National Institute of Chemical Physics and Biophysics

MITOEST 2018

Estonia

Tuesday 24 April 2018 - Wednesday 25 April 2018

Ungern-Sternberg palace, Kohtu 6, Tallinn

National Institute of Chemical Physics and Biophysics

Keemilise ja Bioloogilise Füüsika Instituut

Book of Abstracts

Welcome message

In behalf of scientific organizing committee, I am delighted to welcome you to the conference MITOEST 2018 in Tallinn, in a building of the Estonian Academy of Sciences.

Recent insight has revealed the importance of inter and intracellular structural organization and reprogramming of energy metabolism for metabolic regulation at the level of large number of pathologies. Changes in cell bioenergetics are one of the first signs of cell pathology. The MITOEST 2018 will bring together scientists interested in the cellular bioenergetics and mitochondrial metabolism.
The conference is organized by the National Institute of Chemical Physics and Biophysics, Laboratory of Bioenergetics. The conference is also dedicated to the 25th anniversary of Laboratory of Bioenergetics founded by academic of Estonian Academy of Sciences Valdur Saks.

Wishing you fruitful and enjoyable conference!
Head of the organizing commitee
Tuuli Käämbre

The conference will be financially supported by institutional development program ASTRA (project no: 2014-2020.4.01.16-0041) financed from European Union Regional Development Fund.

Scientific Committee

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Mitochondria and cell research

- Exercise physiology
- Aging
- Neurodegeneration
- Diabetes
- Cancer

- O_2 consumption
- H_2O_2 production
- ATP production
- Membrane potential
- pH, Ca^{2+}, NO

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COST Action CA15203
MitoEAGLE

Evolution **A**ge **G**ender **L**ifestyle **E**nvironment

Mission

- Improve our knowledge on mitochondrial function in health and disease with regard to **E**volution, **A**ge, **G**ender, **L**ifestyle and **E**nvironment
- Interrelate studies across laboratories with the help of a MitoEAGLE data management system
- Provide standardized measures to link mitochondrial and physiological performance to understand the myriad of factors that play a role in mitochondrial physiology

Conference Program

Tuesday, 24 April

8:30 - 9:15 Registration & hanging posters

9:15 - 9:30 Opening

Chairs: Uwe Schlattner and Tuuli Käämbre

9:30 - 10:10 Invited speaker: Petras Dzeja - Phosphotransfer networks and metabolic signaling circuits

10:10 - 10:50 Invited speaker: Uwe Schlattner - The mitochondrial nucleoside diphosphate kinase NME4/NM23-H4 has dual functions in bioenergetics and signaling death decisions

10:50 - 11:20 Coffee break

11:20 - 12:00 Invited speaker: Maria Luisa Genova - Respiratory supercomplexes: physiological and pathological implications

12:00 - 12:30 Emirhan Nemutlu - ^{18}O-assisted mass spectrometry for mitochondrial dynamics evaluation

12:30 - 12:45 Alexander Gritsuk - Mitochondrial oxidation in rat spleen following the short- and long-term dietary ^{137}Cs intake

12:45 - 13:00 Alexander Koval - The eventual role of mitochondrial DNA guanine quadruplexes in sensitivity to ionizing radiation

13:00 - 14:00 Lunch & poster session

Chair: Emirhan Nemutlu

14:00 - 14:30 Invited speaker: Gennady Yegutkin - Cellular ATP turnover and its modulation during inflammation, hypoxia and tumorigenesis

14:30 - 14:50 Mart Roosimaa - Mitochondria-related molecular changes in sarcopenia

14:50 - 15:10 Sona Hubackova - Selective elimination of senescent cells by mitochondrial targeting is regulated via ANT2

15:10 - 15:40 Invited speaker: Marko Vendelin - Studies of creatine deficient mice: from respiration to EC coupling

15:40 - 16:10 Piotr Koprowski - Regulation of activity of mitochondrial potassium channels

16:10 - 16:30 Coffee break

16:30 - 18:30 Entertaining Old Town walking tour. Tour starts in front of the building of the Estonian Academy of Sciences (Kohtu str. 6) and ends in front of the restaurant Kaerajaan

18:30 Dinner in restaurant Kaerajaan (17 Town Hall Square)

Wednesday, 25 April

Chair: Jukka Kallijärvi

9:30 - 10:00 Invited speaker: Erich Gnaiger - Chemiosmotic control of the proton leak: the fundamental distinction of force and pressure

10:00 - 10:40 Invited speaker: Rafael Moreno-Sanches - Control of the NADPH supply and GSH recycling for oxidative stress management in hepatoma mitochondria

10:40 - 11:20 Invited speaker: Varda Shoshan-Barmatz - VDAC1 as a Target: Crossing the Aisle from Cancer to Neurodegeneration and Diabetes

11:20 - 11:50 Coffee break

11:50 - 12:30 Invited speaker: Vladimir Veksler - Mitochondria as a target for sex differences in pathologies

12:30 - 13:00 Invited speaker: Sara Rodriguez-Enriquez - Mitochondrial function as therapeutic target site for metastatic cancers

13:00 - 13:20 Andre Koit - Standard static markers do not explain breast cancer metabolic alterations

13:20 - 14:20 Lunch & poster session

Chair: Andre Koit

14:20 - 14:50 Invited speaker: Jakub Rohlena - Reactivation of dihydroorotate dehydrogenase by respiration restores tumor growth of mitochondrial DNA-depleted cancer cells

14:50 - 15:20 Invited speaker: Malgorzata Tokarska-Schlattner - Doxorubicin cardiotoxicity: mechanistic insight by phosphoproteomics

15:20 - 15:50 Invited speaker: Jukka Kallijärvi - A novel spontaneous CYTB variant exacerbates respiratory chain complex III deficiency in mice

15:50 - 16:10 Kattri-Liis Eskla - Hypothermia augments stress response in mammalian cells

16:10 - 16:40 Jayasimman Rajendran - Alternative oxidase prevents lethal mitochondrial cardiomyopathy by restoring respiration

16:40 - 16:45 Poster Awards

16:45 Closing

Contents

Abstracts of oral presentations in the order of appearance

Phosphotransfer networks and metabolic signaling circuits

P. Dzeja

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A network and circuit view of the cellular energetic system provides new perspective for understanding dynamics of energy metabolism in sustaining energy homeostasis, metabolic monitoring and signaling response. Emerging metabolomics and specifically stable isotope based phosphometabolomics, allows capturing the complexity of system-wide metabolite alterations and the dynamics of metabolic networks. As a member of cellular phosphotransfer network, adenylate kinase isoform (AK1-AK9) subnetwork facilitates mitochondrial energetic communication with cellular ATP-consuming and ATP-sensing components in different compartments including cell nucleus. Moreover, adenylate kinase monitors cellular energetic (ATP/ADP) imbalances and instigates AMP signaling to AMP-sensitive protein kinase (AMPK) and ATP-sensitive potassium channels (K-ATP) to adjust cell energy metabolism and function. Through a series of spatially linked enzymatic reactions adenylate kinase facilitates propagation of nucleotide signals in the intracellular and extracellular space. Evidence is accumulating that adenylate kinase AK2 facilitates nucleotide exchange in the narrow and crowded mitochondrial intra-cristae space. Deletion of mitochondrial intermembrane isoform AK2 in mice is embryonically lethal. AK2-/- mouse embryonic fibroblasts have severely disrupted mitochondrial cristae structure and display low growth and proliferation potential. Knockdown of AK2 using siRNA approach disrupts cardiomyocyte mitochondrial biogenesis and network formation. Heterozygous AK2+/- hearts have lower ability to maintain adenine and guanine nucleotide pools under ischemia and abnormal metabolomic response to treadmill exercise compared to wild type. In humans AK2 deficiency arrests hematopoietic stem cell differentiation, ATP export from mitochondria and cell nucleus energetics leading to immunodeficiency and hearing loss. Cultured cells survive if a single phosphotransfer enzyme – AK2 or CKmit is present in the intermembrane-intracristae space. AK2 deficiency in mice increases significantly CKmit expression to compensate for the loss of phosphotransfer capacity. Conditional liver, heart and whole body AK2-/- knockout mice have significant mitochondria, immune cell and metabolic abnormalities. Thus deficiency of catalyzed phosphotransfer in the mitochondrial cristae space disrupts continuum of energy flow and breaks resilience of cellular energetic system.

The mitochondrial nucleoside diphosphate kinase NME4/NM23-H4 has dual functions in bioenergetics and signaling death decisions

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Several mitochondrial proteins have dual functions in bioenergetics and death signaling. We have shown that this is also the case for the major mitochondrial isoform of the NME/nucleoside diphosphate kinase [NDPK] family, called NME4, NM23-H4 or NDPK-D [1,2]. NME4 is mostly located in the mitochondrial intermembrane space, bound to the inner membrane. The phosphotransfer activity of NME4 locally regenerates GTP for fueling the dynamin-related GTPase OPA1 [3] and yields ADP for stimulating respiration [4], both mechanism involving nucleotide channeling [5]. This will affect OPA1 functions in mitochondrial shape and fusion dynamics. In addition, NME4 has transmembrane lipid transfer activity that is able to externalize cardiolipin [CL] from the inner membrane to the mitochondrial surface, following either mitophagic [6] or apoptotic triggers [7]. This CL transfer relies on NME4/CL interaction, CL-dependent crosslinking of inner and outer mitochondrial membranes by the symmetrical, hexameric NME4 structure, and a putative NME4-based CL transfer pathway. Externalized CL then serves as an “eat me” signal for mitophagy by recruiting LC3-exposing autophagosomes, or in form of oxidized CL as a scaffold for recruiting and functionally altering different pro-apoptotic proteins. One factor inducing NME4-assisted CL transfer seems to be the loss of NME4/OPA1 complexes [6], possibly triggered by the known proteolytic cleavage of OPA1 after collapse of the inner membrane potential.

