could restore axonal mitochondrial distribution in ADOA RGCs, ultimately interrupting the pathogenetic cascade that leads to blindness. To this end, we seek to perform a high content imaging-based drug screening to identify compounds rescuing axonal mitochondrial content in ADOA RGCs. Such large-scale experiment however highlighted the technical bottleneck that is to work with primary mouse RGCs. Indeed, isolating primary RGC from mice cannot provide the sufficient amount of biological material that is necessary. To meet this demand, we have generated the first immortalized RGC line which will also facilitate our *in vitro* studies overall.

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P42. iNPC-derived dopaminergic neurons attained from PARK2 mutated patient fibroblasts unveil an impaired interplay between mitochondrial functions and circadian clockwork

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Mutations in mitochondrial quality control related gene, like PARK2, have been associated with almost half of the autosomal recessive forms of early onset Parkinson's disease (PD). PARK2 gene encodes for Parkin, an E3 ubiquitin ligase involved in mitophagy-mediated removal of damaged mitochondria. Recent studies point out a link between mitochondrial function and the cell molecular circadian clock [1,2]. The aim of this study was to investigate the interplay between mitochondrial bioenergetics and dynamics and the cell-autonomous circadian clock in fibroblasts obtained from PD patients carrying PARK2 mutation, and from these iPSC-derived, induced Neural Progenitor cells (iNPCs) and iNPCs-derived dopaminergic neurons. We demonstrated that in vitro-synchronized control fibroblasts displayed autonomous rhythmic oscillations of both mitochondrial OxPhos and dynamics. Conversely, in the PD-fibroblasts a robust dampening of these oscillations was observed [3]. A similar scenario emerged in PD iNPCs-derived dopaminergic neurons showing a heavy impairment of OxPhos activity and of its circadian oscillatory profile compared to healthy neurons. Notably, analysis of the core clock genes resulted in deregulated expression patterns in all PD-cells. The findings obtained support a reciprocal interplay between the circadian clock genes machinery and the mitochondrial functions, point to a Parkin-mediated mechanism of regulation and unveil a hitherto unappreciated level of complexity in the pathophysiology of PD. Refs.

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P43. The effects of statins on the bioenergetic activity of mitochondria isolated from the rat's brain

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The aim of this study is to elucidate the direct effects of two commonly used blood cholesterol lowering drugs used to treat cardiovascular diseases, atorvastatin and simvastatin, on respiratory function, membrane potential and reactive oxygen species formation in mitochondria isolated from rat brain. Both hydrophobic statins, the calcium-containing atorvastatin and simvastatin, induced a loss of outer mitochondrial membrane integrity, an increase in hydrogen peroxide formation, and a decrease in maximum (phosphorylating and uncoupled) respiratory rate and membrane potential. In addition, both statins reduced the efficiency of oxidative phosphorylation (coupling parameters: ADP/O ratio and respiration control ratio) in isolated rat brain mitochondria. Changes induced by statins indicate impaired function of brain mitochondria at the level of ATP synthesis and at the level of the respiratory chain, presumably complexes I and III. The effects induced by ATOR appear to be more potent than those induced by simvastatin at a given concentration. The effect of calcium-containing atorvastatin on rat brain mitochondria was highly calcium-dependent and caused disruption of mitochondrial calcium homeostasis. The results indicate that hydrophobic statins that cross the blood-brain barrier, widely used as an anti-atherosclerotic agent, have a direct negative effect on isolated rat brain mitochondria.

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P44. Biochemical characterization of functional differentiated SHSY5Y cells as an *"in vitro"* cellular model in neuroscience research

