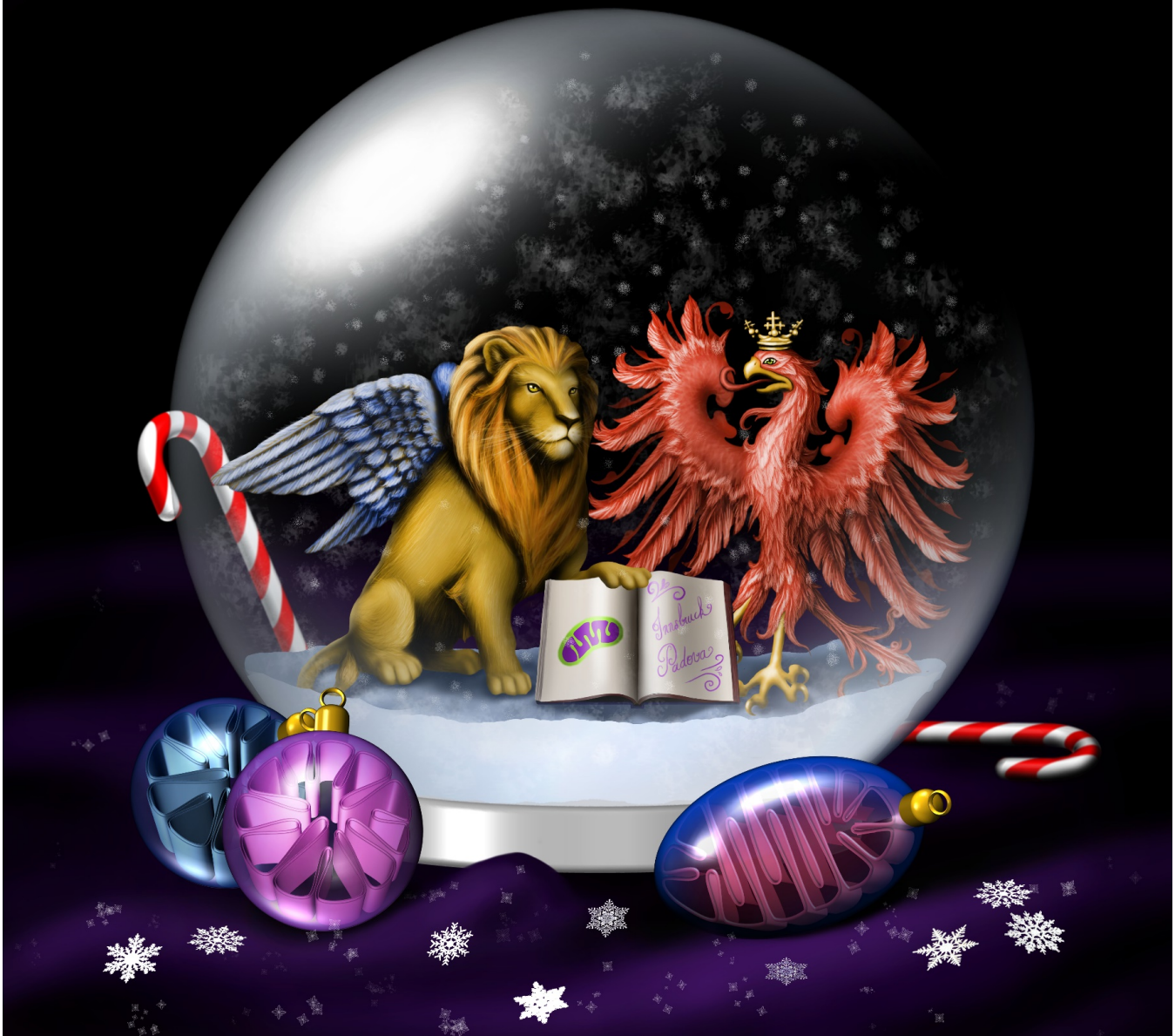
Joined symposium on mitochondrial function of the Universities of Padua and of Innsbruck

Mitochondrial Function in Health and Disease

- Abstract Book -



16th-17th December 2016

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- About the meeting -

First joint meeting of the universities of Padua and Innsbruck on
"Mitochondrial Function in Health and Disease"
16.12.2016-17.12.2016 in Innsbruck

Keynote Lectures

Elena Ziviani: "To eat or not to eat: mitophagy in ageing and neurodegeneration"
Erich Gnaiger: "The World as a Laboratory: Exploring Mitochondrial Fitness"
Maria Eugenia Soriano: "Exploring the role of OPA1-MIC60 containing complexes in the regulation of cristae and cristae junctions biogenesis"
Marco Sandri: "Mitochondrial quality control in muscle physiology and ageing"
Pidder Jansen-Duerr: "Mitochondria and aging"

Host Professors

Christoph Schwarzer
Francesco Ferraguti

Organization, Design and Abstract Book

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http://www.bioblast.at/index.php/Mito_Xmas_Meeting_2016_Innsbruck_AT

- Program -

Day 1 - Friday, 16.12.2016

13.45-14.00	Arrival and registration
14.00-14.15	Introduction - Francesco Ferraguti
Keynote 1	*Chairs: Vincenzo Ciminale, Helmut Klocker*
14.15-14.45	The World as a Laboratory: Exploring Mitochondrial Fitness - Erich Gnaiger
Session I: Mitochondria in Cancer	*Chairs: Vincenzo Ciminale, Helmut Klocker*
14.45-15.00	Direct pharmacological targeting of a mitochondrial ion channel selectively kills melanoma cells *in vivo* - Luigi Leanza
15.00-15.15	Increased Opa1 levels aggravate B cell lymphoma - Dijana Samardzic
15.15-15.30	Exploring the role of mitochondrial dynamics in melanocytes and melanomas - Akiko Omori
15.30-17.00	Coffee Break & **Poster Session (A)**
Keynote 2	*Chairs: Ildikò Szabò , Dominik Pesta*
17.00-17.30	To eat or not to eat: mitophagy in ageing and neurodegeneration - Elena Ziviani
Session II: Methods on Mitochondrial Membranes	*Chairs: Ildikò Szabò , Doninik Pesta*
17.30-17.45	Methods for evaluation of mitochondrial membrane potential with multi-sensor high-resolution respirometry: potentiometry vs fluorometry - Zuzana Sumbalova
17.45-18.00	The impact of mitochondrial phospholipid remodeling on mitochondrial function - Markus Keller
18.00-18.15	Cyclophilin D stabilizes F_OF_1-ATPase dimers and cristae shape to modulate permeability transition pore - Ruben Quintana-Cabrera
18.15-19.30	Christmas Market
19.30	Dinner

Day 2 - Saturday, 17.12.2016

Keynote 3 *Chairs: Keiko Iwata, Nadia Stefanova*

9.15-9.45	Mitochondria and aging - Pidder Jansen-Duerr

Session III: Mitochondria in Aging and in the Brain *Chairs: Keiko Iwata, Sinead Rooney*

9.45-10.00	Prevention of mitochondrial oxidative damage: Novel insights into the activation of the pro-oxidant and pro-death function of p66Shc - Jakob Troppmair
10.00-10.15	The mitochondria and cristae shaping protein Opa1 impinges on fat browning to control insulin sensitivity - Camilla Bean
10.15-10.30	MicroRNAs as modulators of mitochondrial remodeling and apoptosis - Francesca Grespi
10.30-10.45	Determination of mitochondrial copy number as versatile tool in epidemiology - Federica Fazzini
10.45-11.00	Involvement of mitophagy in the elimination of damaged mitochondria during the process of UVB-induced senescence - Maria Cavinato

Keynote 4 *Chairs: Vanina Romanello, Michael Ausserlechner*

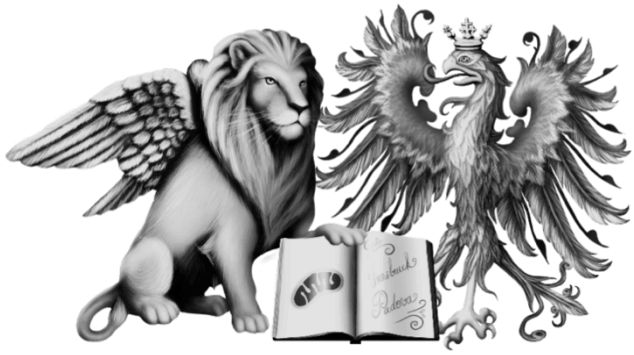
11.00-11.30	Mitochondrial quality control in muscle physiology and ageing - Marco Sandri
11.30-13.00	Buffet & **Poster session (B)**

Session IV: Mitochondria in Blood and Heart *Chairs: Vanina Romanello, Michael Ausserlechner*

13.00-13.15	The mitochondrial shaping protein Optic Atrophy 1 (OPA1) controls angiogenesis- Stephanie Herkenne
13.15-13.30	Searching specific targets of ischemic damage of cardiac mitochondria using O2k-Fluorometry- Carolina Doerrier
13.30-13.45	Effects of systemic iron perturbations on mitochondrial activity and on cellular metabolism *in vivo* - Chiara Volani
13.45-14.00	Mitochondrial reactive oxygen species prime T-cell acute lymphoblastic leukemia to cell - Gloria Scattolin
14.00-14.15	mTORC inhibition increases ROS levels and induces cell death in T-ALL cells - Micol Silic-Benussi

Keynote 5 *Chairs: Vanina Romanello, Michael Ausserlechner*

14.15-14.45	Role of Mic60 and OPA1 complexes in the formation of CJ - Maria-Eugenia Soriano
14.45-15.00	Conclusions & Departure *Christoph Schwarzer*



~ Keynote Lectures ~

Keynote 1

The World as a Laboratory: Exploring Mitochondrial Fitness

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A variety of lifestyles developed in human populations to cope with the environmental and socioeconomic conditions in the inhabited areas of our world. Extremes at high altitude and latitude impose stress conditions which require adjustments in physiological performance or limit permanent settlements. Modern strength and endurance training regimes may be closely linked to a variety of traditional life styles. Diversity is nature's treasure and the subject of comparative physiology [1].

The Polar Inuit of Thule and Qaarnaak in Greenland are among the northernmost populations. This human heritage of a culture and physiological type is endangered not only by a historical politically forced limitation of their territory, but by the current effects of global environmental pollution and climate change, causing social destabilization and a shift towards an unhealthy sedentary in contrast to the traditional active life style of Inuit hunters.

The uncoupling hypothesis for mitochondrial haplogroups of arctic populations suggests that lower coupling of mitochondrial respiration to ATP production was selected for in favour of higher heat dissipation as an adaptation to cold climates through a higher mitochondrial proton leak [2]. Our studies show that mitochondrial coupling control in skeletal muscle of Inuit haplogroups is identical to Danes from western Europe haplogroups, such that biochemical coupling efficiency was preserved across variations in muscle fibre type and lifestyle [3].