[1] Schlattner et al. [2017] Lab. Invest. [epub 2017 Oct 16].
[2] Schlattner et al [2015] Naunyn Schmiedebergs Arch. Pharmacol. 388, 271-8.
[3] Boissan et al. [2014] Science 344, 1510-5.
[4] Tokarska-Schlattner et al. [2008] J. Biol. Chem. 283, 26198-207.
[5] Zala et al. [2017] F1000 Research 6:724.
[6] Kagan et al. [2016] Cell Death Diff. 23, 1140-51.
[7] Schlattner et al. [2013] J. Biol. Chem. 288, 111-121.

Respiratory supercomplexes: physiological and pathological implications

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It was discovered over 50 years ago that the mitochondrial respiratory chain is constituted of a series of protein complexes embedded in the inner mitochondrial membrane [1]. Recent experimental evidence has ascertained that the major respiratory complexes involved in energy conservation are assembled as stoichiometric supramolecular units (supercomplexes, SCs) based upon specific, though dynamic, interactions between individual enzyme complexes. Respiratory SCs have been revealed and characterized in several tissues in many organisms. However, the functional role of SCs is less well defined, and still open to discussion (cf. [2] for review). Several lines of evidence favor the concept that electron transfer from Complex I to Complex III operates by channeling of electrons through Coenzyme Q molecules bound to the SC I_1III_2, in contrast with the previously accepted hypothesis that the transfer of reducing equivalents from Complex I to Complex III occurs via random diffusion of the Coenzyme Q molecules in the lipid bilayer. On the contrary, electron transfer from Complex II to Complex III and from Complex III to Complex IV seems to operate, at least in mammals, by random diffusion of intermediate substrates between the partner enzymes. Furthermore, another property provided by the SC I_1III_2 assembly is the control of generation of reactive oxygen species by Complex I [3], which might be important in regulation of signal transduction from mitochondria. Some physiological and pathological implications of the supercomplex assembly of the respiratory chain will be discussed in this presentation.

References:

[1] Green D.E., Tzagoloff A., (1966): The mitochondrial electron transfer chain, Arch. Biochem. Biophys. 116, 293-304.

[2] Lenaz G., Tioli G., Falasca A.I., Genova M.L., (2017): Respiratory supercomplexes in mitochondria, *in Mechanisms of Primary Energy Transduction in Biology (Ed. M. Wikström)* The Royal Society of Chemistry (RSC), London (UK), vol. 12, pp. 296-337.

[3] Maranzana E, Barbero G, Falasca AI, Lenaz G, Genova ML, (2013): Mitochondrial respiratory supercomplex association limits production of reactive oxygen species from complex I, Antioxid Redox Signal. 19, 1469-1480.

^{18}O-assisted mass spectrometry for mitochondrial dynamics evaluation

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Metabolomic profiling of disease states mandates comprehensive and simultaneous systematic fingerprinting of multiple metabolites. These are to be identified and quantified along with their cellular and systemic variations in response to diseases, drugs, toxins and lifestyle, as well as in the context of genetic or environmental challenges. Characterization of metabolic phenotype requires knowledge not only of metabolite levels but also of their turnover rates from which metabolic fluxes. Especially, many metabolites are present in low concentrations and associated with high flux/turnover rates through the metabolite pools, significant changes in metabolic flux could occur without apparent changes in metabolite concentration. Therefore, dynamic metabolomic profiling and flux measurements are essential for a complete understanding of metabolic phenotypes. Future next generation metabolomic screening requires development of technologies for high throughput and robust monitoring of metabolite levels, their turnover rates and flux distribution in large scale metabolic networks.

The analysis including not only metabolomic concentration but also metabolomic turn over rates is called fluxomics. Stable isotope (^{13}C, ^{15}N and ^{18}O) tracer-based metabolomic technologies allow simultaneous determinations of metabolite levels and their turnover rates with subsequent evaluation of metabolic network dynamics. ^{18}O stable isotope traces, which have many advantages than the others such as short labeling time, a unique information on phosphor metabolism and cell signaling. Stable isotope ^{18}O-based technology uniquely allows simultaneous measurements of metabolite levels and their turnover rates in cell, tissue, organ and plasma by mass spectrometry (GC-MS and LC-qTOF-MS) and ^{31}P NMR. In this presentation, the latest development on ^{18}O labeling ratio analysis of phosphometabolites and non- phosphometabolites using mass spectrometry will be presented. The details of analytical method developed in our laboratory and precautions for analysis and sample preparations will also be given in the presentation.

Keywords: ^{18}O-labeling, fluxomic, phosphometabolomic, GC-MS, High resolution mass spectrometry, LC-qTOF-MS

This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) (Project no: 116Z292).

Mitochondrial oxidation in rat spleen following the short- and long-term dietary ^{137}Cs intake

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Spleen is important organ for immunity and hemopoiesis, and is also very sensitive to radiation. Living in territories, contaminated with ^{137}Cs radionuclides, is accompanied with the risk of ^{137}Cs incorporation and development of pathology.

Simulation of ^{137}Cs incorporation was carried out by feeding the experimental animals for a period from 1 week to 2 months with radioactive food (dried mushrooms with ^{137}Cs activity of 39 kBq/kg and wild boar meat with ^{137}Cs activity of 600 kBq/kg). The control group of animals received a corresponding “clean" diet. 7 experimental groups were formed with the accumulation levels of 60, 170, 290, 420, 600, 1500 and 17000 Bq/kg, which corresponds to absorbed radiation doses of 1.5, 2.5, 4 , 8, 11, 170, 1950 mcGy. Dosimetry control was performed on a scintillation gamma spectrometer LP 4900B (Finland). On the last day of the experiment the animals were decapitated, and the spleen was extirpated, cooled, washed, stored in cool Hanks’ solution (0-2°C), and plungered. In the resulting preparation the oxygen consumption, as well as other parameters of tissue respiration, were analyzed with polarography method (PU-1 polarograph, Belarus) and Clark electrode in the thermostated 2 ml cell, 25°C. [1]

At a short-term intake of ^{137}Cs (incorporation levels 170, 290 and 420 Bq/kg), a dissipative, wasteful energy type is formed in the spleen of animals, which manifests itself as a sharp stimulation of respiratory activity of splenocytes on the background of oxidative phosphorylation (OP) uncoupling. An increased role of endogenous succinic acid at the level of incorporation of 600 Bq/kg and subsequent inhibition of succinate oxidation promotes its endogenous pool increase upon accumulation of 600 Bq/kg, which is accompanied by fatty acids β-oxidation intensification.

After Increased and prolonged (during 4 weeks) contamination with ^{137}Cs up to 1500 and 17000 Bq/kg the changes in tissue respiration and OP of the spleen of both groups of animals varies insignificantly. The absence of dose-dependent effects in the low-dose level, and non-monotonous (bimodal) dependence of radiation effects are supposed.

In our opinion, the incorporated ^{137}Cs impacted the membrane complex of splenocytes, also mitochondrial one. The OP uncoupling co-occurred with the decreased respiratory activity, whereas at lower levels of ^{137}Cs incorporation the uncoupling process occurred after the spleen respiratory activity stimulation.

[1] Gritsuk A.I., Kader A., Koval A.N., Sergeenko S.M., Svergun V.T., (2008): The influence of vitamins A, E, C upon the respiration activity of the lymphocytes of the spleen. Voprosy pitaniia, Vol. 77 (1), 26-29.

The eventual role of mitochondrial DNA guanine quadruplexes in sensitivity to ionizing radiation

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Mitochondrial DNA is known to be prone to damage by reactive oxygen species, produced among all during ionizing radiation. This effect can be explained by weak reparation systems in mitochondria, high density of information written in mitochondrial DNA, compared to the nuclear one.

The mitochondrial heavy guanine-rich strand (H-strand) may form guanine quadruplexes (G4) DNA, characterized by planar stacks of 4 guanines interacting by nonconventional Hoogsteen hydrogen bond. These formations can be found in both DNAs: nuclear and mitochondrial ones. And in mitochondrial DNA their occurrence is 10-fold higher compared to the nuclear. Usually mitochondrial G4 DNA can be found near the sites of deletion [2].

To find out the possible sites of G4 in human mitochondrial DNA we used *gquad* package for R program [1], which was designed for prediction of G quadruplexes and other non-B DNA motifs. There were found 28 G4 sites (67 – if overlapping was considered). The CO1 gene contains the most G4 sites. Other researcher found 270 G4 motifs in human mitochondrial DNA [2].

In our experiments we found the increased frequency of common deletion in rat myocardium mitochondrial DNA following 0.5 Gy total-body gamma-irradiation. The percentage of common deletion in intact rats was 3.52±0.26, and in irradiated animals on the 3^{rd} and 90^{th} days it significantly increased accordingly to 6.36±0.86 and 7.13±0.45. With an increase in the irradiation dose up to 1 Gy, the percentage values were 3,52±0,26 for intact rats, 4,24±0,61 on 3^{rd}, and 5,04±1,16 on 90^{th} day after irradiation, but the differences were not statistically significant.

We can suppose the presence of significant G4 sites in mitochondrial DNA make it prone to damaging action of ionizing radiation. This can be explained by accumulation of ^{137}Cs in G4-rich regions of mitochondrial DNA, also by genomic instability in the vicinity of G4 sites.

References:

[1] https://cran.r-project.org/package=gquad

[2] Bharti S.K., et al. (2014): DNA sequences proximal to human mitochondrial DNA deletion breakpoints prevalent in human disease form G-quadruplexes, a class of DNA structures inefficiently unwound by the mitochondrial replicative Twinkle helicase - The journal of biological chemistry. Vol. 289, No. 43. - 29975–29993.