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Human neuroblastoma SHSY5Y cell line is an important cellular model in neuroscience field. SHSY5Y cells can be differentiated into a neuronal-like phenotype by treatment with various agents, including retinoic acid (RA) [1]. However, treatment with RA results in the generation of a mixed population of differentiated (DCs) and undifferentiated cells (UDCs). Here we optimized a protocol to obtain a homogenous population of neurons, using the cell proliferation inhibitor Floxuridine (FuDr), combined with RA. Upon RA-FuDr-treatment, we detected a proliferation block in UDCs and the exhibition of a morphological phenotype characteristic of neuronal in DCs. The acquisition of neuronal-like properties were confirmed by the expression level of neuronal specific markers, evaluated by real time PCR, and by the measure of functional activity evaluated by whole-cell patch-clamp. To further characterize our model, we performed a lipid analysis by MALDI-TOF/MS, which showed a significant alteration in the cardiolipin (CL) acyl-chain composition, with a higher levels of CL species containing longer fatty acid tails (CL 74:7) in DCs. Since CL acyl chains composition is critical for mitochondrial metabolism [2], we analysed bioenergetic parameters. Using a metabolic fluxes analyzer (SeaHorse), we observed a high coupled and maximal respiration, associated with an increased mitochondrial ATP production in DCs, stating a bioenergetic metabolism based on oxidative phosphorylation (OXPHOS). Since the high OXPHOS capacity has been associated with an increase of cAMP-dependent supercomplex assembly [3], we evaluated cAMP level and supercomplex formation, observing an increased level of cAMP and a rearrangement of the supercomplex assembly in DCs. Overall, this optimised protocol allowed us to obtain a homogenous functional population of neuronal cells characterized by a shift towards a mitochondrial energy phenotype that could be linked to the rearrangement of the supercomplex assembly.

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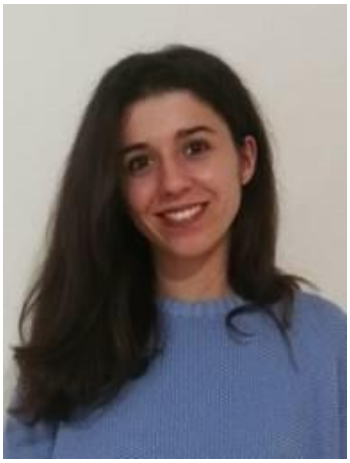

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P45. OPA1 ablation in the heart causes mitochondrial dysfunction and cardiac hypertrophy through inactivation of autophagic process

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Mitochondrial morphological and ultrastructural changes occur during autophagy. In the heart, both inactivation or hyperactivation of autophagy are detrimental for the cardiac homeostasis and whether the mitochondrial cristae biogenesis and fusion protein optic atrophy 1 (OPA1) has a role in this process remains to be elucidated.

Here we show that acute *Opa1* genetic ablation in the mouse heart impairs mitochondrial function and autophagic clearance. Myocardial *Opa1* deletion in adult mice results in cardiac hypertrophy associated with an impaired autophagic process. Mechanistically, we found that in OPA1 cardiac knock-out (OPA1 CKO) mice the autophagic signaling cascade is impaired and, particularly AMPK phosphorylation is inhibited. Further confirming that in OPA1 CKO hypertrophic process was linked to an impaired autophagy, we observed that intermittent fasting lead to a re-activation of the signaling cascade with a clear amelioration of the contractility and a reduction of both cardiac mass and fibrosis.

Our data demonstrate that downregulation of OPA1 plays an important role in mediating the development cardiac hypertrophy and restoration of autophagy attenuates dysfunction in OPA1 CKO hearts.

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P46. The landscape of tether-spacers in mitochondria-endoplasmic reticulum contacts in isoproterenol-induced heart failure

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Mitochondria-endoplasmic reticulum contact sites (MERCs) are emerging as a player in heart failure (HF) progression[1], but their molecular landscape and functional importance remain to be elucidated. We used a FRET-based indicator of ER-mitochondria proximity (FEMP)[2] to inspect mitochondria-ER contact sites in HL-1 cardiomyocytes and confirmed that FEMP could be adenovirally delivered to HL-1 cardiomyocytes where it reliably reports ER-mitochondria proximity. We will use this system, as well as MERCs proteomics and unbiased RNAseq *in vivo* models of transverse aortic constriction and isoproterenol-induced HF to screen for MERCs components altered during the progression of HF. In parallel, we analyzed in silico a GEO dataset (GSE195466) of genes up-or down-regulated in mouse failing hearts and filtered it for mitochondrial localization from the IMPI list. This analysis identified several mitochondrial candidates, including *Ccnb1, Lgals3, Mtfr2*, and *Cdk1*. Among the identified genes, *Lgals3, Rab32*, and *Pdk4* have been reported as the components of MERCs. *Lgals3* and *Rab32* appeared upregulated, whereas *Pdk4* was downregulated in the failing hearts. Our data identify a handful of candidates and delineate an experimental strategy for studying the role of MERCs in HF.