Unexpectedly, total capacity of oxidative phosphorylation (OXPHOS) in the leg of the Inuit hunters was lower compared to untrained Danes. In line with this apparent 'mitochondrial paradox', total OXPHOS capacity decreased in the Danes during 42 days of active skiing on the sea ice in northern Greenland. The Inuit had a higher capacity to oxidize fat substrate in skeletal muscle which increased in Danes approaching the level of the Inuit. A common pattern emerges of mitochondrial acclimatization and evolutionary adaptation in humans at high latitude and high altitude [3-4]: In these environments, economy of locomotion is optimized by preservation of biochemical coupling efficiency at modest mitochondrial density, when V_{O2max} and sustained submaximum performance are not dependent on peripherally increased capacities of oxidative phosphorylation.

Contribution to K-Regio project MitoFit and Horizon 2020 COST Action MITOEAGLE.

1 Johansen K (1987) The August Krogh Lecture: The world as a laboratory. Physiological insights from nature's experiments. In: Advances in physiological research (McLennan H, Ledsome JR, McIntosh CHS, Jones DR, eds). Plenum Publishing Corporation:377-96
2 Ruiz-Pesini E et al (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303: 223-226.
3 Gnaiger E et al (2015) Mitochondrial coupling and capacity of oxidative phosphorylation in skeletal muscle of Inuit and caucasians in the arctic winter. Scand J Med Sci Sports 25 (Suppl 4):126–34.
4 Marconi C, Marzorati M, Cerretelli P (2006) Work capacity of permanent residents of high altitude. High Alt Med Biol 7:105-115.

Keynote 2

Playing hide and seek: Inhibition of the Parkin-antagonizing deubiquitinase USP8 corrects a *Drosophila* PINK1 mutant model of Parkinson's disease

von Stockum S(1,2), Sanchez-Martinez A(3,4), Nardin A(1), Chakraborty J(1), Schrepfer E(1,5), Ferrari V(1), Da Rè C(1,6), Cusumano P(1,6), Costa R(1,6), Bubacco L(1), Whitworth AJ(3,4), Scorrano L(1,5), Ziviani E(1,2)
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During mitophagy, impaired in genetic Parkinson's disease (PD), the kinase PINK1 recruits the E3 ubiquitin ligase Parkin to damaged mitochondria. Increased Parkin levels correct PINK1 deficient *Drosophila* PD models, suggesting that targeting the Parkin-opposing deubiquitination enzymes (DUBs) can yield the same result. Here we show that genetic and pharmacological inhibition of the Parkin-opposing DUB USP8 rescues a *Drosophila* model of PD. A targeted RNA interference screening for DUBs antagonizing Parkin ubiquitination of its key mitochondrial target Mitofusin (MFN) identified six DUBs among which USP8 caught our attention. Biochemical and functional analyses indicated that USP8 impinges on mitochondria dysfunction via Parkin. *In vivo*, genetic or pharmacological inhibition of USP8 corrected mitochondrial dysfunction and locomotor performance in a *Drosophila* PINK1 mutant model of PD, by impinging on the numerous MFN functions that in the fruit fly include both promotion of fusion and ER-mitochondria crosstalk. Our data identify a novel therapeutic target antagonizing pathologic symptoms in a model of PD.

Keynote 3

Mitochondria and aging
Jansen-Duerr P

Keynote 4

Mitochondrial quality control in muscle physiology and ageing

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2 Venetian Institute of Molecular Medicine, 35129 Padova, Italy.

The cellular basis of age-related tissue deterioration remains largely obscure. The ability to activate compensatory mechanisms in response to environmental stress is an important factor for survival and maintenance of cellular functions. Autophagy is activated both under short and prolonged stress and is required to clear the cell of dysfunctional organelles and altered proteins. The removal of mitochondria via mitophagy requires an efficient mitochondrial shaping machinery. We report that autophagy and mitochondrial dynamics in muscles declines with ageing and their inhibition correlates with age-dependent muscle loss and weakness. Specific autophagy inhibition in muscle has a major impact on muscle strength, ultimately affecting the lifespan of animals. Inhibition of autophagy also exacerbates aging phenotypes in muscle, such as mitochondrial dysfunction, oxidative stress, and profound weakness. Mitochondrial dysfunction and oxidative stress directly affect acto-myosin interaction and force generation. Inhibition of mitochondrial fission or fusion inhibits or enhances mitophagy, respectively. Both fusion and fission when specifically blocked in muscles shorten life span of animals. Therefore, mitochondrial quality control is critical both for muscle function and when impaired it systemically reverberates to whole organism affecting animal health. .

Keynote 5

Exploring the role of OPA1-MIC60 containing complexes in the regulation of cristae and cristae junctions biogenesis

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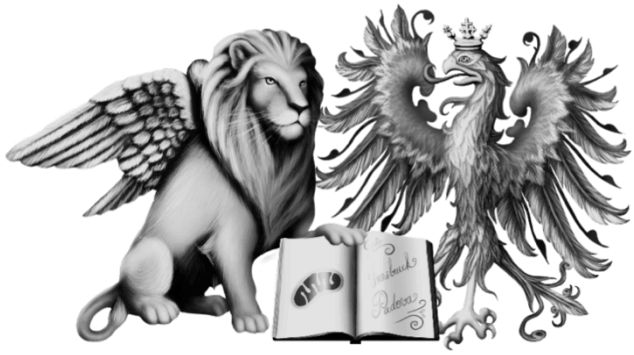
The mitochondrial cristae and cristae-inner boundary membrane junctions (CJ) architecture are controlled by the mitochondrial contact site and cristae organizing system (MICOS) and by Optic atrophy 1 (OPA1), but whether and how they interplay is unknown. Proteomics, native gel electrophoresis and immunoprecipitation indicates that the MICOS component MIC60 and OPA1 physically interact in mitochondrial high molecular weight complexes targeted during cristae remodeling. A combination of genetic epistatic analysis, electron microscopy and tomography, biochemistry and physiology places OPA1 upstream of MIC60 in the control of CJ number and stability, whereas OPA1 defines cristae and CJ width independently of MIC60. Accordingly, MIC60 does not control apoptotic cristae remodeling, cytochrome c redistribution and mitochondrial apoptosis. We provide a unifying model for mammalian cristae biogenesis where OPA1 independently specifies width of cristae and CJ, and MIC60 requires OPA1 to define CJ number and stability.



- Oral Presentations -

Session I: Mitochondria in Cancer

Direct pharmacological targeting of a mitochondrial ion channel selectively kills melanoma cells *in vivo*

Leanza L(1), Romio M(2), Becker KA(3), Azzolini M(4,5), Venturini E(3), Mattarei A(2), Carraretto L(1), Urbani A(1), , Kadow S(3), Biasutto L(4,5), Zoratti M(4,5), Gulbins E(3,8), Paradisi C(2), Szabo I(1,5)

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Mitochondria are important oncological targets due to their crucial role in apoptosis. Our work identifies a novel therapeutic tool that simultaneously exploits both the high expression of the potassium channel Kv1.3 in the mitochondria of various types of cancer cells and the characteristic altered redox state of malignant cells, thereby leading to the selective elimination of even chemoresistant pathological cells by two mitochondria-targeted Kv1.3 inhibitors. Here we show that direct inhibition of Kv1.3 by two novel, mitochondria-targeted drugs alters mitochondrial function and leads to ROS-mediated death of even chemoresistant cells and independently of p53 status. In orthotopic mouse models of melanoma the compounds reduced tumor size by more than 90%. Instead, treatment of the animals with an antioxidant prevented the in vivo effect of the drugs. Our work thus provides direct evidence that specific pharmacological targeting of a mitochondrial channel can lead to ROS-mediated selective apoptosis of cancer cells in vivo, without causing significant side effects. Indeed, the strong tumor-reducing effects observed in melanoma and pancreatic ductal adenocarcinoma preclinical models are not accompanied by immune-depression, cardiac toxicity or histological alteration of healthy tissues. These findings thus offer the perspective of a major advance in the pharmacological treatment of melanoma.

Session I: Mitochondria in Cancer

Increased Opa1 levels aggravate B cell lymphoma

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2 Dept of Biology, Univ. of Padova, Padova, Italy
3 Dept of Cancer biology, Dana – Farber Cancer Institute, Boston, MA, USA

Mitochondria are key organelles that amplify apoptosis. Deregulation of apoptosis is a typical hallmark of cancer. Changes in the shape and in the ultrastructure of the organelle contribute to the progression of apoptosis. They are controlled by a family of proteins that include, amongst others, the inner membrane GTPase Opa1. When cells express mutated inactive Opa1 they are more susceptible to apoptosis, whereas cellular and animal models of Opa1 overexpression display a resistance to apoptosis.
We aimed to elucidate what is the role of Opa1 in the acquisition and maintenance of the cancer phenotype, in cellular (DLBCL), and animal lymphoma models (Eμ-myc / Opa1^{tg} animals).
BCR vs. OxPhos DLBCL cell subsets differ in the overall ratio between long and short Opa1 forms. The relative balance between these forms is necessary for Opa1 function, and here we show that the balance between these forms is maintained in the OxPhos subset, whereas in the BCR subset short forms accumulate. Immunological, histopathological and survival analysis of mice revealed that Eμ-myc / Opa1^{tg} mice have a shorter life span and develop a stronger tumorigenic phenotype compared to Eμ-myc mice over time.
Here we present evidence that mitochondrial morphology, metabolism, and ultrastructure are different between the BCR and the OxPhos DLBCL subsets that display different levels of Opa1. We also show evidence that overexpression of Opa1 is contributing to the development of cancer in Eμ-Myc transgenic animals. Our data indicates a role for Opa1 in DLBCL features, and tumor progression in vivo.

Session I: Mitochondria in Cancer

Exploring the role of mitochondrial dynamics in melanocytes and melanomas

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Melanin, the pigment that colors the skin, hair and eyes, is made by cells called melanocytes. Melanin serves multiple purposes, one of which is skin protection against UV irradiation (1). However, melanocytes can become origins of melanoma, the most aggressive type of skin cancer with high mortality. Despite recent advances in cancer research, there are only few effective treatments for melanoma. It has been shown that aging symptoms, such as hair graying, are caused by defective self-maintenance of melanocyte stem cells (MSC). In fact, Bcl2 deficiency causes selective apoptosis in the MSC and accelerates hair graying. The anti-apoptotic Bcl2 protein suppresses cytchorome c release from mitochondrial cristae (2,3). These reports suggest that mitochondria play important roles for melanocyte/MSC survival.
Mitochondrial fission and fusion (mitochondrial dynamics) play critical roles in maintaining functional mitochondria. Mitochondrial dynamics are regulated by ubiquitously expressed Dynamin-related GTPases. Dynamin related protein1 mediates fission, while Mitofusin 1 and 2 in the outer mitochondrial membrane and Optic atrophy (Opa1) mediate fusion. However, the involvement of mitochondrial dynamics on melanocyte regulation and growth of melanoma is poorly known
Despite the well-known role of Bcl2, the anti apoptotic role of Opa1, which keeps cristae structures tight and suppresses cytochrome c release, is less well established in melanocytes and melanoma. Remarkably, we found a significant increase of Opa1 in several cancer melanoma cell lines. We also observed the gray hair syndrome in melanocyte specific Opa1 mutants. These data suggest important functions of Opa1 in melanocytes and highlights a new potential therapeutic target for melanoma.