Cellular ATP turnover and its modulation during inflammation, hypoxia and tumorigenesis

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Current models of cellular purine turnover depend of functional interactions between distinct processes, including (i) the release of endogenous ATP and/or ADP, (ii) triggering of signaling events via a series of nucleotide- and nucleoside-selective receptors, (iii) ectoenzymatic interconversion of extracellular nucleotides, (iv) cellular uptake of nucleotide-derived adenosine and other nucleosides via equilibrative nucleoside transporters and finally, (v) intracellular interconversion of the transported nucleosides into ATP through complex phosphotransfer reactions [1]. Recent data further extend this model by demonstrating the complex implication of both extrinsic and intrinsic mechanisms into a tuned adenosine-dependent control of energetic balance and signal transduction pathways in tumor cells [2] and in vascular endothelial cells [3]. Despite the significant progress in our understanding of the purinergic machinery as a multistep cascade, current knowledge on the whole sequence of "release-signaling-inactivation" remains rather limited. I summarize recent advances in this rapidly evolving field, with particular emphasis on ATP-inactivating and –regenerating pathways at such (patho)physiological states as inflammation, hypoxia, vascular remodeling and tumorigenesis.

References:

[1] Yegutkin, G.G. (2014): Enzymes involved in metabolism of extracellular nucleotides and nucleosides: Functional implications and measurement of activities, Crit. Rev. Biochem. Mol. Biol. 49, 473-497.

[2] Virtanen, S.S., Kukkonen-Macchi, A., Vainio, M., Elima, K., Härkonen, P.L., Jalkanen, S., Yegutkin, G.G. (2014) Adenosine inhibits tumor cell invasion via receptor-independent mechanisms, Mol. Canc. Res. 12, 1863-1874.

[3] Losenkova, K., Zuccarini, M., Helenius, M., Jacquemet, G., Gerasimovskaya, E., Tallgren, C., Jalkanen, S., Yegutkin, G.G. (2018) Endothelial cells cope with hypoxia-induced depletion of ATP via activation of cellular purine turnover and phosphotransfer networks, BBA Mol. Basis Dis.1864, 1804-1815.

Mitochondria-related molecular changes in sarcopenia

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Sarcopenia is termed as the age-associated loss of skeletal muscle mass and function leading to muscle fragility [1]. It is multifactorial disease and mitochondria are of great importance regarding to this disorder. Identification of new factors and molecular mechanisms that control skeletal muscle development and physiology has essential involvement for improving human health. On this basis, the purpose of the present study was to find out molecular changes of cellular energy metabolism in aged muscle to understand how mitochondria are linked to pathogenesis of sarcopenia in elderly. The biopsies from *m. vastus lateralis*. of young subjects (20-30 years) and old subjects with sarcopenia (70-80 years) were studied by real-time PCR, spectrophotometric and oxygraphic methods. Our studies revealed decreased mitochondrial respiration normalized to tissue wet weight (-36%, $p<0.05$) which was diminished by normalization to citrate synthase activity (-14%, NS). No significant changes of mitochondrial to nuclear DNA ratio was observed in different developmental stages of muscle cells (myoblasts, myotubes, mature myocytes) from old subjects compared to young ones. Mitochondrial DNA content was consistent with citrate synthase activity promising to assume unchanged mitochondrial biogenesis. This statement seems to be supported by 1) unchanged expression of transcription factors PPARγ and PGC1α which are known to be responsible for mitochondrial biogenesis and 2) similar expression level of mitochondrial respiration chain components encoded by nuclear DNA in sarcopenic muscles (old subjects). At the same time, the expression of mitochondrial kinases (e.g. CKMT2) and muscle-specific CKM gene was significantly decreased ($p<0.05$) in *m. vastus lateralis* of them. Thus, it can be concluded that decreased oxidative phosphorylation in ageing-induced sarcopenia was caused by decreased expression of energy metabolism-regulating kinases, namely the components of CK, AK and HK energy transfer system. Taken together, these changes certainly lead to decreased cellular energy supply in sarcopenic skeletal muscle.

References:

[1] Cruz-Jentoft, A. J., Landi, F., Topinková, E., Michel, J. P. (2010): Understanding sarcopenia as a geriatric syndrome, Curr Opin Clin Nutr Metab Care, 13, 1-7.

Selective elimination of senescent cells by mitochondrial targeting is regulated via ANT2

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Cellular senescence is a form of cell cycle arrest that limits the proliferative potential of cells, including tumour cells. However, inability of immune cells to subsequently eliminate senescent cells from the organism may lead to inflammation, carcinogenesis or development of age-related diseases.

Mitocans are agents with anti-cancer activity that induce apoptosis of malignant cells via targeting mitochondria. We have developed several highly specific mitocans with selective mitochondrial uptake driven by high mitochondrial potential of cancer cells. Although these agents were intended to eliminate malignant cells, their potential efficacy in targeting cells with increased mitochondrial potential, such as senescent cells, make them intriguing candidates for senolytic agents.

We found that MitoTam, unlike conventional anti-cancer agents, not only kills cancer cells without inducing senescence *in vitro* and *in vivo*, but also selectively eliminates both malignant and non-cancerous senescent cells. In naturally aged mice treated with MitoTam, we observed a significant decrease of senescent markers in all tested organs compared to non-treated animals. Mechanistically, we found an important role of adenine nucleotide translocator 2 (ANT2) in survival of cells treated with MitoTam. Restoration of ANT2 in senescent cells resulted in their resistance to MitoTam, while its downregulation in non-senescent cells sensitized these cells to both MitoTam and oligomycin A as well as CCCP, which underscores the crucial importance of the interplay between ANT2, ATP synthase and the level of mitochondrial potential in maintenance of mitochondrial structure and integrity. The key finding presented here show that simultaneous interference with mitochondrial integrity and ATP homeostasis in senescent cells leads to their effective removal.

The ability to pharmacologically eliminate senescent cells opens the door to study their role in a wide range of relevant (patho)physiological settings and brings a new strategy for the treatment of age-related pathologies or senescence-associated tumorigenesis.

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Studies of creatine deficient mice: from respiration to EC coupling

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Creatine kinase (CK) system is known as a major regulator of high- energy phosphoryl buffering and transfer system between mitochondria and ATPases. Functional coupling between CK and adenine nucleotide translocase, on mitochondrial side of energy transfer system, coupling between ATPases and CK, ATP-sensing KATP-channel and CK, have been demonstrated by others earlier suggesting a major role of CK system in energy transfer in the heart. Disruption of the creatine kinase (CK) system in hearts of CK-deficient mice leads to changes in the ultrastructure and regulation of mitochondrial respiration. We expected to see similar changes in two lines of creatine-deficient mice, which lack one of the enzymes on creatine synthesis pathway: guanidinoacetate methyltransferase (GAMT) or arginine-glycine amidinotransferase (AGAT). According to our earlier data on respiration of permeabilized cardiomyocytes recorded at different conditions, intracellular mitochondrial organization, and other parameters in GAMT knockout mice, with no significant differences found between knockouts and their wildtype littermates, there are no major adaptations to creatine deficiency in these mice. Here, we present our analysis of mitochondrial respiration and positioning on AGAT knockouts and look into the differences in excitation- contraction coupling between mice with and without functional creatine kinase system.

Regulation of activity of mitochondrial potassium channels

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Several potassium channels have been found in the inner mitochondrial membranes of various cells. One of them is mitochondrial large conductance calcium activated potassium channel (mitoBK$_{Ca}$), which is formed by so called DEC splice variant of KCNMA1 gene. The other, Kir1.1b, is a splice variant of KCNJ1 gene, which forms a mitochondrial potassium channel inhibited by ATP (mitoK$_{ATP}$). Both channels reside in the mitochondrial inner membrane, providing a way to regulate mitochondrial membrane potential and ROS production. These channels retain basic pharmacological properties known for their splice variant residing in the plasma membrane, making them a difficult target for specific modulation by drugs.

The knowledge of the molecular identity of mitochondrial channels is relatively new and even less is known about their specific protein partners. However, physical interactions with other mitochondrial proteins could modify the functioning of mitochondrial channels including modulation of their activity, as it was demonstrated by our group in the case of mitoBK$_{Ca.}$ We showed by means of blue native electrophoresis that mitoBK$_{Ca}$ interacts with mitochondrial respiratory chain and activity of mitoBK$_{Ca}$ was modulated by respiratory chain substrates [1]. Therefore, characterization of interactoms of mitochondrial channels plays an important role in our current studies. We use proximity biotynylation (Bio-ID), which allows for identification of transiently and weakly interacting proteins.

In our preliminary studies, we have identified potential candidates for Kir1.1b partners, which were known to be associated with the inner mitochondrial membrane. Some of these proteins indicate for functional network that might indirectly regulate activity of mitochondrial channels e.g. via gasotransmitters like H_2S.

This work was supported by the Polish National Science Center, grants no. 2015/19/B/NZ1/02794 to P.K. and 2015/17/B/NZ1/02496 to A.S.

[1] Bednarczyk, P., Wieckowski, M.R., Broszkiewicz, M., Skowronek, K., Siemen, D., Szewczyk, A., (2013): Putative Structural and Functional Coupling of the Mitochondrial BK$_{Ca}$ Channel to the Respiratory Chain, PLoS One 8(6):e68125.