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P47. Performing discovery-based proteomics using a novel Opa1-TurboID stable cell line to determine the metabolically dependent Opa1 protein interactome

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Cristae are dynamic subcompartments of mitochondria involved in respiratory efficiency, control of cytochrome c release and cellular proliferation. Optic Atrophy 1 (OPA1), a dynamin-related IMM protein, is a central regulator of mammalian cristae shape that is retrieved in oligomeric complexes modulated by changes in nutrients and metabolism. However, whether and how OPA1 connects fuel availability to cristae dynamics is unexplored. To address this question we generated an OPA1-TurboID chimera that allows us to study the OPA1 interactome upon metabolic changes. The OPA1-TurboID fusion protein correctly localizes to the inner mitochondrial membrane, facing the intermembrane space and displays biotinylation activity. Once expressed in Opa1-/- cells, OPA1-TurboID restores mitochondrial ultrastructure and fusion, confirming that OPA1-TurboID can vicariate OPA1 and that it can therefore be used in our proteomics workflow to study the molecular determinants of the connection between metabolism and mitochondria ultrastructure. We next immortalized Opa1 fl-fl Murine Embryonic Fibroblasts and used a lentivirus to stably express Opa1-TurboID in our cells. We next cultured our Opa1-TurboID expressing cells in different metabolic conditions (DMEM high glucose, HBSS, Fatty Acids alone, Amino Acids alone, or in a physiologic like cell culture medium called Metablomax) while inducing biotinylation. We next collected protein lysates, trypsin digested the peptides, and performed streptavidin magnetic bead pulldowns to identify biotinylated peptides using liquid chromatography mass spectrometry. Samples are now being run through the LC-MS and we will soon uncover and report the metabolically dependent Opa1 protein interactome.

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P48. Specific OPA1 inhibitors that enhance apoptotic release of cytochrome c and cell death

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The GTPase activity of the dynamin-related mitochondrial protein Optic Atrophy 1 (OPA1) controls cristae remodeling, cytochrome c release and apoptosis. OPA1 is hence being increasingly identified as a vulnerability of cancer cells. From a high throughput screening of 10,000 drug-like compounds for OPA1 GTPase inhibition, we identified MYLS22 as the most promising hit. This compound is not mitochondriotoxic, but it increases cytochrome c release in response to proapoptotic stimuli. MYLS22 causes mitochondrial fragmentation, cristae remodeling and it sensitizes a panel of cancer cells to drug treatment. In vitro, MYLS22 binds recombinant OPA1 GTPase and did not inhibit recombinant Dynamin 1 GTPase activity. In cells, it does not display any additional effect over OPA1 deletion, further substantiating its specificity. Structural activity relationship (SAR) analysis of the OPA1 inhibitor turned out into a series of MYLS22 derivatives with improved water solubility. Furthermore, three of these compounds exhibit significantly enhanced inhibitory effects on OPA1 GTPase activity *in vitro*, as well as mitochondrial fragmentation ability. In conclusion, MYLS22 and its derivatives are the first-in-kind specific OPA1 inhibitors that exert anti-cancer properties.

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P49. Bioenergetic Profile and Redox Control during in vitro Osteogenesis of Dental Pulp Stem Cells

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Human dental pulp stem cells (hDPSCs) are an easily accessible source of stem cells suitable for regenerative medicine applications. For achieving their therapeutic potential, it is necessary to retain their stemness properties *in vitro*. Redox signaling and energy metabolism are known to be involved in controlling the balance between self-renewal and proliferation/differentiation of stem cells [1]. In this study we investigated metabolic and redox changes occurring during in *vitro* hDPSCs osteoblast (OB) differentiation and tested on them the impact of the antioxidant Trolox.

Following induction of OB differentiation, we observed a significant down-regulation of the expression of the mitochondrial respiratory chain complexes since the early phase of the process and a reduction of the basal intracellular peroxide level in the late phase of differentiation. The former finding was functionally confirmed evaluating the metabolic fluxes by the Seahorse technology, that in addition to decreased OCR resulted also in dampened glycolysis-related ECAR. These observations indicated relevant changes in the metabolic profile of OB differentiating hDPSCs unexpectedly characterized by a lower energy-generating phenotype.

Trolox treatment markedly delayed OB differentiation of hDPSCs assessed as ALP activity, mineralization capacity, expression of stemness and osteoblast marker genes (Nanog, Lin28, Dspp, Ocn, Runx2) and activation of ERK1/2. In addition, the antioxidant partly prevented the inhibitory effect on cell metabolism observed following OB induction, intriguingly the efficacy of Trolox treatment depended on the time-window of its administration highlighting the early-middle phases of the process as important in regulating expansion/differentiation of HDPSCs.