1 Schneider MR et al. The hair follicle as a dynamic miniorgan. 10;19(3):R132-42. 2009 Curr Biol.
2 Sorriano ME et al. The interplay between BCL-2 family proteins and mitochondrial morphology in the regulation of apoptosis. 687:97-114. 2010 Adv Exp Med Biol.
3 Nishimura EK et al. Mechanisms of hair Graying: Incomplete Melanocyte Stem Cell Maintenance in the Niche. 37. 2005 Science.

Methods for evaluation of mitochondrial membrane potential with multi-sensor high-resolution respirometry: potentiometry vs fluorometry

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3 Department of Visceral, Transplant and Thoracic Surgery, Daniel Swarovski Research Laboratory, Medical University Innsbruck, Austria

Under physiological conditions mitochondrial (mt) membrane potential ($\Delta\Psi_{mt}$) is maintained within a healthy range compatible with major mt functions [1]. In pathophysiological states, elevated values of $\Delta\Psi_{mt}$ may be associated with increased production of reactive oxygen species, while diminished $\Delta\Psi_{mt}$ values compromise mt ATP generation and Ca^{2+} retention capacity. Absolute values of $\Delta\Psi_{mt}$ depend on available substrates and the prevailing coupling state of a mt preparation [2]. Details of the interrelationship between respiratory states and $\Delta\Psi_{mt}$ can be studied using high-resolution respirometry (HRR) combined with potentiometric or fluorometric detection of $\Delta\Psi_{mt}$.

Methods for measurement of $\Delta\Psi_{mt}$ depend on the addition of a reporter ion. For the potentiometric approach, the tetraphenylphosphonium ion (TPP^+) is frequently used, which can be readily detected using an ion sensitive electrode system (OROBOROS ISE-Module). For fluorometric detection of $\Delta\Psi_{mt}$, fluorescent dyes Safranin [3] or TMRM [4] can be applied with detection of their fluorescence by the OROBOROS O2k-Fluo LED2-Module. Unfortunately, at commonly applied concentrations of 1.5 - 2 µM all these probes interfere with mt respiration to some extent. Inhibition by TPP^+ was found to be the lowest (< 3%) among these three dyes in mouse brain mitochondria. TPP^+ could be successfully used for the simultaneous measurement of respiration and $\Delta\Psi_{mt}$ with NADH-linked substrates (N) and N combined with succinate (NS) [5], whereas limited sensitivity of potentiometric method at low $\Delta\Psi_{mt}$ rendered it unsuitable in combination with succinate (S). In comparison, fluorometric methods using TMRM or Safranin appeared more sensitive in the range of low $\Delta\Psi_{mt}$, but at the cost of considerable inhibition of mt respiration particularly when employed with N-linked substrates (~30 %).

From the potentiometric TPP^+ experiments absolute values of $\Delta\Psi_{mt}$ [mV] can be calculated, since the signal of the TPP^+ electrode corresponds to the concentration of free TPP^+ outside the mitochondria. In contrast, the fluorescence signal obtained with Safranin or TMRM consists of a mixture of the signal from free and bound probe and thus cannot be considered as a defined dye concentration convertible to mV. For future applications a method can be established for transformation of the fluorescence signal to $\Delta\Psi_{mt}$ for each type of mitochondria and protein concentration, which has to be kept constant in any set of experiments.

Both fluorometric and potentiometric measurements can be a valuable tool to distinguish differences in $\Delta\Psi_{mt}$ between experimental groups. Optimization of the experimental approach is possible by selecting the fluorometric or potentiometric approach and corresponding dye according to the specific questions to be addressed and the tissue- and species-specific mitochondrial properties.

Supported by Action Austria-Slovakia (SZ) and K-Regio project MitoFit (GE).

1 Nicholls DG. Simultaneous monitoring of ionophore- and inhibitor-mediated plasma and mitochondrial membrane potential changes in cultured neurons. J Biol Chem. 2006 May 26;281(21):14864-74.
2 Sumbalova Z, Fasching M, Gnaiger E (2011) Substrate control in mitochondrial respiration and regulation of mitochondrial membrane potential. Abstract Mitochondrial Medicine Chicago.
http://wiki.oroboros.at/index.php/Sumbalova_2011_Abstract_Mitochondrial_Medicine
3 Krumschnabel G, Eigentler A, Fasching M, Gnaiger E (2014) Use of safranin for the assessment of mitochondrial membrane potential by high-resolution respirometry and fluorometry. Methods Enzymol 542:163-81.
4 Scaduto RC Jr, Grotyohann LW (1999) Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. Biophys J 76:469-77.
5 Sumbalova Z, Fasching M, Gnaiger E (2012) Evaluation of mitochondrial respiration and membrane potential in mouse brain homogenate. Mitochondr Physiol Network 17.12:61 http://wiki.oroboros.at/index.php/Sumbalova_2012_Abstract_Bioblast

parameters, and consequently to elucidate the largely uncharacterized pathobiochemistry of these diseases.

Session II: Methods on Mitochondrial Membranes

The impact of mitochondrial phospholipid remodeling on mitochondrial function

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Mitochondria are highly organized into the outer mitochondrial membrane and the extensively folded cristae structure of the inner mitochondrial membrane (IMM). These membranes are vital for providing the embedding environment of membrane bound complexes such as the respiratory chain and the mitochondrial protein import machinery. Main components of mitochondrial membranes are glycerophospholipids, among which the mitochondria specific cardiolipins are abundant with up to 20% in the IMM.

Cardiolipins represent unique lipids with a glycerol bridged diphosphatidylglycerol backbone that can be substituted with up to four acyl chains. The generated wedge like structure is optimal for facilitating the curved cristae, while the backbone acts as proton trap and buffers pH fluctuations generated in mitochondria.

The biosynthesis of cardiolipins is facilitated in a multi-step, multi-compartment manner, initiated from phosphatidic acid in the endoplasmic reticulum and finally forming cardiolipins in the IMM. As the biosynthesis enzymes are unspecific in respect to the acyl side chains of their substrates, a post-biosynthetic side-chain remodeling process is required to generate a mature and functional cardiolipin profile.

Patients with impaired cardiolipin remodeling process are for example suffering from the x-linked Barth Syndrome, or the MEGDEL syndrome. In affected individuals the impaired membrane assembly causes mitochondrial dysfunctions resulting in common symptoms such as 3-methylglutaconic aciduria, cardiomyopathy, and muscle weakness.

We recently developed a lipidomics platform that allows us to comprehensively quantify alterations in respective mitochondrial lipid compositions, to connect these data with mitochondrial functional parameters, and consequently to elucidate the largely uncharacterized pathobiochemistry of these diseases.

Cyclophilin D stabilizes F_0F_1-ATPase dimers and cristae shape to modulate permeability transition pore

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The permeability transition pore (PTP) represents a major trigger of mitochondrial-mediated cell death, accounting for necrosis and tissue damage subsequent to a wide spectrum of insults, such as cardiac or brain ischemia. Although F_0F_1-ATPase dimers have been recently proposed as a core constituent of PTP, its complete molecular identity and interactors are still under debate. However, there is wide consensus on the role of cyclophilin D (CyPD) as a positive PT modulator, yet the mechanism by which it regulates PTP remains obscure.
Here we provide evidence that the matrix protein CyPD stabilizes ATPase dimers in mitochondria. Upon genetic or pharmacological interference with CyPD, F_0F_1-ATPase dimers are loss, preventing PT opening. As a result of reduced ATPase dimerization cristae collapse and mitochondrial bioenergetics is compromised, as revealed by a higher susceptibility to membrane potential loss and matrix acidification upon complex III blockage.
In sum, CyPD stabilization of F_0F_1-ATPase dimers determines mitochondrial cristae structure and bioenergetics, favoring PTP opening in stress conditions. Importantly, our findings unveil a structural role for CyPD in the formation of PTP, challenging novel approaches in the prevention of PTP-mediated cell death.

Session III: Mitochondria in Aging and in the Brain

Prevention of mitochondrial oxidative damage: Novel insights into the activation of the pro-oxidant and pro-death function of p66Shc

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Excessive production of reactive oxygen species (ROS) has been causally linked to cell death resulting in loss of cognitive or organ function. Diseases range from neurodegeneration, stroke, diabetes, to ischemia/ reperfusion injury (IRI). Antioxidants failed in the clinical setting. Also the direct inhibition of mitochondrial and non-mitochondrial ROS producing systems is not clinically feasible. Among those sources p66Shc is unique as its knockout did not affect normal development while it prevented or ameliorated ROS-induced pathophysiological changes. p66Shc normally resides in the cytoplasm. Previous work suggested that activation of the pro-oxidant and pro-death function of p66Shc required phosphorylation on serine 36 (S36) followed by mitochondrial import and PKCβ has been proposed as S36 kinase. Due to the lack of inhibitors of its oxidoreductase function we pursue a strategy to inhibit p66Shc by interfering with its upstream activation. To this end we initiated a detailed analysis of the mechanisms controlling p66Shc activity and function.

We confirmed the requirement of PKCβ for ROS production and cell death but not for p66ShcS36 phosphorylation. The search for a bona Prevention of oxidative damage fide S36 kinase lead to JNK1/2, whose involvement was confirmed through the use of inhibitors and JNK1/2-deficient cells. Moreover, expression of a S36E mutant in p66Shc-deficient rescued ROS production. Additionally, we identified S139, T206 and S213 as the critical PKCβ target sites regulating the pro-oxidant and pro-death function of p66Shc. JNK1/2 and PKCß are normally activated under cellular stress and targeting them offers a novel therapeutic approach to prevent diseases associated with excessive ROS production.

The mitochondria and cristae shaping protein Opa1 impinges on fat browning to control insulin sensitivity

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Mitochondria-shaping proteins modulate bioenergetics, apoptosis, Ca2+ signalling and autophagy. The inner membrane pro-fusion and cristae shaping protein Optic atrophy 1 (Opa1) protects multiple tissues from damage by regulating cytochrome c release and mitochondrial respiratory efficiency, but whether this is mirrored by systemic changes in intermediary metabolism is unknown. Here we identify Opa1 as a key regulator of insulin sensitivity and adipose tissue function. Controlled Opa1 overexpression in the mouse reduces weight, improves glucose metabolism and insulin sensitivity, by reducing fat depots and favoring brownization of white adipose cells in vivo and in vitro. Adipocyte-specific Opa1 deletion triggers a lipodystrophic phenotype with hyperglycemia, insulin resistance, brown adipose tissue whitening and hepatosteatosis. Our findings identify the genetic and metabolic basis for Opa1 role in a lean and insulin-sensitive phenotype, paving the way for novel therapeutic strategies to treat obesity and diabetes.