Chemiosmotic control of the proton leak: the fundamental distinction of force and pressure

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The protonmotive force and the proton leak belong to the most fundamental components of theoretical and experimental bioenergetics. While the interest in membrane potential and proton gradients has peaked by 1974, publications increased exponentially on mitochondrial research during the last 20 years, without accompanying advancement of the physicochemical foundations. '*.. the coupling membrane has a low permeability to ions generally and not only to protons, so that the electron transport and ATPase systems could be coupled through the sum of the* ***electrical pressure difference*** *and the* ***osmotic pressure difference*** *(i.e. the* ***electrochemical potential difference****) of protons that would thus be conserved across the membrane*' (Mitchell 1966 [1]). Why should mitochondrial physiologists and biochemists suspect that there is a need for clarification of the nature of forces, potentials and pressures and their relationships to the fluxes? For understanding the non-linear ('non-ohmic') relationship between the proton leak and the protonmotive force, two concepts need to be addressed, (*1*) the distinction between continuous potential gradients across a membrane connecting compartments, *versus* discontinuous potential differences between compartments separated by a membrane; and (*2*) the contrast between force and pressure. The protonmotive force is conceived as the discontinuous electrochemical potential difference between the negatively charged matrix and the positively charged outer compartment. The advancement of proton diffusion towards equilibrium is compensated for by proton pumping fueled by electron transfer at steady-state. In continuous diffusion along the geometric direction of a concentration gradient, the vector force is the chemical potential gradient which is counteracted by the resistance of diffusion. Flux across a unit membrane area perpendicular to the direction of the gradient is not only a function of the force which controls the velocity of the diffusing particle, but also of the local concentration of the diffusing particles (Einstein 1905 [2]). The chemical potential gradient [$J \cdot mol^{-1} \cdot m^{-1}$] times the local concentration [$mol \cdot m^{-3}$] yields the chemical pressure gradient, $d_d\boldsymbol{\Pi} \cdot d\boldsymbol{z}^{-1}$ [$J \cdot m^{-3} \cdot m^{-1}$ = $Pa \cdot m^{-1}$], and flux across the unit area [$mol \cdot s^{-1} \cdot m^{-2}$] is a linear function of the pressure gradient, but varies as a non-linear function of the force. When applied to a discontinuous two-compartmental system of constant volume $V = V_A + V_B$ [m^3], flux of diffusion [$mol \cdot s^{-1} \cdot m^{-3}$] is a linear function of the chemical pressure difference, $\Delta_d\Pi$ [$J \cdot m^{-3}$ = Pa], and $\Delta_d\Pi$ is the product of the force (chemical potential difference [$J \cdot mol^{-1}$]) and a concentration term (free activity [$mol \cdot m^{-3}$], comparable to the local concentration of continuous diffusion) [3]. The linear flux-Δ pressure law of diffusion is generalized to include electrochemical pressure, revealing proton flux as a linear and proportional function of chemiosmotic pressure. The principle of chemiosmotic pressure does not contradict complex kinetic models that have been tuned to fit empirical results, but reveals non-linear flux-force relations as the rule, with linear and proportional flux-force dependence as possible although special cases. The fundamental distinction between flux-pressure and flux-force relations provides a solid bridge between thermodynamics and kinetics [3], as an outstanding example of the importance of rigorous terminological and conceptual definitions [4].

1. Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Glynn Research, Bodmin. Biochim Biophys Acta Bioenergetics 1807 (2011):1507-38.
2. Einstein A (1905) Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. Ann Physik 4, XVII:549-60.
3. Gnaiger E (1989) Mitochondrial respiratory control: energetics, kinetics and efficiency. In: Energy transformations in cells and organisms. Wieser W, Gnaiger E (eds), Thieme, Stuttgart:6-17. - http://www.bioblast.at/index.php/Gnaiger_1989_Energy_Transformation
4. MitoEAGLE preprint 2018-04-20(Version 38). Mitochondrial respiratory states and rates: Building blocks of mitochondrial physiology Part 1.
   - http://www.mitoeagle.org/index.php/MitoEAGLE_preprint_2018-02-08

Control of the NADPH supply and GSH recycling for oxidative stress management in hepatoma mitochondria

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To unveil what controls mitochondrial ROS detoxification, the NADPH supply and GSH/GSSG recycling for oxidative stress management was analyzed in cancer and non-cancer mitochondria. Therefore, proteomic and kinetomic analyses were carried out of the mitochondrial (i) NADPH producing enzymes (IDH-2, GDH, ME, TH); and (ii) GSH/GSSG recycling enzymes (GR, GPx-1, GPx-4, TrxR-2) associated to oxidative stress management. The protein contents of the eight enzymes were similar or even higher in AS-30D hepatoma mitochondria (HepM) than in rat liver (RLM) and rat heart (RHM) mitochondria, suggesting that the NADPH/GSH/ROS pathway was fully functional in cancer mitochondria.

The *Vmax* values of IDH-2 were much greater than those of GDH, TH and ME, suggesting that IDH-2 is the predominant NADPH producer in the three mitochondrial types; in fact, GDH reverse reaction was thermodynamically and kinetically favored. In addition, the *Vmax* for IDH-2 was 7-fold higher in HepM than in RLM. The *Vmax* values of GR and GPx were lower in HepM than in RLM, suggesting that the oxidative stress management is compromised in cancer mitochondria. The *Km* values of IDH-2, GR and GPx were all similar among the different mitochondrial types.

With the kinetome data, computational models of this mitochondrial NADPH/GSH/ROS pathway were built and further validated by their accurate prediction of the intermediary metabolite concentrations. Thus, the complex interplay among the different enzymes and metabolites of the pathway can be better understood and the control mechanisms identified. Kinetic modeling revealed that the oxidative stress management was mainly controlled by GR, GPx and IDH-2 in the three mitochondrial types. Modeling also revealed that, due to their lower GPx activity presumably by extensive acetylation, HepM (i) required higher peroxide concentrations to achieve reliable steady-state fluxes and metabolite concentration; and (ii) endured higher peroxide concentrations without collapsing their GSH/GSSG ratios. Then, to specifically prompt lower GSH/GSSG ratios under oxidative stress thus compromising cancer mitochondria functioning, GPx should be re-activated.

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VDAC1 as a Target: Crossing the Aisle from Cancer to Neurodegeneration and Diabetes

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VDAC1 is a multi-functional protein that mediates the fluxes of nucleotides and metabolite, hemes, cholesterol and ions, including Ca^{2+}, across the outer mitochondrial membrane (OMM). VDAC1 is considered as a hub protein, interacting with over 150 proteins that regulate the integration of mitochondrial functions with other cellular activities. Thus, VDAC1 serves as a mitochondrial gatekeeper, controlling metabolic and energetic cross-talk between mitochondria and the rest of the cell and a key player in mitochondria-mediated apoptosis. Indeed, VDAC1 has been shown to be involved in many diseases including cancer, neurodegenerative diseases as Alzheimer's disease (AD), Parkinson's disease, ALS, and type 2 diabetes (T2D).

We have developed VDAC1-based strategies for treating cancer and other diseases involving dysregulated metabolism and/or apoptosis and in which VDAC1 is over-expressed. We have developed VDAC1-specific siRNA, VDAC1-based peptides and small molecule as VDAC1 inhibitors and validated their effects both in vitro and in mouse models for specific diseases.

We demonstrated that in cancer, depleting VDAC1 using si-RNA resulted in reprogrammed metabolism, while VDAC1-based peptide promote apoptosis. Both treatments lead to a multi-pronged attack on cancer hallmarks, inhibiting cell proliferation, tumor growth, invasion and angiogenesis, and inducing cancer stem cells differentiation. As VDAC1 over-expression was shown to lead to apoptotic cell death, and VDAC1 is overexpressed in neurodegenerative disease as AD, we developed new VDAC1-specific small molecules, VBIT-4 and VBIT-12, interfering with VDAC1 pro-apoptotic action and prevented apoptosis and associated processes, with no effect on cells under physiological conditions. The novel VDAC1 inhibitors improved cognition, learning and memory of Alzheimer's disease-like model mice, and in type-2 diabetes db/db mouse model, they decreased blood glucose and increased blood insulin. Thus, VDAC1 is a novel, yet unexploited target, providing an innovative conceptual framework for new therapeutic paradigms for treating cancer, neurodegeneration, diabetes, heart disease and aging.

Mitochondria as a target for sex differences in pathologies

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It is increasingly acknowledged that a sex and gender specificity affects the occurrence, development, and consequence of a plethora of pathologies. Mitochondria are considered as the powerhouse of the cell because they produce the majority of ATP but they also participate in many other functions like steroid hormone synthesis, reactive oxygen species production, ionic regulation, and cell death. Adequate cellular energy supply and survival depend on mitochondrial life cycle, a process involving mitochondrial biogenesis, dynamics, and quality control via mitophagy. It appears that mitochondria are the place of marked sexual dimorphism involving mainly oxidative capacities, calcium handling, and resistance to oxidative stress. In turn, sex hormones regulate mitochondrial function and biogenesis. Mutations in genes encoding mitochondrial proteins are the origin of serious mitochondrial genetic diseases. Mitochondrial dysfunction is also an important parameter for a large panel of pathologies including neuromuscular disorders, encephalopathies, cardiovascular diseases (CVDs), metabolic disorders, neuropathies, renal dysfunction etc. Many of these pathologies present sex/gender specificity. Here we review the sexual dimorphism of mitochondria from different tissues and how this dimorphism takes part in the sex specificity of important pathologies mainly CVDs and neurological disorders.

One of the important examples is cardiotoxicity induced by doxorubicin, widely used as an antineoplastic agent. We have shown that in rats, doxorubicin treatment resulted in sex differences characterized in males but not on females by (1) important weight loss and decrease in survival rate, (2) strong alterations of myocardial function, (3) decrease in energy signaling pathways, (4) downregulation of mitochondrial biogenesis, (5) decrease in cardiolipin content, (6) decrease in mitochondrial DNA content, and (7) and alteration of mitochondrial respiration. The study shows that cardiac mitochondrial dysfunction seems to be critical site of sex differences in cardiotoxicity. In general, the advantage of preserved mitochondrial function in females involves the positive action of estrogens but may also be driven by the evolutionary selection of mitochondria in the female background. Further research should consider both sexes not only to better understand the pathophysiology of the diseases but also to prompt personalized therapeutic interventions. Taking into account sex specificity in ageing and pathologies would allow developing more focused drugs and therapeutic strategies. Studies are thus necessary for delineating the consequences of mitochondrial sex specificity in the pathophysiology of chronic diseases and to elaborate new therapeutic interventions.