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P50. The pro-oncogenic protein IF_1 does not inhibit ATP synthase physiological activity and confers a proliferative advantage on tumor cells exposed to stress conditions

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Many cancer cells overexpress IF_1, the endogenous protein that inhibits the hydrolytic activity of ATP synthase when the mitochondrial membrane potential ($\Delta\mu_{H+}$) falls, as occurs in the anoxic areas of solid tumors [1]. IF_1 has also been shown to be involved in mitochondrial cristae structure organization and resistance to apoptosis [2]. Here, we exploited three different cancer cell models: human cervical carcinoma (HeLa), osteosarcoma (143B) and colon carcinoma (HCT116) expressing different levels of IF_1. We generated IF_1-silenced clones for each cell model to characterize their bioenergetics and investigate IF_1 mechanisms of action. We analyzed oligomycin-sensitive ATP synthesis rate, oxygen consumption rate and mitochondrial membrane potential. Overall our results establish that IF_1 does not affect the ATP synthesis rate (OXPHOS) in cancer cells. Moreover, we have investigated the capability of IF_1 to promote survival and proliferation in cells exposed to uncoupling conditions. We exposed IF_1-silenced and parental cells to FCCP that collapsed $\Delta\mu_{H+}$. Under this condition, IF_1-expressing cells showed a higher energy charge than IF_1-knockdown cells and presented an increased mitophagy balanced by mitochondrial biogenesis. After washing out the uncoupler, the IF_1 expressing cells proliferate at a higher rate than related IF_1-knochdown clones. In conclusion, our study demonstrates that IF_1 does not inhibit the physiological function of ATP synthase, but by inhibiting its ATP hydrolytic activity it confers a proliferative advantage to cancer cells exposed to stress conditions.

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P51. NME4 elevation promotes OPA1 activity to accelerate pancreatic carcinogenesis

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Understanding how mitochondrial function and morphology impact tumor progression could unravel new vulnerabilities of malignant cells, allowing the development of novel therapeutical approaches. In this context, PDA is one of the deadliest cancers worldwide and it is projected to be the second highest contributor to cancer-related deaths by 2030. To dissect the contribution of mitochondria to pancreatic cancer progression, we combined organelle immunopurification with mass spectrometry to examine changes in the mitochondrial proteome of human PDA cell lines. We found the upregulation of mitochondrial proteins involved in nucleotide synthesis and processing. In particular, NME4 is a nucleotide diphosphate kinase involved in the regulation of mitochondrial morphology and able to increase GTPase activity of the master regulator of cristae shape and biogenesis, OPA1. In line, mitochondrial cristae width significantly decreases during pancreatic cancer progression, as observed in multiple human cell lines and in mice expressing oncogenic KRAS in the pancreas (KC mice). Notably, OPA1 overexpression in KC mice accelerates pancreatic cancer progression, causing the formation of neoplastic lesions with high grade dysplasia. This phenotype is associated with an increased cellular proliferation and histone acetylation, both in the normal pancreas and during pancreatic cancer progression. This work documents dynamic changes of mitochondrial ultrastructure during carcinogenesis, defines molecular underpinnings and suggests the possibility that OPA1 promotes tumor progression through the regulation of the epigenome.

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P52. A genome-wide screening identifies conserved regulators of mitochondrial fission

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Mitochondrial fission is crucial for mitochondrial function, apoptosis, mitophagy, and mitochondrial segregation during mitosis. While core mitochondrial fission factors have been elucidated and characterized, it is unclear if additional molecules participate or are main players of the fission process. To solve this question, we setup genome wide, high content imaging (HCI) screenings to identify suppressors of mitochondrial fragmentation in mouse embryonic fibroblasts (MEFs) deficient for the crucial fusion effector Optic atrophy 1 (Opa1), and in human fibroblasts carrying the inactivating OPA1^{S545R} mutation to generate a list of hits conserved across higher and lower mammals. In proof of principle experiments, ablation of the key fission effector Drp1 in Opa1$^{-/-}$ MEFs restored mitochondrial elongation, independently of any observable mitochondrial fusion. A HCI screening in Opa1$^{-/-}$ MEFs transfected with pooled siRNAs targeting >19,000 genes identified 1679 genes that restored mitochondrial elongation. Bioinformatic analysis revealed that the potential hits were enriched for genes annotated as ER, Golgi, cytoplasmic vesicles, cytoskeleton and mitochondria. Indeed, ~10% of these hits were predicted as mitochondrial based on an Integrated Mitochondrial Protein Index (IMPI) score >0.7. Orthogonal assays validated that two candidates, one retrieved in both screenings and a second one found only in the screening performed in MEFs, modulate mitochondrial fission by acting at different steps of the mitochondrial fission process.