Session III: Mitochondria in Aging and in the Brain

MicroRNAs as modulators of mitochondrial remodeling and apoptosis

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Opa1 is a dynamin-related protein of the inner mitochondrial membrane mediating fusion and cristae remodeling, a key mechanism in apoptosis. Indeed, Opa1 is mutated in Autosomal Dominant Optic Atrophy (ADOA) a genetic disorder characterized by a high degree of phenotypic variability. Nevertheless, triggers determining the severity of the disease are still unknown. Intriguingly, emerging evidence highlight a link between mitochondrial fusion/fission and microRNAs, well-known epigenetic regulators. This suggests that miRNAs might participate in modulation of penetrance of diseases affecting mitochondrial shape. Accordingly, we performed an in silico screening to identify miRNAs putatively targeting the 3'UTR of Opa1. Our preliminary data demonstrate that specific miRNAs can directly modulate Opa1 levels, thus influencing mitochondrial shape and apoptosis. We identified specific cell death triggers inducing miRNAs expression, indicating that candidates are important at the beginning of the apoptotic cascade. For this reason, we hypothesize that the differential penetrance of ADOA is mediated by miRNAs and this feature can be exploited therapeutically. Indeed, our project is proposing an innovative mechanism of modulating ADOA penetrance that has never been investigated before. We believe that increasing levels of Opa1, even if mutated, by modulation of miRNAs by AntagomiRs, could ameliorate the phenotype of ADOA patients. In conclusion, our study could provide new potential therapeutic targets for a currently untreatable disease.
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Determination of mitochondrial copy number as versatile tool in epidemiology

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Alterations of mitochondrial DNA (mtDNA) copy number appear to be associated with several pathologies including encephalopathies and neuropathies as well as the process of aging [1-2].
The aim of this study was to set up a reliable quantitative PCR based assay for mitochondrial DNA copy number determination meeting quality requirements for mtDNA specificity.
We established a duplex quantitative PCR assay that allows for targeting a single copy nuclear gene (ß2-microglobulin) and the mtDNA (t-RNA Leu) simultaneously. The use of a plasmid containing both targets in a 1:1 ratio was used to normalize against differences in emission intensities of the fluorescent dyes VIC and FAM.
QPCR on the serial dilution of the calibrator plasmid revealed that the FAM dye emission signal exceeded the VIC signal, resulting in a ΔCT value of up to 1.2 cycles corresponding to more than a double amount of molecules. Using the plasmid calibrator with internal positive controls reduced the intra-assay variability from 21% (uncorrected) to 7% (plasmid corrected). We evaluated the applicability of the method by using DNA samples that were isolated with different methods and revealed significantly different numbers of mtDNA copies (copy number ratio: salting out/magnetic beads = 1.65).
We developed a sensitive and robust assay for mitochondrial copy number detection relative to nuclear DNA. The use of the dual insert calibrator plasmid allows for correction against unequal emission intensities of the differently fluorescence labelled targets. Furthermore, we discovered that the diverse extraction methods selectively isolate different DNA molecules within a sample.

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Involvement of mitophagy in the elimination of damaged mitochondria during the process of UVB-induced senescence

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Skin aging is the result of two types of aging, “intrinsic aging” an inevitable consequence of physiologic and genetic determined changes and “extrinsic aging” which is dependent on external factors like exposure to sunlight, smoking, dietary habits among others. UV and other forms of ionizing radiation cause skin injury through the generation of free radicals and other oxidative byproducts as well as DNA damage. The oxidized proteins are generally degraded by the ubiquitin-proteasome system or by autophagy, and alterations on these pathways lead to accumulation of damaged molecules. The activity of proteasomes and autophagic organelles is regulated by overlapping signals, and regulatory cross-talk between both quality control systems has been described. Likewise, excessive ROS production leads to impairment of mitochondria with consequences that are related to several age-related diseases as well as to the physiology of normal aging. We have previously demonstrated that inhibition of proteasomal degradation of damaged proteins and activation of autophagosome formation are early events in UVB-induced senescence of human dermal fibroblasts (HDF), dependent on UVB-induced accumulation of reactive oxygen species (ROS). Here we show that UVB treatment of HDFs leads to impaired mitochondrial function, damage to mitochondrial structure and disruption of mitochondrial network and that these damaged organelles are eliminated by mitophagy. Under these conditions, mitophagy receptor Bnip3L/Nix is differentially regulated in fibroblasts. Elimination of Bnip3L/Nix increases cell proliferation and inhibits mitophagy activation upon UVB treatment. These findings have potential implications for fundamental as well as translational research into skin aging, and in particular photoaging.

Session IV: Mitochondria in Blood and Heart

The mitochondrial shaping protein Optic Atrophy 1 (OPA1) controls angiogenesis

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Mitochondria not only synthesize most of the cellular ATP, but they are also centrally placed in intermediate metabolism Ca2+ signaling, redox homeostasis and apoptosis. The multifunctional inner mitochondrial membrane mitochondrial fusion protein Optic Atrophy 1 (OPA-1) is placed at the crossroad of fusion, cristae biogenesis, metabolism, apoptosis and regulation of cardiomyocyte differentiation, yet the role of mitochondrial dynamics in angiogenesis, the physiological process through which new blood vessels form from pre-existing ones, has not been addressed. Here we show that Opa1 is a crucial component of the angiogenetic program. Upon endothelial cells angiogenic stimulation, mitochondria elongate and OPA-1 level increase. Genetic Opa1 ablation signals retrogradely from mitochondria to the nucleus to modify angiogenic genes expression and therefore inhibit all features of angiogenesis. Conditional Opa1 ablation substantiates its role in mouse and zebrafish angiogenesis and in lymphangiogenesis mediated tumor metastatization. Thus, Opa1-dependent mitochondrial dynamics is a targetable component of angiogenesis.

Searching specific targets of ischemic damage of cardiac mitochondria using O2k-Fluorometry

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Ischemia-reperfusion damage during the transplant process occurs mainly in three steps: (i) warm ischemia (WI), (ii) cold ischemia, (iii) reperfusion. WI is defined as the time interval between a tissue remaining at body temperature after blood supply has been reduced or interrupted. Oxidative stress is considered to be one of the main causes of injury during ischemia-reperfusion.

In the present work we used high-resolution respirometry (O2k-Fluorometer; OROBOROS, Innsbruck, Austria) [1] to investigate simultaneously respiration and hydrogen peroxide production (H_2O_2) of mitochondria isolated from the hearts of C57BL/6 mice. By using inhibitors of the main mitochondrial H_2O_2 scavengers (DNCB for glutathione and AF for thioredoxin) we evaluated the total H_2O_2 production compared to net H_2O_2 production in the absence of these inhibitors [2].

OXPHOS and ETS respiratory capacities of isolated mitochondria (normalized per mg mt-protein) were decreased after 1-h WI of the excised heart. A significant injury of the outer mt-membrane is consistent with ischemia-induced mt-permeability transition [3], which can explain a general respiratory defect. In addition, application of a newly developed substrate-uncoupler-inhibitor titration (SUIT) protocol [4] revealed a specific defect of fatty acid β-oxidation (FAO) [5] H_2O_2 flux based on the Amplex red assay more than doubled after application of AF and DNCB inhibitors to the controls. The glutathione and thioredoxin antioxidant system did not protect mitochondria after WI from this increased H_2O_2 production. Taken together, standardized respiratory SUIT protocols combined with SOPs in the fluorometric assay of H_2O_2 production offer a sensitive diagnostic tool for comprehensive OXPHOS analysis.

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Effects of systemic iron perturbations on mitochondrial activity and on cellular metabolism in vivo

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Mitochondria are dynamic organelles, involved in different cellular functions, including oxidative phosphorylation, where iron is a fundamental co-factor (1). Besides being central part of mitochondrial I-IV complexes in the electron transport system, iron also regulates the TCA (tricarboxylic acid cycle) by modulating mitochondrial aconitase (2, 3). Hence, imbalances of iron homeostasis could affect mitochondrial activity and cellular metabolism (4). Nevertheless, little is known on that. Therefore, we aimed at investigating the impact of alterations of iron homeostasis on mitochondrial function, and on peripheral blood metabolites, in order to potentially identify distinctive signatures.
Mitochondrial respiration was studied in liver samples of 10-week old FVB mice and C57BL/6N mice, receiving either normal- or high iron (25 g/kg)-diet two weeks before being sacrificed. Livers were homogenized and mitochondrial respiration was assessed by means of high resolution respirometry (OROBOROS Instruments, Austria). Peripheral blood was collected, and metabolomics analysis was performed by using liquid chromatography-mass spectrometry (LC-MS).
Our ongoing experiments indicate that dietary iron supplementation affects the phosphorylation system in the mouse liver of both FVB and C57BL/6N mice. The analysis of peripheral blood metabolites is currently under investigation, and might provide useful information on changes in the overall metabolism.

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Session IV: Mitochondria in Blood and Heart

Mitochondrial reactive oxygen species prime T-cell acute lymphoblastic leukemia to cell death by engaging the OMA1-OPA1 axis

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Approximately 20% of T-cell acute lymphoblastic leukemia (T-ALL) patients do not respond to current therapy, and their clinical outcome is dismal.
In the present study we show that leukemic cells isolated from T-ALL patients as well as cell lines stabilized in vitro or propagated in vivo as xenografts exhibit high levels of mitochondrial reactive oxygen species (ROS). Interestingly, raising mitochondrial ROS using NS1619, a small molecule that opens the mitochondrial BK K+ channel, induced death of leukemic cells from T-ALL patients and T-ALL cell lines but not of primary normal thymocytes or PBMC. These effects were enhanced by blunting ROS-scavenging pathways with dehydroepiandrosterone (DHEA), an inhibitor of the pentose phosphate pathway (PPP). The combination of NS1619 and DHEA led to proteolytic processing of OPA1, an inner mitochondrial membrane protein that controls cristae remodeling, cytochrome c release and apoptosis. OPA1 cleavage was dependent upon both ROS and the OMA1 mitochondrial protease. Furthermore, OPA1 cleavage induced by treatment with NS1619 and DHEA primed T-ALL cells to apoptosis induced by TNF-Related Apoptosis Inducing Ligand (TRAIL).
These findings suggest that engaging the OMA1-OPA1 axis by raising mitochondrial ROS may prove to be an effective strategy for apoptotic priming of refractory T-ALL, which poses a major clinical challenge at present.