Mitochondrial function as therapeutic target site for metastatic cancers

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The metabolic reprogramming in metastatic cancer cells is related to an elevated glycolytic rate mediated by the activation of some transcriptional factors (HIF-1α) and oncogenes (c-MYC, h-RAS). In malignant cancers, the glycolysis activation has been frequently associated with an impaired mitochondrial function. However, it has been recently demonstrated that metastatic cancer cell lines grown as monolayers or microspheroids show a functional oxidative phosphorylation (OxPhos) [1] which provides more that 75% of the ATP required for growth and other cell functions. As a higher electronegative mitochondrial membrane potential has been observed in cancer cells *vs.* non-cancer cells, it seems plausible to target cancer mitochondria with lipophilic cations such as casiopeina II-gly, rhodamines and vitamin E-phosphonium derivatives. Indeed, these lipophilic cations at submicromolar, therapeutic doses inhibited cancer cell growth, decreased mitochondrial protein contents and abolished OxPhos enzyme activities and flux, with no apparent effect on non-cancer cells. Therefore, anti-mitochondrial therapy emerges as a complementary and successful strategy for metastatic cancer treatment.

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References:

[1] Moreno-Sánchez, R.; Marín-Hernández, A.; Saavedra, E.; Pardo, J. P.; Ralph, S. J.; Rodríguez-Enríquez, S. (2014) Who controls the ATP supply in cancer cells? Biochemistry lessons to understand cancer energy metabolism. Int. J. Biochem. Cell Biol. 50, 10-23.

Standard static markers do not explain breast cancer metabolic alterations

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Genomic, proteomic and other static predictive biomarkers have shown limited benefit in large patient cohorts in cancer studies. As a result, current precision medicine is able to select only minority of patients for treatments and majority of them are left aside. Therefore, without questioning the role of genomic methods, it is evident that additional tools are necessary to make further segregation among the majority of patients who currently are predicted to be non-responders or are not correctly placed into prognostic groups. Here, using functional analysis of mitochondrial metabolism in breast cancer cell lines and human samples, we show that metabolic function of this organelle cannot be predicted based on static properties like abundance of certain central enzymes or even number of mitochondria. In addition, multivariate tests reveal whether mitochondria in a given sample prefer pyruvate or glutamine as the main carbon source and how these ratios relate to prognosis of the disease. Taken together, functional biomarkers can become highly valuable for understanding underlying disease biology and have prospective to become usable for segregating patients into specific predictive and prognostic subgroups.

Reactivation of dihydroorotate dehydrogenase by respiration restores tumor growth of mitochondrial DNA-depleted cancer cells

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Cancer cells without mitochondrial DNA (mtDNA) need to reconstitute oxidative phosphorylation (OXPHOS) by acquisition of host mitochondria to form tumors (1,2), but the reasons why functional respiration is crucial for tumorigenesis remain unclear. Using time-resolved analysis of the initial stages of tumor formation by mtDNA-devoid cells and genetic manipulations of OXPHOS components, we now show that pyrimidine biosynthesis, supported by the respiration-linked dihydroorotate dehydrogenase (DHODH), is strictly required to overcome cell cycle arrest, while mitochondrial ATP generation is dispensable for tumorigenesis. Primed DHODH is present in mtDNA-devoid cells and becomes fully active by complex III/IV respiration after mitochondrial transfer, or by the introduction of an alternative oxidase. Conversely, DHODH deletion interferes with tumor formation even in cells with functional OXPHOS, whereas disruption of mitochondrial ATP synthase has little or no effect. Collectively, our results show that pyrimidine biosynthesis via DHODH is the essential pathway that links respiration to tumorigenesis.

1. Tan, A. S., Baty, J. W., Dong, L. F., Bezawork-Geleta, A., Endaya, B., Goodwin, J., Bajzikova, M., Kovarova, J., Peterka, M., Yan, B.*, et al.* (2015). Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. Cell Metab *21*, 81-94.
2. Dong, L. F., Kovarova, J., Bajzikova, M., Bezawork-Geleta, A., Svec, D., Endaya, B., Sachaphibulkij, K., Coelho, A. R., Sebkova, N., Ruzickova, A.*, et al.* (2017). Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. Elife *6*.

Doxorubicin cardiotoxicity: mechanistic insight by phosphoproteomics

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Doxorubicin belongs to the most efficient anticancer drugs, but its use is limited by a risk of severe cardiotoxicity, which is not completely understood. We addressed potentially involved signaling mechanisms by analyzing doxorubicin-induced changes in the Langendorff-perfused rat heart using non-biased and targeted phosphoproteomics. 2D-gel-MS analysis revealed significant drug-induced changes in the phosphoproteome. An overrepresentation of mitochondrial proteins (>40%) suggested this compartment as a prime target. Identified proteins were mainly involved in energy metabolism (e.g. pyruvate dehydrogenase), sarcomere structure/function (e.g. desmin) and chaperone-like activities, thus explaining energy imbalance and myofibrillar disorganization observed in cardiotoxicity. Targeted analysis of AMP-activated protein kinase (AMPK) signaling revealed its paradox inhibition by doxorubicin, despite combined energetic, oxidative, and genotoxic stress which should activate this pathway. Such negative feedback increases cellular energy deficits and could contribute to the pathological cardiac phenotype.

A novel spontaneous CYTB variant exacerbates respiratory chain complex III deficiency in mice

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BCS1L, frequently mutated in respiratory chain complex III (CIII, cytochrome bc_1) deficiency, is required for Rieske iron-sulphur protein (RISP) assembly into CIII. Knock-in mice carrying a homozygous *Bcs1l*$^{p.S78G}$ patient mutation display growth restriction, hepatopathy, kidney tubulopathy and premature lethality. We observed over four times longer survival (35d vs. >150d) of *Bcs1l*$^{p.S78G}$ mice in C57Bl/6JCrl as compared to an in-house C57BL/6JBomTac background. We performed whole genome sequencing and identified a novel homoplasmic mtDNA variant (*m.14904G>A*, *Cytb*$^{p.D254N}$) in the short-lived colony. The variant affects a highly conserved negatively charged amino acid in the RISP-interacting region of the CYTB subunit of CIII. We show that the mtDNA haplotype dictated the survival as homozygous *Bcs1l*$^{p.S78G}$ progeny of females carrying *Cytb*$^{p.D254N}$ mitochondria survival to median P38 whilst those of females carrying wild-type mitochondria to P151. *Cytb*$^{p.D254N}$ further decreased the low CIII activity and respiration in *Bcs1l*$^{p.S78G}$ mice, explaining their rapid decline. Remarkably, *Cytb*$^{p.D254N}$ alone decreased CIII activity and respiration in liver mitochondria, and the weight of juvenile mice. In published crystal structures of CIII the Fe-S domain of RISP brushes the *ef* loop segment of CYTB and docks to either the CYTB or CYTC1 subunit to promote electron transfer. However, BNGE analysis showed that the low RISP steady-state level in CIII in *Bcs1l*$^{p.S78G}$ mitochondria was not further compromised by *Cyb*$^{p.D254N}$. Seeking an alternative mechanism, we performed fully atomistic classical molecular dynamics simulations of cytochrome bc_1. These showed restricted conformational flexibility of the mutant *ef* loop, potentially affecting RISP kinetics. To test this, we utilized isolated *Rhodobacter capsulatus* cytochrome bc_1 complex and show that the mutation stiffened the ef loop with consequent longer occupancy of positions towards the Qo site by the RISP Fe-S domain. To the best of our knowledge, this is the first case of a mitonuclear epistasis directly affecting any mitochondrial enzyme. Given that no technology to introduce specific mutations to mtDNA exist, we envision that the mice carrying *Cytb*$^{p.D254N}$ will become a valuable tool in studies of CIII function.

Hypothermia augments stress response in mammalian cells

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Clinical hypothermia is considered as one of the most effective intervention for a range of hypoxic ischemic pathologies of the central nervous system, however, the mechanisms that underlie its protective effects are uncertain. In order to increase the effectiveness of therapeutic hypothermia, we took a step back and asked whether hypothermia could modulate the responsiveness of mechanisms coping with cellular stressors.

We used systems approach based on genome-wide expression screens, reporter assays and biochemical studies, and found that hypothermia leads to the activation of molecular systems enhancing oxidative stress and hypoxic stress tolerance, while not activating unfolded protein and inflammatory responses.

Our study demonstrates for the first time that mild hypothermia (32°C) activates major transcription factors Nrf2 and HIF1A, which orchestrate adaptive responses to hypoxic stress. Currently, it is widely accepted that therapeutic effects of hypothermia are due to thermodynamic effects of metabolic depression. However, our results suggest that hypothermia activates specific signaling pathways leading to increased stress tolerance during oxygen restriction.

The results of this study gives a new insight to basic science and small animal based research and highlights the importance of the cellular stress response systems as potential targets of clinical hypothermia.

Alternative oxidase prevents lethal mitochondrial cardiomyopathy by restoring respiration

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Mitochondrial respiratory chain (RC) deficiencies are among the most prevalent of inborn errors of metabolism and often fatal, but largely lack treatments and therapeutic strategies. Upon RC blockade by toxins or disease-causing mutations, ectopic alternative oxidase (AOX) can restore electron flow, reduce reactive oxygen species (ROS) and rescue lethality in fruit flies, mammalian cells, and mice. We crossed viable RC complex III (CIII) deficient *Bcs1l* mutant (c.232A>G knock-in) mice, displaying multiple visceral manifestations and early death, with transgenic mice broadly expressing *Ciona intestinalis AOX*. The mutant mice expressing AOX were viable and remained growth-restricted but displayed dramatically increased median survival from P210 to P590. Respirometry showed that AOX was active in the symptomatic mutant mice, but not in *Bcs1l* wild-type mice, suggesting responsiveness to CIII dysfunction in the affected tissues. At P200, their kidney disease, and astrogliosis of the somatosensory cortex, but not hepatopathy, was partially rescued. Remarkably, AOX permanently rescued cardiac histopathology and dysfunction, which extended mice median survival to P590. In Heart, AOX mediated respiration normalized total CI+CII-linked respiration and slightly improved CIII activity. Indirect reactive oxygen species (ROS) measurements showed no changes in global ROS and it was not the main cause of cardiac dysfunction. Transcriptomics and targeted metabolomics showed significant presymptomatic (P150) changes in the mutant heart and these were largely prevented by AOX. In summary, AOX rescue effect in different organs related to their pathometabolism, and respiratory by-pass was sufficient to prevent cardiac energetic crisis and decompensation in the *Bcs1l* mutant model of fatal CIII deficiency.