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P53. MCJ/DNAJC15 mitochondrial chaperonine increases vulnerability to ferroptosis of chemoresistant ovarian cancer cells

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Ovarian cancer (OC) is the most lethal gynecological neoplasm due to its extremely silent invasive capacity, characterized by high mortality frequently caused by therapeutic failure and chemoresistance onset. Recent studies have indicated that the chemotherapy response can be affected by metabolic status and mitochondrial bioenergetic efficiency, that regulates ferroptosis susceptibility enhancing reactive oxygen species levels and lipid peroxidation. Emerging insights aim to connect ferroptosis activation with OC growth and chemoresistance occurrence. Interestingly, the OC therapeutic resistance has been associated with the silencing of *DNAJC15* gene that encodes for the mitochondrial co-chaperonine MCJ, a negative regulator of the electron transport chain (ETC) function. Hence, the main aim of this study is to evaluate MCJ as modulator of both ETC function and chemoresistance. Our data indicated that the MCJ overexpression is able to enhance the cells respiratory capacity, increase the sensitivity to cisplatin and reduce the *in vitro* tumorigenic properties of chemoresistant OC cells. Further, the MCJ overexpression impacts on the glutathione-associated antioxidant defense with ferroptosis activation in cisplatin-resistant cells. Overall, we speculate that the MCJ overexpression may modulate the mitochondrial metabolism, increasing oxidative stress and vulnerability to ferroptosis, which in turn leads to the reduction of both cisplatin-resistance and tumorigenicity of OC cells. The dissection of these mechanisms may unveil new molecular players and insights for this silent killer disease.

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P54. A genome wide screening to identify mediators of cellular senescence induced by loss of the mitochondrial fission protein Fis1

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Senescence is a complex stress response state of the cell, mainly characterized by a stable cell cycle arrest in G1/G2 phase and a relevant secretome release (a.k.a. Senescence Associated Secretory Phenotype) [1]. Mitochondria are dynamic intracellular organelles and key players in many aspects of cell biology. This functional versatility is tightly coupled to constant reshaping of the mitochondrial network by fusion and fission (division) events [2]. Mitochondria are also key players in cellular senescence: in senescent cells, the highly interconnected and elongated mitochondria because of low expression of the fission proteins Drp1 and Fis1 [4,5] display low ATP production [1,3]. Additionally, downregulation of Fis1 leads to extensive mitochondrial elongation and senescence. Altogether these data represent a proof of concept of a direct correlation between sustained mitochondrial elongation and senescence induction, but the underlying mechanism is unknown. To address this point, we decided to perform a genome-wide RNAi screening based on high content imaging, to identify genes essential for senescence induced by loss of Fis1. We will present data on the establishment of the screening pipeline using $Fis1^{flx/flx}$ mouse adult fibroblasts (MAFs) where genetic deletion of Fis1 induces accumulation of the beta galactosidase marker of senescence that can be readily imaged. Our screening is expected to highlight the connection between mitochondrial dynamics and the establishment of senescence.

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P55. Defining a process of mitochondrial quality maintenance based on lateral separation of aggregated proteins

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Activation of mitochondrial fusion and fission is one of the countermeasures in response to mitochondrial dysfunction. Fusion allows functional complementation and neutralization by mixing the content between damaged and healthy mitochondria. Fission helps the segregation of abnormal content by asymmetric division of individual mitochondria, which is generating mitochondrial fragments that can be degraded by autophagy. However, the molecular mechanism underlying intra-mitochondrial sorting during mitochondrial fission is still unknown. Here, we generated cell models visualizing the dynamics of intra-mitochondrial unfolded proteins to clarify how the distributions of unfolded and folded proteins are controlled. Live tracking of mitochondrial fission using our cell model revealed that soluble protein and unfolded proteins can be selectively loaded into each of daughter mitochondria. Moreover, the characterization of single mitochondrion including aggregates of unfolded protein suggested that mitochondrion was impaired in different manners, bioenergetics or oxidative, depending on the sub-mitochondrial localization of the unfolded protein, IMS or Matrix, respectively. Our visualized models of mitochondrial IMS/Matrix unfolded proteins are expected to bring not only further understandings of the molecular mechanisms underlying intra-mitochondrial sorting but also findings of pathophysiological threat to mitochondrial proteostasis.