mTORC inhibition increases ROS levels and induces cell death in T-ALL cells

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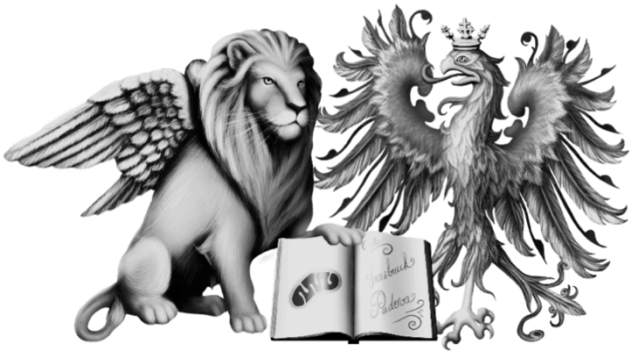
Approximately 20% of T-cell acute lymphoblastic leukemia (T-ALL) patients do not respond to current therapy, and their clinical outcome is dismal.
The PI3K/Akt/mTOR oncogenic pathway is commonly found hyper-activated in T-ALL. In addition to controlling cell growth and autophagy, mTORC1 influences mitochondrial activity and oxidative metabolism by controlling the interaction between YY1 and PGC1α.
In previous studies, we showed that T-ALL cells exhibit high levels of mitochondrial reactive oxygen species (ROS). ROS are powerful signalling molecules that can induce apoptosis through p53 activation, but may also increase cancer cell survival through PTEN oxidation, which results in an increased Akt activity.
In the present study, we aimed at testing the possible cross-talk between mTOR and ROS in T-ALL. Interestingly, in vitro studies revealed that the mTORC1-inhibitor Everolimus increased ROS levels and induced cell death both in T-ALL cell lines and patient-derived T-ALL xenografts (PDTALL) but not in primary normal thymocytes. In addition, Everolimus increased the dexamethasone sensitivity of the glucocorticoid-resistant PDTALL19 in vitro. The finding that Everolimus-induced cell death was reduced by pre-treatment with the ROS scavenger N-acetyl-cystein (NAC) indicates that this effect was ROS-dependen. In vivo experiments carried out using PDTALL cells showed that Everolimus significantly reduced the number of leukemic cells and increased the survival of treated-mice.
These studies indicate a connection between mTOR and ROS, and suggest that mTORC1 inhibition may prove to be effective to overcome dexamethasone-resistance of refractory T-ALL patients.



~ Posters ~

Poster A

1 **Anja Weber:**
Loss of PTEN in prostate cancer cells leads to a switch to succinate-mediated mitochondrial respiration associated with increased expression of NaDC3 and HIF-1alpha

2 **Annalisa Serafini:**
A novel role for the Parkinson's disease gene LRRK2 as endoplasmic reticulum-mitochondria tether identified by a genome wide high content screen

3 **Bernd Schöpf:**
Mitochondrial Function in Primary Prostate Cancer

4 **Donna D'Agostino:**
p13, a mitochondrial protein coded by human T-cell leukemia virus type 1

5 **Elisa Barbieri:**
An alternatively spliced mitochondrial Fission 1 variant participates in mitochondrial elongation during autophagy

6 **Elisa Lidron:**
MCU knock-down impairs skeletal muscle and motor neuron development in zebrafish embryos

7 **Elisa Penna:**
Impact of CCDC90B on mitochondrial functions

8 **Erich Gnaiger:**
MitoFit and MITOEAGLE –towards a global data bank on mitochondrial physiology

9 **Fernanda Cerqueira:**
The Deubiquitinating (DUB) protein Ubiquitin carboxyl- terminal hydrolase L1 (UCH-L1) regulates Mitofusin-2 levels and mitochondrial function in mammalian cell lines and in D. Melanogaster

10 **Gerhard Krumschnabel:**
Establishment of a reference sample for High-Resolution Respirometry

11 **Giovanni Rigoni:**
Nucleoids and mtDNA dynamics regulation by mitochondrial ultrastructure

12 **Gregor Ömer:**
Mass spectrometric characterization of cardiolipins reveals detailed insights into the structural diversity of mitochondrial phospholipids

Poster B

13 **Ildikò Szabò:**
Targeting a mitochondrial potassium channel as a new way to treat Chronic Lymphocytic Leukemia

14 **Javier Espino:**
Mitochondrial [Ca^{2+}] measurements using a novel very-low Ca^{2+} affinity aequorin-based probe

15 **Keitaro Shibata:**
Linear Motor Proteins Induce Mitochondrial Shape Change

16 **Laura Morbiato**
Purification of and characterization of human recombinant COQ4 and its putative Zn binding site mutants

17 **Laura Martorano**
OXPHOS complex impairment and mitochondrial DNA depletion modify Hypoxia signaling pathway activity in zebrafish

18 **Lorenza Iolanda Tsansizi**
PPM1K, novel insights in connecting metabolism and autophagy in the heart

19 **Luiz Felipe Garcia-Souza**
Assessment of fatty acid oxidation in mouse brain and liver mitochondria

20 **Maria Andrea Desbats**
CoQ biosynthetic proteins are physically and functionally related to respiratory supercomplexes in mammalian cells

21 **Roberta Peruzzo**
Novel psoralen-derivatives with increased solubility in cancer treatment

22 **Sabrina Neururer**
A project to develop a standardized minimum dataset to describe mitochondrial data

23 **Sofia Zanin**
Loss-of-function mutations in the SIGMAR1 gene cause distal hereditary motor neuropathy by impairing ER-mitochondria tethering and Ca^{2+} signaling

24 **Vanina Romanello**
Contribution of mitochondrial dynamics to age-related muscle loss

25 **Verena Laner**
OXPHOS and ETS capacity in permeabilized fibresof canine superathletes

Poster A - 1

Loss of PTEN in prostate cancer cells leads to a switch to succinate-mediated mitochondrial respiration associated with increased expression of NaDC3 and HIF-1alpha

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Metabolic changes are a hallmark of prostate cancer cells. In order to reveal novel targets that could be used for therapeutic intervention, we performed a comprehensive metabolic analysis of different human and murine prostate cancer cell lines.
In line with previous studies we found that loss of phosphatase and tensin homolog (PTEN), a major driver of prostate cancer cells, is associated with high glycolytic activity, increased lactate production and elevated expression of hexokinase 2 (HK2). This increased glycolytic activity corresponds with weak activity of pyruvate dehydrogenase (PDH), which drives pyruvate into oxidative phosphorylation, and high expression of the PDH inhibitor PDK1 (pyruvate dehydrogenase kinase). Mitochondrial routine respiration was elevated in all PTEN- compared to PTEN+ cells. While digging deeper into these pathways we showed that high lactate production in PTEN- cells leads to a switch in mitochondrial respiration towards complex II. We found that PTEN- PCa cells favour succinate as substrate for oxidative phosphorylation while lowering the capacity to use glutamate and pyruvate. In addition, we found that the Na(+)-dependent dicarboxylate transporter NaDC 3, responsible for succinate uptake into cells, is elevated in PTEN- compared to PTEN+ cells and that HIF-1alpha, stabilized by succinate, is increased in cells lacking PTEN.
Our data show that uptake of succinate via the NaDC 3 transporter could be an efficient way to overcome hypoxia and to fulfill the energy requirements in PTEN- PCa cells and that intervening with this pathway may offer a new way for treatment of PCa.

Poster A - 2

A novel role for the Parkinson's disease gene LRRK2 as endoplasmic reticulum-mitochondria tether identified by a genome wide high content screen

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The contact sites that are formed between mitochondria and Endoplasmic Reticulum (ER) are involved in several relevant cellular processes such as lipid and Ca^{2+} homeostasis. Despite some of the proteins involved in the structural maintenance and regulation of the contact sites between the two organelles have been discovered, the overall molecular identity of these protein complexes is not fully understood. Here we demonstrate that Parkinson's disease (PD)-associated gene leucine rich repeat kinase 2 (LRRK2) is an ER-mitochondria tether, identifying a previously unknown physiological function for this protein. Based on the variations in basal and maximal FRET values of a FRET ER-Mitochondria proximity probe (FEMP) expressed in mouse embryonic fibroblasts transduced with lentiviral particles carrying shRNAs targeting the whole murine genome, we performed two replicates of a genome wide high content screening identifying 250 potential tethers. A network of genes associated to PD emerged from pathway analysis of the potential tethers, among which LRRK2 appear to be a top candidate, due to its known localization at both outer mitochondrial membrane and ER. Subcellular fractionation experiments showed that LRKK2 localized mostly in ER and mitochondria associated membranes (MAMs). As expected for a tether, levels of ER-mitochondria juxtapposition were decreased in LRRK2-/- cells. ER-mitochondria proximity was fully rescued by reintroduction in LRRK2-/- cells of wt protein, but not of the familial PD-associated mutants. In conclusion, LRRK2 is involved in tethering between ER and mitochondria and its PD associated mutations impair interorganellar juxtaposition and communication.

Poster A - 3

Mitochondrial Function in Primary Prostate Cancer

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Reprogramming of energy metabolism is a hallmark of cancer. Mutations in the mitochondrial DNA (mtDNA) might contribute to cancer development and progression. We analyzed mitochondrial respiration of fresh malignant and non-malignant prostate tissue samples obtained from 50 prostate cancer patients via High-Resolution Respirometry (HRR), determined mtDNA copy numbers by duplex qPCR, sequenced the whole mtDNAs using Next-Generation Sequencing (NGS) and analyzed expression of mitochondria-related genes in a subset of 16 cases by RNA-sequencing. HRR uncovered a shift of respiratory activity from mitochondrial complex I to complex II accompanied by a substrate shift toward higher respiratory activity elicited especially by succinate and pyruvate. The mutation load was significantly higher in tumor tissue compared to the non-malignant counterpart. Heteroplasmy levels of potentially deleterious mutations in mtDNA genes correlated significantly with reduced complex I respiration capacity. RNA-seq revealed a signature of differentially expressed metabolic enzymes in tumors exhibiting a severe compared to a mild complex CI mt-phenotype. The gene signature corresponded to observed altered substrates effects on respiration, e.g. increased pyruvate and citrate and decreased glutamate oxidation.

Poster A - 4

p13, a mitochondrial protein coded by human T-cell leukemia virus type 1

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Human T-cell leukemia virus type 1 (HTLV-1) is a complex retrovirus that causes a T-cell neoplasm termed adult T-cell leukemia/lymphoma and a neurodegenerative disease termed tropical spastic paraparesis/HTLV-associated myelopathy. HTLV-1 expresses several regulatory and accessory proteins, including p13, an 87-amino acid protein that is targeted to the inner mitochondrial membrane through the action of an arginine- and leucine-rich mitochondrial targeting signal (MTS). In mitochondria, p13 opens a K+ selective channel, resulting in an inward K+ current that enhances the activity of the respiratory chain, increases production of ROS and leads to membrane depolarization and mitochondrial fragmentation. These effects are linked to the protein's impact on cell turnover, which include activation of primary T-cells and reduced proliferation and sensitization to death signals in transformed cells. Further investigation of the factors influencing the intracellular localization and activity of p13 showed that it accumulates both in mitochondria and in the nucleus when expressed at high levels. Results of mutational analyses indicated that a serine residue located near the MTS modulates p13's activities, as its substitution with alanine abrogated mitochondrial fragmentation and loss of membrane potential, while substitution with aspartic acid led to pronounced fragmentation and collapse of membrane potential and partial localization of the protein to the cytosol and nucleus. Current studies are aimed identifying possible posttranslational modifications on the serine residue that might play a role in controlling p13's function.