Abstracts of poster presentations in the order of presenting author

Neurotrophic factor and ER residual protein DmManf interacts genetically with genes involved in the mitochondria and the ubiquinone synthesis pathway

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Mesencephalic Astrocyte-derived Neurotrophic Factor (MANF) belongs to an evolutionarily conserved family of neurotrophic factors. Previous studies have shown MANF support and protect brain dopaminergic system in non-cell-autonomous manner[1,2]. However, MANF has also been shown to function intracellularly in the endoplasmic reticulum[3,4]. As yet identified the MANF orthologues are the only neurotrophic factors with conserved amino acid sequence in invertebrates[5]. Still, the knowledge on the interacting partners of MANF and the involved signaling pathways is very limited.

Here we employed the Drosophila genetics to screen for potential interaction partners of Drosophila Manf (DmManf). Our screen involved about 2000 UAS-RNAi RNA silencing lines. In result, we discovered novel genetic interactions of DmManf with genes known to function in the mitochondria[6]. We further verified those interactions by applying DmManf overexpression and mutant backgrounds. Also we applied transgenic flies carrying UAS-mitoTimer transgene to follow the rate of mitogenesis. We will present our recent data from these experiments.

In conclusion, DmManf genetically interacts with several mitochondria-related genes. Our data supports the functional importance of these evolutionarily significant proteins and provides new insights for the future studies.

References:

[1] Petrova PS, Raibekas A, Pevsner J, Vigo N, Anafi M, Moore MK, Peaire AE, Shridhar V, Smith DI, Kelly J, Durocher Y, Commissiong JW., (2003): MANF: a new mesencephalic, astrocyte-derived neurotrophic factor with selectivity for dopaminergic neurons. J Mol Neurosci. 20:173–87.

[2] Lindholm P, Voutilainen MH, Laurén J, Peränen J, Leppänen V-M, Andressoo J-O, Lindahl M, Janhunen S, Kalkkinen N, Timmusk T, Tuominen RK, Saarma M., (2007): Novel neurotrophic factor CDNF protects and rescues midbrain dopamine neurons in vivo. Nature 448:73–7.

[3] Apostolou A, Shen Y, Liang Y, Luo J, Fang S. Armet, a UPR-upregulated protein, inhibits cell proliferation and ER stress-induced cell death. Exp Cell Res. 2008;314:2454–67.

[4] Palgi M, Greco D, Lindström R, Auvinen P, Heino TI. Gene expression analysis of Drosophila Manf mutants reveals perturbations in membrane traffic and major metabolic changes. BMC Genomics. 2012;13:134.

[5] Palgi M, Lindström R, Peränen J, Piepponen TP, Saarma M, Heino TI. (2009): Evidence that DmMANF is an invertebrate neurotrophic factor supporting dopaminergic neurons. Proc Natl Acad Sci U S A 106:2429–34.

[6] Lindström R, Lindholm P, Palgi M, Saarma M, Heino TI, (2017): In vivo screening reveals interactions between Drosophila Manf and genes involved in the mitochondria and the ubiquinone synthesis pathway. BMC Genet 18(1):52

Mitochondrially encoded humanin turned out to be related with sarcopenia

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Recent findings show that along with its protective function in the cell, mitochondrially-encoded peptide humanin can promote mitochondrial biogenesis, increase ATP level and respiratory rate in pancreatic β-cell line [1]. Even more, it has been proposed that humanin improves the crosstalk between endoplasmic reticulum and mitochondria, representing a novel function for this peptide [2]. It suggests, that reduced expression of humanin may contribute to some aging-related diseases where effective maintenance of mitochondrial functionality is gradually lost.

The decrease in mass and strength of muscles of elderly people is aptly termed sarcopenia. The present study was aimed to find out the effect of aging-related sarcopenia on humanin gene (MT-RNR2) expression in human *m. vastus lateralis*. The level of mRNA, transcribed from this gene was assessed by using real-time PCR method. The study revealed that mRNA expression was significantly lower ($p<0.05$) in the muscle of elderly people (69-79 years) compared to young ones (21-25 years), indicating 2,5-fold difference. Relative expression of MT-RNR2 appeared to be associated with plasma IGF-1 concentration of female subjects studied. It suggests that IGF-1 may regulate humanin level in skeletal muscle.

The results of the work allow to conclude:

1) Decreased humanin expression in the muscle could contribute to pathogenesis of sarcopenia, probably because of insufficient cellular protection resulting from diminished amount of mitochondria and reduced ATP production in the cells.

2) Pathophysiological changes in sarcopenic muscle may also result from disturbed communication between mitochondria and sarcoplasmic reticulum, where humanin has been demonstrated to have an important functional role.

This study complemented the current understanding of humanin function in aging- related diseases.

References:

[1] Qin, Q., Jin, J., He, F. et al. (2018): Humanin promotes mitochondrial biogenesis in pancreatic MIN6 β-cells, Biochem Biophys Res Commun 497, 292–297.

[2] Sreekumar, P.G., Hinton, D.R., Kannan. R. (2017): Endoplasmic reticulum-mitochondrial crosstalk: a novel role for the mitochondrial peptide humanin, Neural Regen. Res. 12, 35–38.

Paternal mtDNA is transmitted in several generations of laboratory mice

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Inheritance of mutant mtDNA and its distribution among organs in prenatal development is of key importance for onset of OXPHOS ('oxidative phosphorylation') diseases, featuring the failure of energy metabolism in affected tissues. Modeling OXPHOS diseases in animals might help disclosing their pathogenesis, clarifying the regularities of mtDNA distribution among tissues. Previously we obtained laboratory mice that developed from zygotes injected with human mitochondria. Human mtDNA was transmitted by transmitochondrial (TM) mice along the maternal lineage down to the 6^{th}-8^{th} generation. Of 42 males analyzed in F1, 25 (59.5±7.6%) showed the capacity to transmit foreign mtDNA to embryos. Of 19 males analyzed in F2, foreign mtDNA was transmitted to embryos by 14 males (73.7±10.1%). In F3 12 males were analyzed, and 7 of those (58.3±14.2%) transmitted foreign mtDNA to embryos. In F4 the respective figures were 8 (analyzed) and 7 (transmitted; 87.5±11.7). Hence, a stable and frequently repeated phenomenon of paternal inheritance mtDNA is observed.

Modern application of GC-MS method to find new drug target for Breast cancer treatment

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According to presence or absents of hormone receptors breast cancer can divide into subtypes: Luminal A, Luminal B, HER2 positive and triple negative. Unfortunately, regular drug which used to treat Luminal or HER2 breast cancer types is not efficient for triple negative breast cancer patients. Triple negative breast cancer type is usually aggressive with high metastatic rate. In 2011, Hanahan and Weinberg proposed that cells energy metabolism should be a new therapeutic target for cancer treatment [1].

Adenylate kinase is ubiquitous enzyme critical in cell nucleotide metabolism and metabolic signaling. The energetic role of adenylate kinase is related to its ability to facilitate transfer and distribute energy from both γ- and β-phosphoryls of the ATP molecule between different cellular compartments thus increasing energetic efficiency. Beside ATP, intracellular energy distribution depends on the ability to transfer phosphoryls to GTP, UTP and CTP reflecting activity of protein, carbohydrate and lipid biosynthesis and cell growth, respectively.

Stable isotope ^{18}O-based dynamic phosphometabolomic approach was used to study adenylate kinase metabolic flux by measuring ATP β-phosphoryl turnover and ^{18}O-labeling of nucleotide triphosphate where γ-phosphoryls reflecting high-energy phosphoryl distribution. γ- and β-phosphoryl turnover rates were detect by GC-MS. In current study 7 different cell types were used. HepG2 (liver) and 293T (kidney) where β-phosphoryl turnover rate is high [2] and MEF (mouse embryonic fibroblasts) and primary astrocytes where β-phosphoryl turnover rates is low as well as two breast cancer cell lines MCF7 (luminal A) and MDAMB231 (triple negative) and MCF10A is used as a control.

Labeling experiments indicate that in cells such as HepG2 and 293T, the rate of label incorporation into ATP β-phosphoryls, which is a measure of adenylate kinase velocity, closely approximates that of γ-ATP reflecting cellular ATP turnover rate. This indicates that cells efficiently using both γ- and β-ATP high-energy phosphoryls and that majority of phosphoryls utilized in cells are processed through the adenylate kinase-catalyzed phosphotransfer system. In other type of cells, MEF and primary astrocytes, β-ATP[^{18}O] labeling was by 20-30% lower compared by γ-ATP[^{18}O] reflecting diminished adenylate kinase flux. In breast cancer cells ATP synthesis and adenylate kinase flux is altered compared to normal breast epithelial cells (MCF10A). Nevertheless, the levels of main nucleotide triphosphates were higher in cancer than in normal breast epithelial cells. Relative amount of label incorporation into γ-ATP[^{18}O], γ-GTP[^{18}O], γ-UTP[^{18}O] and γ-CTP[^{18}O], reflecting energy distribution to biosynthetic processes, was 100%, 12%, 9% and 3% respectively. Thus stable isotope phosphometabolomics permits assessment of changes in dynamics of cellular energetic and metabolic signaling circuits.