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P56. Mitochondria: a time to sleep, a time to wake up

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Regulation of metabolism is emerging as a major output of circadian clock circuitry in mammals. Accordingly, mitochondrial oxidative metabolism undergoes both in vivo and in vitro daily oscillatory activities. In previous studies we showed that both glycolysis and mitochondrial oxygen consumption displayed a similar time-resolved rhythmic activity in synchronized HepG2 cell cultures, which translated in overall bioenergetic changes documented by measurement of the ATP level [1]. Moreover, we also demonstrated that pyruvate was a major source of reducing equivalents to the respiratory chain and that its oxidation was driven by the mtCa^{2+}-mediated rhythmic reversible phosphorylation of the pyruvate dehydrogenase complex [2].

Evaluation of the mRNA transcription of mtDNA encoded genes as well as of mitochondrial transcription factors underwent oscillatory profile in synchronized HepG2 cells. However, the absence of significative changes in the OxPhos components and syncronous variations of the mitophagy-promoting factors suggest a coordinated linkage between mitochondrial biogenesis and organelle quality control. This conclusion was also supported by assessment of mitochondrial dynamics parameters.

Intriguingly, silencing of Bmal1, a master regulator of the canonical circadian core-clock system, dampened only partially the above-reported oscillatory profiles. All together our findings unveil a hitherto unexplored complex interplay between mitochondria physiology and multiple daily clockwork networks [3].

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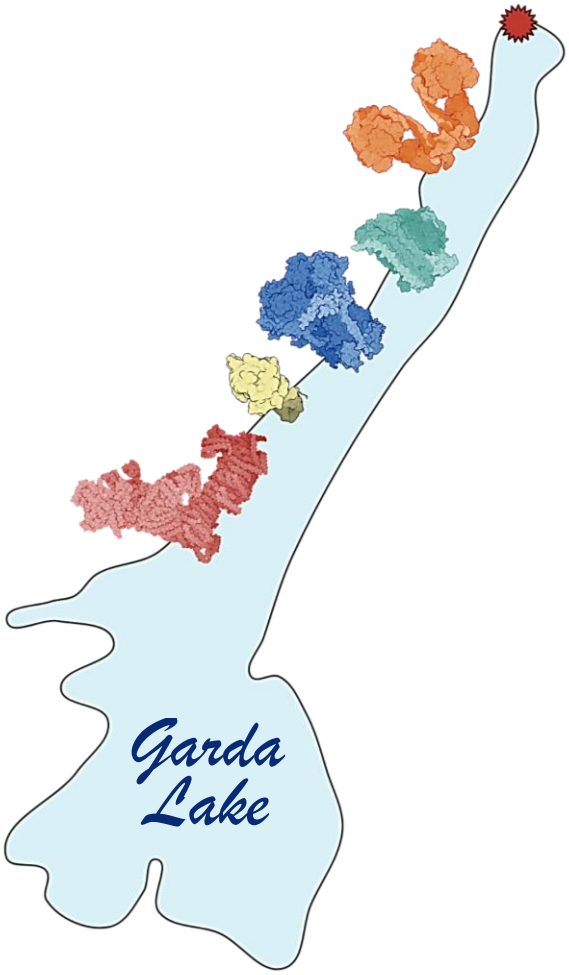

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Magrin Federico

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Messina Angela

Miglietta Stefano

Mohammed Sami Elamin Mustafa

Mollica Maria Pina

Morris Silke

Muraca Valentina Pia

Nastasi Martina Roberta

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Schiavone Marco

Scorrano Luca

Scrima Rosella

Semenzato Martina

Seydel Anke

Shinjo Satoko

Signorile Anna

Solaini Giancarlo

Sollazzo Manuela

Takeda Keisuke

Tamma Mirko

Timon-Gomez Alba

Tioli Gaia

Tommasin Ludovica

Tonazzi Annamaria

Trevisan Elena

Vinelli Federica

Wojcicki Krzysztof

Zambello Ludovica

Zamberlan Margherita

Zancani Marco

Zanini Giada

Zanna Claudia

Thank you