Poster A - 5

An alternatively spliced mitochondrial Fission 1 variant participates in mitochondrial elongation during autophagy

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The role of the outer mitochondrial membrane protein Fission 1 (FIS1) as a mitochondrial receptor for the pro-fission dynamin related protein 1 (DRP1) has been recently challenged and Fis1 has been conversely implied in mitophagy, but the molecular mechanisms governing FIS1 involvement in mitochondrial morphology and autophagy are unclear. Here we show that both human and mouse FIS1 genes give rise to splicing variants with opposite effect on mitochondrial morphology. FIS1 variant 1 or 3 trigger fragmentation whereas variant 2 induces mitochondrial elongation. Upon starvation, FIS1 variant 2 expression is up-regulated in a protein kinase A-dependent manner and its specific knockdown inhibits autophagy associated mitochondrial elongation. Thus, FIS1 is alternatively spliced to modulate mitochondrial morphology during autophagy.

Poster A - 6

MCU knock-down impairs skeletal muscle and motor neuron development in zebrafish embryos

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Ca^{2+} is a fundamental signalling molecule which decodes a variety of extra-and intra-cellular inputs and regulates diverse biological processes, from egg fertilization to organogenesis and tissue specific function, such as contraction in skeletal muscle and neuronal firing in brain.
Mitochondria are one of the most important targets and regulators of cellular Ca^{2+} signalling. In 2011, the molecular complex responsible for the entry of Ca^{2+} in mitochondria, the mitochondrial Ca^{2+} uniporter (MCU), was identified by our and Mootha's groups, opening the path for the biochemical and molecular characterization of the mechanisms underlying mitochondria contribution to Ca^{2+} signalling.
Our work aims to explore the contribution of MCU and mitochondrial Ca^{2+} dynamics in the regulation of vertebrate development and organogenesis implementing the zebrafish (danio rerio) as a model organism. Our experimental strategy consists in knocking down drMCU expression during zebrafish embryonic development. Western blot analysis reveals an efficient MCU knocking down after 48-72 hpf, accompanied by reduced Ca^{2+} uptake in morphant embryo cells. The down regulation of MCU is extraordinarily maintained up to 8 dpf, suggesting a strong maternal contribution to MCU expression during early stages. Despite MCU morphant fish develop without gross morphological abnormalities, to a deeper analysis they present an altered skeletal muscle structure and a compromised motor neuron differentiation. Concluding, our data indicate a role of MCU in early zebrafish development and in particular we found that MCU is required for the differentiation and maturation of skeletal musculature and of motor neuron network.

Poster A - 7

Impact of CCDC90B on mitochondrial functions

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Mitochondria are the masters of cellular energy metabolism and are crucially involved in a number of metabolic and signal transduction pathways. Several pathological conditions are associated with dysfunction of the oxidative phosphorylation (OXPHOS), impairment of the Electron Transport Chain (ETC) and ROS production. In this work we investigated the role of an uncharacterized mitochondrial protein named CCDC90B. CCDC90B is ubiquitously expressed and broadly conserved until yeasts. Its ortholog in S. cerevisiae encodes for a protein called FMP32, recently described as an assembly factor for cytochrome c oxidase (COX) [1]. However, in mammals an additional isoform named CCDC90A is present and has been recently described to regulate mitochondrial Ca^{2+} uptake (and hence it is also known as MCUR1) [2]. Here we investigate how CCDC90B impacts on both OXPHOS and organelle calcium handling in human cells. We demonstrated that CCDC90B can physically interact with its isoform CCDC90A. However, we didn't detect any major functional interaction with all the known members of the Mitochondrial Calcium Uniporter (MCU) complex, although the downregulation of CCDC90B causes a defective organelle Ca^{2+} uptake. Most importantly, we found that both silencing and ablation of CCDC90B decrease mitochondrial membrane potential and O_2 consumption rate (OCR). Overall, this work describes a novel mitochondrial protein that, unlikely its closely related isoform, it plays no direct role in the regulation of mitochondrial Ca^{2+} uptake but rather controls the activity of the ETC.

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Poster A - 8

MitoFit and MITOEAGLE – towards a global data bank on mitochondrial physiology

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A lack of physical activity associates with decreased mitochondrial capacity and is a major cause underlying metabolic dysregulation and preventable diseases in modern societies. In contrast, an active lifestyle supports enhanced mitochondrial capacities and reduces the risk of degenerative diseases. Despite of this well-known relation between health and mitochondrial function, there is no regimented, quantitative system, or database organised to routinely test, compare and monitor mitochondrial capacities within individuals or populations. Using high-resolution respirometry, the MitoFit and MITOEAGLE initiatives will develop novel lab standards and diagnostic methods for monitoring of a mitochondrial fitness score. To this end, SOPs will be worked out regarding sample preparation, respiratory evaluation and data documentation. Fresh and cryopreserved cells obtained noninvasively from blood samples will serve as models, the latter allowing samples to be collected for later analysis, thereby broadening the scope for respirometric investigations.
This approach will then be expanded to all sorts of human tissues and cells of interest and assess aspects relating to Evolution, Age, Gender, Lifestyle and Environment (EAGLE) as essential background conditions characterizing the individual patient, subject, study group, and/or species. The huge scope of this endeavour requires an international network of laboratories capable of generating the necessary number of consistent data to address the complexity of EAGLE. Coping with the mass of the expected data necessitates a dedicated MITOEAGLE knowledge management network developing harmonization protocols towards generating a rigorously monitored data repository on mitochondrial respiratory function. The resulting MITOEAGLE data management system will enable to interrelate results of a large number of studies, to interpret pathological phenotypes, and to set results into the multidimensional context of EAGLE.

Poster A - 9

The Deubiquitinating (DUB) protein Ubiquitin carboxyl- terminal hydrolase L1 (UCH-L1) regulates Mitofusin-2 levels and mitochondrial function in mammalian cell lines and in D. Melanogaster

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The ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is a cytosolic deubiquitinating (DUB) protein, highly expressed in neurons and beta-cells, which substrates are not well known. UCH-L1 is linked to a familiar case of Parkinson`s Disease (PD), therefore its gene is classified as PARK5. Curiously, UCH-L1 is being associated to type 2 Diabetes, and it is found reduced in beta cells from Diabetic donors. Although mitochondrial dysfunction is central in PD and Diabetes, mitochondrial proteins were not reported, so far, to be a target of UCH-L1.

Given the similarities and conserved pathways between beta cells and neurons, we proposed to investigate the role of UCH-L1 on mitochondrial dynamics and function in neuroblastoma SY5Y-SH and beta cell line INS1 and we are using D. Melanogaster as an *in vivo* model.

Knocking down UCH-L1 in both cell lines SY5Y-SH and INS1, resulted in mitochondrial fragmentation, reduced mitofusin-2 levels, and higher mitochondrial spare capacity, without any increment in mitochondrial mass. Interestingly, the knockdown of UCH, the UCH-L1 homologue, in D. Melanogaster reduced Marf levels, and resulted in significantly higher maximum respiratory capacity in isolated mitochondria, suggesting changes in respiration are not coupled to any direct morphology alteration promoted by this DUB.

To test if UCH-L1 activity affects Mfn2 ubiquitiation, we quantified Mfn2 ubiquitination levels in cells where UCH-L1 was depleted. Upon UCH-L1 KD, Mfn-2 levels were drastically reduced. The addition of the proteasome inhibitor MG-132 prevented Mfn2 degradation in UCH-L1 KD cells. Under these conditions we measured increased levels of ubiquitylated Mfn2 compared to the control cells (non-UCH-L1 depleted, also treated with MG-132). Pull down assays showed an interaction between Mfn-2 and UCH-L1 when cells were treated with MG-132 or when cross-linking with formaldehyde was performed, suggesting the interaction between both molecules is transient and enhanced under circumstances of accumulation of ubiquitylated Mfn2 (i.e. MG-132 treatment).

This study suggests UCH-L1 is a DUB which regulates mitochondrial function and Mfn2 is a potential target.

Poster A - 10

Establishment of a reference sample for High-Resolution Respirometry

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Mitochondrial respiratory function may be assessed and analyzed in great detail applying high-resolution respirometry (HRR), which is thus a valuable tool for the diagnostic assessment of function and dysfunction of oxidative phosphorylation related to a wide range of metabolic and other diseases. In order to allow for a valid comparison of data among research groups it appears necessary to agree on certain standards relating to sample treatment and measurements protocols, but an ultimate tool for data validation and comparison would be obtained with an available common references sample. Here, we report on efforts on establishing such a reference sample, which is functionally stable over time and can be distributed across the globe to other labs and could provide the basis of standard proficiency tests within and between reference laboratories. We chose a widely applied human cell line, HEK293T cells, which can be examined using reference substrate-uncoupler-inhibitor titration (SUIT) protocols, and evaluated conditions for cell storage for later and reproducible respirometric assessment. We found that after storage of these cells in standard freeze medium at -80°C cell viability remained largely preserved and that respirometric parameters showed an initial decrease in the first two weeks of storage but remained fairly constant thereafter for 3, but not for 6 month. Storing already permeabilized cells led to even better preservation of respiratory function for at least 3 month. First experiments after storage of cells in dry ice for 48h indicated similarly preserved mitochondrial function and altogether these results suggest that HEK293T cells may be regarded promising candidates for a respirometric reference sample.

Poster A - 11

Nucleoids and mtDNA dynamics regulation by mitochondrial ultrastructure

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Alteration of mitochondrial ultrastructure has emerged as phenotypical marker of dysfunction. In particular, changes in cristae shape and number regulate the respiratory efficiency of the cell and the release of proapoptotic factors. Moreover, in the last years a growing number of studies have observed a loss of mtDNA associated to mitochondrial related disorders but whether and how mtDNA and nucleoids regulation is influenced by mitochondrial ultrastructure is unknown. To address this question we studied nucleoids distribution and mtDNA copynumber in cellular models where the mitochondrial ultrastructure has been altered. We show that nucleoids distribution vary depending on mitochondrial ultrastructure and accordingly also mtDNA copy number. Furthermore, we have identified a mitochondrial complex that decrease with nucleoids suggesting a relevant role of this complex for nucleoids and mtDNA stability. Our results suggest that nucleoids and mtDNA stability is regulated by mitochondrial ultrastructure.

Poster A - 12

Mass spectrometric characterisation of cardiolipins reveals detailed insights into the structural diversity of mitochondrial phospholipids

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The mitochondrial glycerophospholipid class of cardiolipins possess a unique structure, as they can carry up to four acyl side chains and are able to trap protons by phosphate-glycerol bicyclic resonance structure. They are almost exclusively found in the inner mitochondrial membranes where they are functionally involved in maintaining oxidative phosphorylation by stabilising transmembrane proteins, buffering fluctuations of the proton gradient, protecting mtDNA, and mediating oxidative damage - related apoptotic signalling events. Interestingly, a highly regulated tissue-specific fatty acyl side chain composition of cardiolipins is required to establish maximum functional efficiency. The exact patterns of acyl substitutions are established in a post-biosynthetic remodelling process requiring the Barth Syndrome linked protein tafazzin.