[1] Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.

[2] Dzeja, P. and A. Terzic, *Adenylate kinase and AMP signaling networks: metabolic monitoring, signal communication and body energy sensing.* Int J Mol Sci, 2009. **10**(4): p. 1729-72.

Distribution of foreign mtDNA in early development of transmitochondrial embryos

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Maternal inheritance of mitochondrial DNA (mtDNA) so far was regarded as the only possible way of transmittance in mammals. Previously we obtained transmitochondrial (TM) mice that carried human mtDNA along with the murine mitochondrial genome (Sokolova *et al*. 2004 [1], Bass *et al*. 2006 [2], 2010[3]). Prerequisites for persistence and distribution of foreign mtDNA in TM mice were revealed in their early development, studying 1-, 2-, 4- and 8-cell embryos obtained by mating TM+ males (F0-F4) with wild- type intact females. The percentage of TM+ embryos among one-cell embryos was 7.6±0.76% (92 of 1212), and for two-cell, 4-cell and 8-cell embryos it was 8.1±1.5%, 32.4±5.7% and 30.6±2.2% (132 of 411), respectively. This increase is highly significant according to chi-square test with Yates' correction (29.12, $p<0.001$) and bilateral Fisher's test ($p<0.001$), providing an indirect evidence of foreign mtDNA replication before the blastocyst stage.

[1] Sokolova, V., Kustova, M., Arbuzova, N., Sorokin, A., Moskaliova, O., Bass, M., Vasilyev, V. (2004). Obtaining mice that carry human mitochondrial DNA transmitted to the progeny. *Mol. Reprod. Devel*., 68: 299—307.

[2] Bass, M, Sokolova, V., Kustova, M., Grachyova, E., Kidgotko, O., Sorokin, A., Vasilyev, V. (2006) Assaying the probabilities of obtaining maternally inherited heteroplasmy as the basis for modeling OXPHOS diseases in animals. *Biochim. Biophys. Acta*. 1757: 679—685.

[3] Bass,M., Sokolova, V., Kustova, M., Kidgotko, O., Zakharova, F., Vasilyev, V. (2010) Transmitochondrial mice in studying mtDNA distribution and transmission. *In*: Horizons in DNA research. 1: 151—170.

The role of dietary fiber in the energy metabolism of human colon cancer cells

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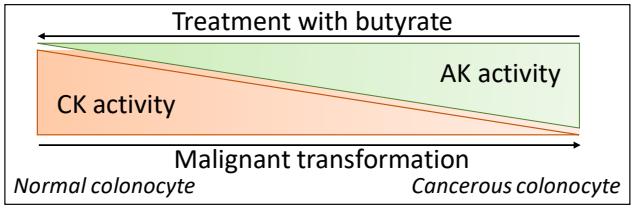
Increased dietary fiber consumption has been associated with various beneficial effects, including improvement of insulin sensitivity, prevention of obesity and reduced risk of colon cancer [1]. In human intestine, dietary fibers undergo fermentation by anaerobic microbiota leading to the production of short-chain fatty acids (SCFAs). Butyrate, a most abundant SCFA, is the main energy source for normal colonocytes and can induce cell growth inhibition and differentiation in colon cancer cells.

In the current study we investigated whether butyrate may also reverse changes in cellular bioenergetics of colon cells caused by malignant transformation. Colon cancer cells (Caco-2 cell line) were treated with 1 mM sodium butyrate for 48 h and the bioenergetics of cells was analysed by inspecting cellular and mitochondrial morphology, measuring mitochondrial respiration and activities of main enzymes involved in the transport of energy-rich phosphoryl.

Treatment of Caco-2 cells with butyrate was accompanied by obvious signs of colonocyte differentiation: reduced proliferation, increased alkaline phosphatase activity, tendency to grow in a monolayer and elongated morphology compared to cells cultured in the absence of butyrate. Although the mitochondrial mass, respiratory capacity and membrane potential remained unaltered, differentiated cells had a more fragmented mitochondrial network with smaller mitochondrial clusters. The activities of main glycolytic enzymes were significantly altered. While the activity of hexokinase was slightly increased in differentiated cells, the lactate dehydrogenase and pyruvate kinase activities were rather decreased. Treatment of cells with butyrate also resulted in re-organization of phosphotransfer system. In our previous work, we have found that creatine kinase (CK) network was significantly downregulated while adenylate kinase (AK) was upregulated in patients with colorectal cancer [2]. In the present study, butyrate was able to re-establish CK/AK homeostasis similar to normal large intestine tissue. The activity of CK was significantly increased. On the contrary, the total activity of AK was reduced due to decreased AK1 activity.



Our data suggest that butyrate exerts a significant effect on the cellular bioenergetics of colon cancer cells. Further research is necessary to more fully address the mechanisms involved in the action of butyrate.

References:

[1] McNabney, S.M., Henagan, T.M., (2017): Short Chain Fatty Acids in the Colon and Peripheral Tissues: A Focus on Butyrate, Colon Cancer, Obesity and Insulin Resistance, Nutrients 9(12), 1348.

[2] Chekulayev, V., et al., (2015): Metabolic remodeling in human colorectal cancer and surrounding tissues: alterations in regulation of mitochondrial respiration and metabolic fluxes, Biochem and Biophys Reports 4, 111-125.

Characterization of the oxidative phosphorylation system in the cytoplasmic hybrid cells harboring mutations m.9185T>C and m.13513G>A

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Mitochondrial diseases are heterogenous multisystem organ disorders which can be caused by alterations in both nuclear and mitochondrial DNA (mtDNA). Defects in the oxidative phosphorylation system (OXPHOS) are presented in many cases. Cytoplasmic hybrid (cybrid) cells are a good model to investigate the influence of mtDNA alterations on cell function [1]. The aim of this study was to characterize functionality of the OXPHOS in the cybrid cell lines harboring mutations m.9185T>C and m.13513G>A.

During our previous work we have revealed two pathogenic mutations associated with Leigh syndrome i.e., m.9185T>C in the gene *MT-ATP6* and m.13513G>A in the gene *MT-ND5* [2]. Two cybrid cell lines harboring mutations m.9185T>C and m.13513G>A and six control group cell lines were developed using osteosarcoma derived rho zero cells and platelets from the Leigh syndrome patients and healthy donors respectively. The established cell lines were used to determine the mitochondrial functionality by high resolution respirometry using Oroboros O2k. Respiratory chain complex I – IV and additionally complex I+III and II+III activities were measured spectrophotometrically in isolated mitochondria as previously described [3] with modifications.

We obtained only homoplasmic clones for the line containing mutation m.9185T>C but for the line containing m.13513G>A mutation we achieved three clones with different amount of mutant molecules - 70% mutant, 50% mutant and 100% wild type (WT) clones. Cell lines containing 50% and 70% of mutation m.13513G>A showed decrease of the OXPHOS complex I, IV and I+III activities with increasing level of heteroplasmy compared to the WT line. Respirometry measurements revealed declined complex I dependent oxygen consumption in the clone with 70% heteroplasmy. Cells with mutation m.9185T>C showed normal OXPHOS enzyme I – IV activities compared to control group cells. Though they showed declined efficiency of the oxidative phosphorylation compared with control cell line with the same haplogroup in the respirometry assay.

High resolution respirometry showed oxidative phosphorylation defects in both mutant cell lines, but only cells with the mutation in the *MT-ND5* gene showed reduced OXPHOS enzyme activities.

References:

[1] Wilkins H.M., Carl S.M., Swerdlow R.H., (2014). Cytoplasmic hybrid (cybrid) cell lines as a practical model for mitochondriopathies, Redox Biol 2: 619-631.

[2] Pelnena D., Burnyte B., Jankevics E., Lace B., Dagyte E., Grigalioniene K., Utkus A., Krumina Z., Rozentale J., Adomaitiene I., Stavusis J., Pliss L., Inashkina I., (2017). Complete mtDNA sequencing reveals mutations m.9185T>C and m.13513G>A in three patients with Leigh syndrome, Mitochondrial DNA A DNA Mapp Seq Anal 12: 1-6.

[3] Feichtinger R.G., Zimmermann F., Mayr J.A., Neureiter D., Hauser-Kronberger C., Schilling F.H., Jones N., Sperl W., Kofler B., (2010). Low aerobic mitochondrial energy metabolism in poorly- or undifferentiated neuroblastoma, BMC Cancer 10: 149.

A novel CYTB variant exacerbates respiratory chain complex III deficiency in mice by restricting RISP movement

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Rieske iron-sulphur protein (RISP) assembly into complex III (CIII, cytochrome bc1 complex) requires functional BCS1L translocase. *Bcs1l* mutant (*Bcs1l*$^{p.S78G}$) mice display growth restriction and hepatorenal manifestations and survive 200 days in the C57Bl/6JCrl background but in a C57BL/6JBomTac-derived background only 35 days [1-3]. From the short-lived colony, we identified a novel homoplasmic mtDNA variant, *Cytb*$^{p.D254}$, that explained the survival difference. The variant replaces a conserved negatively charged amino acid to neutral asparagine in the RISP-interacting region of the CYTB subunit of CIII. The *Cytb*$^{p.D254N}$ further decreased the low CIII activity of *Bcs1l*$^{p.S78G}$ mice in liver, kidney and skeletal muscle. Remarkably, *Cytb*$^{p.D254N}$ alone altered respiratory chain function and affected the growth of the mice. Computational simulations and functional assays utilizing the isolated *Rhodobacter capsulatus* enzyme demonstrated that the mutation stiffens the CYTB ef loop with consequent longer occupancy of RISP Fe-S domain towards the quinol oxidation site. Given that no technology to introduce specific mutations to mtDNA exist, we envision that this novel mutation will become an invaluable tool in studies of CIII function and dysfunction.