We have recently developed a reversed-phase high performance liquid chromatography – tandem mass spectrometric methodology, enabling the identification and absolute quantification of up to 140 different cardiolipins within biological samples such as bacteria, unicellular eukaryotes and tissues of multicellular organisms. In contrast to most previous approaches, that only focus on few targeted cardiolipin subspecies, we are able to analyse a broad spectrum of cardiolipins, including their respective monolyso- and oxidised/ peroxidised counterparts, resulting in a complete cardiolipidome. Furthermore, by mathematical modelling of MS2 spectra generated by data-dependent fragmentation, it was possible to reveal the specific fatty acyl compositions of individual cardiolipin species, and to distinguish specific intramolecular side chain and double bond distributions.

Thus, the here presented cardiolipidomics approach opens up the possibility to comprehensively characterise this mitochondrial signature lipid and to study its functional roles in a broad range of samples.

Poster B - 13

Targeting a mitochondrial potassium channel as a new way to treat Chronic Lymphocytic Leukemia

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Human Chronic Lymphocytic Leukemia (B-CLL) is the most commonly diagnosed leukemia in the Western World. Therapeutic options to treat this leukemia are very limited. B-CLL is characterized by a clonal accumulation of mature neoplastic B cells that are resistant to apoptosis. Different leukemic cells or cell lines, both myeloid and lymphoid, express/overexpress several potassium channels including shaker type voltage-gated Kv1.3, Kv11.1 (Herg), and calcium-activated KCa3.1, and their pharmacological inhibition has been related to reduced B-CLL proliferation, pointing to ion channels as promising oncological targets in B-CLL. We obtained evidence that Kv1.3 is highly expressed in B-CLL respect to normal B cells both in the plasma membrane and in the inner mitochondrial membrane. We have recently shown that the treatment with mitochondrial Kv1.3 inhibitors actively killed primary B-CLL cells in ex-vivo experiments, by induction of intrinsic apoptosis. Importantly, cells form healthy subjects and even residual normal T lymphocytes of the same patients were unaffected by the drugs, while B-CLL cells were killed. Importantly, B-CLL cell death was observed also when leukemic cells were co-cultured with mesenchymal stromal cells (MSC), which favor tumor cell growth by releasing anti-apoptotic and pro-survival factors. Here we report the first in vivo evidence, that pharmacological targeting of the mitochondrial Kv1.3 by a new mitochondrial targeted inhibitor is sufficient to lead to a massive CD5+/CD19+ elimination in several organs (blood, peritoneal cavity, spleen, bone marrow) in a B-CLL genetic mouse model (EuTCL-1), without inducing side effects and death in healthy immune cells, including cytotoxic T lymphocytes. These results open the possibility to a new therapeutical approach for this disease by directly targeting the mitochondrial channel.

Poster B - 14

Mitochondrial [Ca^{2+}] measurements using a novel very-low Ca^{2+} affinity aequorin-based probe

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Aequorin is a 22-kDa photoprotein produced by the jellyfish Aequorea victoria that has been long utilised for the study of Ca^{2+} signaling (1). It has been also engineered to induce its specific targeting to various cell regions so as to monitor [Ca^{2+}] in different subcellular comparments, e.g., mitochondrial matrix (2). Nevertheless, its potential applicability is somewhat limited owing to consumption or saturation of aequorin throughout the experiment as well as stability of aequorin at physiological temperature. Herein, in an attempt to overcome the aforementioned disadvantages, we have developed a mitochondria-targeted triple-mutated form (Asp119Ala, Gln168Arg and Leu170Ile) of the photoprotein aequorin that enables measurement of [Ca^{2+}] in the millimolar range. In fact, it is shown that addition of extramitochondrial Ca^{2+} to permeabilized HeLa cells triggers an increase in mitochondrial [Ca^{2+}] up to approximately 2 mM. In intact cells, the novel probe allows recording agonist-stimulated mitochondrial [Ca^{2+}] rises without problems derived from aequorin saturation and/or consumption. Notably, in addition to the increased dynamic range, the Gln168Arg and Leu170Ile mutations endowed this new aequorin-based probe with an increase lifetime at 37°C. This also allowed the generation of a cell line stably expressing the probe at very high levels.

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Poster B - 15

Linear Motor Proteins Induce Mitochondrial Shape Change

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Mitochondria are essential organelles that provide cellular ATP by oxidative phosphorylation, and regulate housekeeping processes. They undergo dynamic morphological changing and make a complex network in eukaryotic cells. The dynamics is linked to their functional versatility so deeply that it is important to elucidate the networks and mechanisms by which they change their shape. Motor proteins (kinesin, dynein and myosin) move along the cytoskeleton (microtubule and actin filament) with using the energy of ATP made by mitochondria and can be linked to mitochondria via adapter proteins on the mitochondrial membrane. Mitochondria use force from motor proteins for movement and transformation. However, it is not known how motor proteins regulate the complex morphology of mitochondria. Where are motor proteins during the dynamic shape changing of mitochondria? Are mitochondria pulled and extended by the motor proteins, or by other mechanisms? To answer these questions, we are visualizing the endogenous motor proteins and following them on mitochondria changing the shape in living cells, and try to demonstrate the allocation of roles among the motor proteins in mitochondrial shape change..

Poster B - 16

Purification of and characterization of human recombinant COQ4 and its putative Zn binding site mutants

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Defects in genes involved in coenzyme Q (CoQ) biosynthesis cause primary CoQ deficiency, a clinically heterogeneous disorder with clinical manifestation ranging from fatal neonatal multisytem disorders to adult-onset isolated encephalopathy or nephropathy.
COQ4 codes for an ubiquitously expressed 265 amino acid protein that is peripherally associated with the mitochondrial inner membrane on the matrix side (ref 1,3); the precise function of human COQ4 is not known, but the yeast ortholog Coq4p seems to play a structural role crucial in the stabilization of a multiheteromeric complex including several, if not all, of the CoQ biosynthetic enzymes (1). It has also been proposed that yeast Coq4p could be a zinc protease, based on the presence of the zinc binding motif HDxxH (2). In this view the protein of interest could also play a role in the maturation of other COQ polypeptides.
At the beginning the protein (devoid of the N-terminal mitochondrial targeting sequence) was expressed in E.Coli attached to a 6 His-Tag and a consensus for enterokinase (DDDK). We purified the protein using a Ni affinity cromatografy on an AKTA-FPLC and a gel filtration chromatography, in order to increase the purity. The yield and the purity of recombinant human COQ4 were satisfactory. Biochemical characterization of the protein was carried out using different approaches like: circular dichroism (CD), analysis of zinc content, blue native gel, and electron microscopy.
We found by CD that COQ4 binds zinc, increasing the stability of the recombinant protein. We observed both by BN-PAGE and by gel filtration that our protein forms a high molecular weight complex, a multimeric complex comprised of 5-6 monomers. We would also like to investigate the effective role of the putative Zn binding motif HDxxH; therefore we are going to purify and characterize 3 COQ4 mutants: H155A, D156A, H159A.
Altogether these data provide new insights on the role of COQ4 in CoQ biogenesis. Further work will be aimed at obtaining crystals in order to solve the three-dimensional structure of the protein.

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Poster B - 17

OXPHOS complex impairment and mitochondrial DNA depletion modify Hypoxia signaling pathway activity in zebrafish

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Mitochondria are essential for cell survival and health, utilizing about 90% of the oxygen we breath for OXPHOS. Previous studies showed a strong relationship between mitochondrial alterations and hypoxia signaling pathway, an interesting aspect to be investigated. POLG-related disorders are a group of diseases characterized by the dysfunction of DNA polymerase gamma, an enzyme crucial for mtDNA replication, repair and stability. Danio rerio (zebrafish) is an ideal vertebrate model of human mitochondrial diseases because of its high conservation of physiological processes and genomic structure, transgenic lines availability and embryonic transparency. Using zebrafish embryos, we have performed a transient knock-down of the polg gene, inducing a dilated cardiomyopathy and an increased heart bit rate. Moreover, we have developed a transgenic line able to show in vivo the activation of hypoxia-inducible factor 1 (Hif1) signaling. Taking advantage of this reporter line, we established that Hypoxia pathway is up-regulated in polg morphants. In addition, using a pharmacological approach targeting OXPHOS complexes NADH:ubiquinone reductase (Complex I) and Succinate dehydrogenase (Complex II), we have investigated the effect of mitochondrial dysfunctions on the Hypoxia pathway. We established that Hypoxia pathway is significantly reduced, both in normoxia and in hypoxia conditions, perhaps due to an increase in ROS production. In conclusion, our data suggest the existence of cross-talk mechanisms sensing mitochondrial dysfunction and changing Hypoxia signaling. In addition, our results on polg transient inactivation incourage the use of zebrafish as a suitable model to perform CRISPR/Cas9-mediated mutagenesis of DNA polymerase gamma, an ongoing project in our laboratory.

Poster B - 18

PPM1K, novel insights in connecting metabolism and autophagy in the heart

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Metabolic homeostasis is an integral part of cardiac function and mitochondria are the main sources of metabolites and energy in this highly specialized tissue. Indeed, metabolic remodeling is a hallmark of heart failure, and although there are recent insights on the impact of fatty acids and carbohydrates in it, aminoacid metabolism is not yet studied in this context. Additionally, maintaining a healthy pull of mitochondria in the cell by autophagic degradation of the dysfunctional ones is crucial to cardiomyocyte viability. Data from our lab showed a link between PPM1K, a mitochondrial matrix protein phosphatase involved in branched chain aminoacid (BCAA) catabolism and autophagy. PPM1K is known to be highly expressed in cardiac tissue besides low BCAA catabolism levels, and its ablation leads to cardiac impairment. Thus, we are now testing the hypothesis that the PPM1K-dependent regulation of autophagy impacts in cardiac physiology.

Poster B - 19

Assessment of fatty acid oxidation in mouse brain and liver mitochondria

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Mitochondrial (mt) respiratory control by substrates and inhibitors represents a key aspect of bioenergetics research. Substrate-uncoupler-inhibitor titration (SUIT) protocols are applied for determining respiratory capacities of selected mt-pathways, with fatty acid oxidation (FAO) becoming a particularly hot topic for mt-fitness [1,2]. FAO measurement in mt-preparations requires addition of fatty acids and a NADH-linked substrate (malate) to prevent inhibition by accumulating acetyl-CoA. Malate, however, may stimulate respiration above the level of FAO-OXPHOS capacity mainly due to the presence of mt-malic enzyme (mtME) [3]. Therefore, conventional SUIT protocols require adjustments for accurate determination of FAO capacity.
In the present study we investigated FAO in liver and brain isolated mitochondria (imt) and homogenate (thom) from C57BL/6 mice. Malate concentration was varied in the range of 0.05 to 10 mM. Titration of octanoylcarnitine alone resulted in a modest increase of oxygen consumption in liver imt and thom, suggesting the presence of endogenous substrates. In liver and brain thom, malate titration stimulated respiration in the presence of ADP. FAO was saturated by malate at 0.1 mM (M.1), whereas mtME required 2 mM. FAO capacity is obtained accurately as the increase of respiration when titrating fatty acid after ADP and M.05.
Complex II is not required for the FAO pathway [3]. Respiration of brain mt with octanoylcarnitine and malate was inhibited by 50% by malonate (inhibitor of CII). In liver mt, however, FAO-linked respiration was paradoxically increased by malonate, which then was inhibited by rotenone. This may be explained by mt-malonyl-CoA synthase activity [4] in liver. These results illustrate the requirement of strict quality control of SUIT protocols and critical evaluation of metabolic assumptions [5] made for mitochondria studied in different tissues and species.
Supported by K-Regio MitoFit. Contribution to COST Action MITOEAGLE.

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Poster B - 20

CoQ biosynthetic proteins are physically and functionally related to respiratory supercomplexes in mammalian cells.

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Coenzyme Q (CoQ) biosynthesis is a complex process that requires at least 16 different polypeptides. Most CoQ biosynthetic proteins in yeast are organized in a mitochondrial multienzymatic complex (QBC) necessary for CoQ biosynthesis. Coq4p is a component of the Q complex that lacks enzymatic activity which is yet essential for the stability of the complex. However, little is known about the nature of the QBC in mammals. Here, we report that human COQ proteins are localized in the mitochondrial matrix and interact forming several mitochondrial complexes. Interestingly, COQ4 and other COQ polypeptides were found co-migrating with respiratory supercomplexes (RCS) by 1^{st}BN-PAGE-2^{nd}SDS-PAGE/WB. Moreover, COQ4 immunoprecipitated with proteins from complexes I, III and IV, suggesting that the QBC and RCS are structurally related to each other. Strickingly, cells showing defects in RCS biogenesis presented a disruption of the QBC and a decrease in CoQ levels, meaning that supercomplexes are required for efficient CoQ biosynthesis. Conversely, CoQ deficiency in human cells decreased RCS. Finally, CoQ_{10} supplementation in patient fibroblasts with primary CoQ deficiency increased supercomplexes. Our findings demonstrate the importance of CoQ for supercomplexes biogenesis and suggest the interdependency between CoQ biosynthesis and the respiratory chain organization into RCS in mammalian cells

Poster B - 21

Novel psoralen-derivatives with increased solubility in cancer treatment

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Ion channels are emerging as new oncological targets. Indeed, several ion channels show a different expression pattern in normal and cancer cells. The potassium channel Kv1.3 has a multiple sub-cellular localization, including both in the plasma membrane and in the inner mitochondrial membrane. Pharmacological inhibition of the mitochondrial channel (mtKv1.3), but not of the plasma membrane channel, by membrane permeant blockers, Psora-4, PAP-1 and clofazimine, triggered apoptosis in different cancer cells. Cell death occured even in the absence of Bax and Bak, by inducing mitochondrial membrane depolarization, production of mitochondrial ROS and release of cytochrome c. Downregulation by siRNA of Kv1.3 prevented all these effects, indicating specificity. Since membrane permeant Kv1.3 inhibitors are characterized by poor water solubility, in order to increase their bioavailability as well as their solubility, we have recently synthesized a few more soluble PAP-1 derivatives. The new derivatives have been found to selectively kill cancer cells in vitro and even in vivo in a mouse melanoma preclinical model, without inducing any side effect.

Poster B - 22

A project to develop a standardized minimum dataset to describe mitochondrial data

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Mitochondrial research strongly relies on high quality data. A novel project aims to develop a concept for a minimum data set to describe and exchange mitochondrial data between research groups. The development is based on the MIABIS (Minimum Information About BIobank data Sharing) [1] standard, which was created for sharing meta data referring to biobanks and biomaterial collections. Within the framework of this project, we evaluate, which basic data is needed to describe mitochondrial meta information and if MIABIS could be reused for this purpose. The project consists of five steps: During Step 1 relevant free-text mitochondrial information is analyzed following and extending the definition analysis approach described by Neururer et al. [2]. In Step 2 a typological analysis [2] is carried out in order to identify the relevant data fields. These data fields are mapped to the concepts offered by MIABIS during Step 3. This step shows, to which extent the MIABIS standard can be reused and extended for this project. The concept for a novel mitochondrial data model is developed and described in Step 4. A detailed and expert-based evaluation of this concept is the main objective of Step 5. The project combines different approaches from interdisciplinary fields of research (e.g. computer science, social sciences) and contributes to the current state of the art of mitochondrial knowledge management to enhance mitochondrial research networking.

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2 S. B. Neururer, N. Lasierra, K. P. Peiffer, and D. Fensel, "Formalizing the Austrian Procedure Catalogue: A 4-step methodological analysis approach," J. Biomed. Inform., vol. 60, pp. 1–13, 2016.

Poster B - 23

Loss-of-function mutations in the SIGMAR1 gene cause distal hereditary motor neuropathy by impairing ER-mitochondria tethering and Ca^{2+} signaling

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Distal Hereditary Motor Neuropathies (dHMNs) are clinically and genetically heterogeneous neurological conditions characterized by degeneration of the lower motor neurons. So far, 18 dHMN genes have been identified, however about 80% of dHMN cases remain without a diagnosis.
By a combination of autozygosity mapping, identity-by-descent segment detection and whole-exome sequencing approaches we identified two novel homozygous mutations in the SIGMAR1 gene (p.E138Q and p.E150K) in two distinct Italian families affected by an autosomal recessive form of HMN.
Sigma receptor 1 (δ-1R), a 28 kDa chaperone of the endoplasmic reticulum (ER), localizes at the mitochondria-associated ER membrane (MAM) and is implicated in many aspects of cellular homeostasis in the nervous system, including regulation of ion channels and Ca^{2+} signaling.
Functional analyses in several neuronal cell lines strongly support the pathogenicity of the δ-1R mutations and provide insights into the underlying pathomechanisms involving the regulation of ER-mitochondria tethering, Ca^{2+} homeostasis and autophagy. We demonstrated that σ-1R substitutions behave as "loss-of-function" mutations affecting cell viability and altering Ca^{2+} homeostasis due to a derangement of ER-mitochondrial tethers. Moreover, primary skin fibroblasts from patients homozygous for the E150K mutation showed MAM disorganization and a higher level of autophagy compared to controls.
Our data definitively demonstrate the involvement of SIGMAR1 in motor neuron maintenance and survival by correlating, for the first time in the Caucasian population, mutations in this gene to distal motor dysfunction and highlight the chaperone activity of δ-1R at the MAM as a critical aspect in motor neuron survival and dHMN pathology.

Poster B - 24

Contribution of mitochondrial dynamics to age-related muscle loss

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Loss of muscle mass and force occurs in many diseases such as disuse/inactivity, diabetes, cancer, obesity, renal and cardiac failure and in aging-sarcopenia. In these catabolic conditions the mitochondrial content, morphology and function are greatly affected. A dysfunctional mitochondrial network trigger catabolic signaling pathways which feed-forward to the nucleus to promote the activation of muscle atrophy. Optimised mitochondrial function is strictly maintained by the coordinated activation of different mitochondrial quality control pathways such as mitochondrial fusion and fission. Muscle loss associated with aging-sarcopenia is characterized by alterations in the balance of mitochondrial dynamics. We showed that in humans there is an important age-dependent decrease of mitochondria-shaping proteins that correlate with muscle wasting and weakness that can be counteracted by regular exercise. In agreement, muscle-specific acute deletion of either Opa1 or Drp1 leads to exacerbated muscle loss. Moreover, fusion impairment in muscles reverberates to whole body inducing multi-organ precocious aging and premature death. Given the relevant role of balanced mitochondrial dynamics in muscle atrophy and to dissect the mechanistic insights linking mitochondria to sarcopenia, we investigate here the consequences of simultaneous disruption of both, mitochondrial fission and fusion, and thus freezing mitochondrial dynamics in the adult stage of skeletal muscles. All together, these findings are important for the understanding of the molecular pathways that controls muscle mass. This step is crucial for developing novel and specific therapeutic approaches that could ultimately counteract age-related tissue dysfunction allowing a healthy aging.

Poster B - 25

OXPHOS and ETS capacity in permeabilized fibres of canine superathletes

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Comparative mitochondrial physiology strongly relies on quantitative data sets for comparison of OXPHOS capacities and respiratory control patterns between species and tissues. Combination and interpretation of a wide variety of studies requires standardization of respiratory protocols, implementation of quality control criteria, and consistency of normalization. Previously we described a reference method for the application of a cytochrome *c* threshold as exclusion criterion in mitochondrial OXPHOS analyses [1]. Alaskan sled dogs (*N*=6) were studied 72 to 120 h after finishing a competitive 1,000 mile race within less than nine days. Permeabilized fibres (0.81-1.28 mg ± 0.12 SD wet weight per assay) were prepared from needle biopsies and immediately studied by high-resolution respirometry [2] using 12 chambers in parallel (OROBOROS Oxygraph-2k). Compared to human skeletal muscle fibres, the canine samples were more delicate to handle, highly sticky and appeared to be fragile, disintegrating to various degrees during substrate-uncoupler-inhibitor titration (SUIT) protocols in mt-respiration medium MiR06Cr. Two substrate-uncoupler-inhibitor titration protocols were applied. SUIT1 emphasized pathway control with fatty acid oxidation (F) versus carbohydrate oxidation capacity, whereas the focus of SUIT2 was on coupling control with N-linked substrates. Both protocols were designed to provide a common reference state of NS-linked ETS capacity, in comparison to separate N- and S-linked pathway control states (N versus S).
NS-linked ETS capacity was 262±41 pmol·s^{-1}·mg^{-1} *W*w independent of the presence or absence of 0.2 mM octanoyl carnitine (F). This is the highest value so far reported for mammalian skeletal muscle. Top human endurance athletes have a NS-linked ETS capacity approaching 200 pmol·s^{-1}·mg^{-1} *W*w [3], compared to 153±19 pmol·s^{-1}·mg^{-1} *W*w in competitive racing horses [4].

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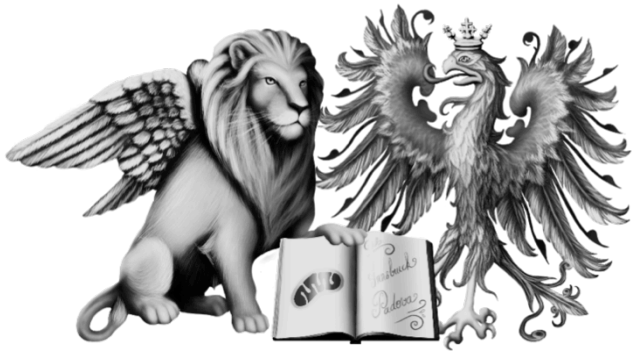

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