References:

[1] Levéen, P., Kotarsky, H., Mörgelin, M., Karikoski, R., Elmér, E., and Fellman, V. (2011): The GRACILE mutation introduced into Bcs1l causes postnatal complex III deficiency: A viable mouse model for mitochondrial hepatopathy. Hepatology 53, 437–447

[2] Davoudi, M., Kotarsky, H., Hansson, E., Kallijärvi, J., and Fellman, V. (2016): COX7A2L/SCAFI and Pre-Complex III Modify Respiratory Chain Supercomplex Formation in Different Mouse Strains with a Bcs1l Mutation. PLOS ONE 11, e0168774

[3] Purhonen, J., Rajendran, J., Mörgelin, M., Uusi-Rauva, K., Katayama, S., Krjutskov, K., Einarsdottir, E., Velagapudi, V., Kere, J., Jauhiainen, M., Fellman, V., and Kallijärvi, J. (2017): Ketogenic diet attenuates hepatopathy in mouse model of respiratory chain complex III deficiency caused by a Bcs1l mutation. Scientific Reports. 7, 957

Energy transfer in development and pathology – valuable information from oxygraphic measurements

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Results of previous studies have demonstrated intracellular diffusion restrictions for energy metabolites, metabolic compartmentalization, metabolite channeling and functional coupling in cells of skeletal and heart muscles [1]. Also, the movement of the adenine nucleotides through mitochondrial outer membrane and voltage dependent anion channels (VDAC) is impeded by interaction of VDAC with specific cytoskeletal proteins [2]. Therefore, energy transport from mitochondria to the energy consumption sites is a vitally important part of the energy metabolism. Creatine kinase (CK), adenylate kinase (AK) and other energy pathways create an opportunity for facilitated energy transport, without ADP and ATP free diffusion on the cytoplasm [3, 4]. In addition, these energy transfer systems provide better feedback between ATP consumption and synthesis. Oxygraphic measurements in saponin permeabilized muscle cells provide a unique opportunity to assess energy transfer pathways in connection their capacity to stimulate oxidative phosphorylation (OXPHOS) in pathology and in the course of lifetime.

Mutations in the wolframin (Wfs1) gene cause Wolfram syndrome (WS), an autosomal recessive neurodegenerative disorder characterized by early juvenile *diabetes mellitus*, progressive optic nerve atrophy, *diabetes insipidus* and deafness. Wfs1 deficient (Wfs1KO) mice develop impaired glucose tolerance [5]. Also, previous studies in cell culture have shown link between Wfs1 deficiency and mitochondrial damage [6].

The regulation of the energy metabolism in Wfs1KO mice heart and skeletal muscles demonstrate sift in the energy pathways preferences. In oxidative heart muscle of the Wfs1KO mice the AK pathway is more active, while the creatine activated respiration is lower than in control animals. On the contrary, in glycolytic *m. rectus femoris* of the Wfs1KO mice the activity of AK pathway is slightly decreased in comparison to control. The coupling of hexokinase to OXPHOS is altered in the muscle of Wfs1KO mice. In the glycolytic *m. gastrocnemius* white the glucose induced respiration is lower in Wfs1KO animals than in control; while in the oxidative heart muscle of Wfs1KO mice this respiration rate is higher. Similarly, during aging in rat heart muscle the CK phosphotransfer pathway efficiency is declined and probably partly compensated by AK and glycolytic pathways.

In conclusion, it is important to understand regulatory pathways in bioenergetics and the order of alterations to support maintenance of muscle performance and start prevention in first signs of pathology.

References:

1 .Saks, V., et al., Intracellular Energetic Units regulate metabolism in cardiac cells. J Mol Cell Cardiol, 2012. 52(2): p. 419-36.
2. Timohhina, N., et al., Direct measurement of energy fluxes from mitochondria into cytoplasm in permeabilized cardiac cells in situ: some evidence for Mitochondrial Interactosome. J Bioenerg Biomembr, 2009. 41(3): p. 259-75.
3. Tepp, K., et al., High efficiency of energy flux controls within mitochondrial interactosome in cardiac intracellular energetic units. Biochim Biophys Acta, 2011. 1807(12): p. 1549-61.
4. Dzeja, P.P. and A. Terzic, Phosphotransfer networks and cellular energetics. J Exp Biol, 2003. 206(Pt 12): p. 2039-47.
5.Noormets, K., et al., Sex differences in the development of diabetes in mice with deleted wolframin (Wfs1) gene. Exp Clin Endocrinol Diabetes, 2011. 119(5): p. 271-5.
6. Koks, S., et al., Silencing of the WFS1 gene in HEK cells induces pathways related to neurodegeneration and mitochondrial damage. Physiol Genomics, 2013. 45(5): p. 182-90.

Different regulation of mitochondrial respiration in human colorectal and breast cancer clinical samples

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Bioenergetics is a fast growing field in cancer research, where many promising outcomes could provide targeted cancer treatment. However, energy metabolism specific literature is still characterized by many contradictions, concluding that cancer cells metabolize their increased glucose uptake via glycolysis rather than more energy efficient oxidative phosphorylation. Furthermore, the majority of these conclusions are the outcome of *in vitro* studies on cell culture models, without taking into consideration the factors arising from the tumor microenvironment giving significant effects *in vivo*. We have quantitatively analyzed the mitochondrial respiration in human post-operational tissue samples in colorectal cancer (HCC), normal colon tissues, colon polyps and in breast cancer (HBC) [1]. We also included MDA-MB 231 and MCF-7 cell cultures. The flux was measured as the rate of oxygen consumption, using a high-resolution respirometry (OROBOROS Instruments, Austria). For the interpretation of our data about the regulation of respiration, we used the model of supercomplex named mitochondrial interactosome (MI) [2]. Our results show that respiration capacity is not lost as a result of tumor formation. Kinetic method can be useful for quantifying the role of components in the MI, the tumor maximal respiration and the regulation of mitochondrial outer membrane permeability. The main rate controlling steps in HBC are Complex IV and adenine nucleotide transporter, but in HCC, complexes I and III. In addition, when results from cultured cells were compared to clinical samples, clear differences were present. Comparing healthy colon, HCC tissue and colon polyps, we found that colon polyps (52,5 ± 12,7; µM ± SEM) show similar Km values to healthy mucosal colon tissue (42 ± 14; µM ± SEM).

Altogether, our data show that mitochondrial respiration and regulation of mitochondrial membrane permeability have substantial differences between these two cancer types when compared to each other, to their adjacent healthy tissue or to respective cell cultures. Further research is in progress to generate a full cancer development model consisting of cell cultures, clinical polyps and malignant versus healthy tissue samples.

[1] Koit A, Shevchuk I, Ounpuu L, Klepinin A, Chekulayev V, Timohhina N, et al. Mitochondrial Respiration in Human Colorectal and Breast Cancer Clinical Material Is Regulated Differently. Oxidative medicine and cellular longevity 2017;2017:1372640.

[2] Timohhina N, Guzun R, Tepp K, Monge C, Varikmaa M, Vija H, et al. Direct measurement of energy fluxes from mitochondria into cytoplasm in permeabilized cardiac cells in situ: some evidence for Mitochondrial Interactosome. J Bioenerg Biomembr 2009;41(3):259-75.

Cell respiration recovery after anoxia in HKC8 cell line

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Cellular respiration has a central role in mammalian cells. Besides providing ATP as energetic currency, it also holds the cellular redox balance and supports vital reactions by using the reduced form of nicotinamide adenine dinucleotide (Sullivan et al. 2015). At times of oxygen deprivation or anoxia this balance is perturbed and metabolic pathways reconfigured (Chouchani et al. 2014; Loscalzo 2016). These reconfigurations are thought to underlie the post ischemic reperfusion injury, but are not fully understood.

We constructed a unique system in which we can measure CO_2 emission and oxygen consumption simultaneously in real time at very high resolution. This gave us an opportunity to study how cells react to anoxic conditions, especially TCA cycle and electron transport chain (ETC). We used two main carbon sources, glucose and glutamine, on HKC8 cells to evaluate their contribution to TCA cycle and ETC (Fan et al. 2013). Measurements were performed on monolayer culture grown on 83mm glass plates.

Taken that on full medium (DMEM with glutamine, glucose and pyruvate) cell respiration is 100% then medium with only glucose isn't enough to fully support cellular respiration which stays at 40%. On the other hand, medium with glutamine raised respiration near the level of full medium (89%), confirming glutamine as a major carbon source in immortalized cell lines.

Regardless of medium (and cell line), under anoxia where no oxygen is present cells still evolved CO_2 at >10% of the normoxia rate, which may indicate continuing TCA cycle activity and reducing reactions. After restoring normoxia CO_2 emission recovery was distinctly slower for cells with glutamine as the only carbon source.

References:

[1] Chouchani, Edward T., Victoria R. Pell, Edoardo Gaude, Dunja Aksentijević, Stephanie Y. Sundier, Ellen L. Robb, Angela Logan, et al. 2014. "Ischaemic Accumulation of Succinate Controls Reperfusion Injury through Mitochondrial ROS." *Nature* 515 (7527): 431–35. https://doi.org/10.1038/nature13909.

[2] Fan, Jing, Jurre J Kamphorst, Robin Mathew, Michelle K Chung, Eileen White, Tomer Shlomi, and Joshua D Rabinowitz. 2013. "Glutamine-Driven Oxidative Phosphorylation Is a Major ATP Source in Transformed Mammalian Cells in Both Normoxia and Hypoxia." *Molecular Systems Biology* 9 (December): 712. https://doi.org/10.1038/msb.2013.65.

[3] Loscalzo, Joseph. 2016. "Adaptions to Hypoxia and Redox Stress: Essential Concepts Confounded by Misleading Terminology." *Circulation Research* 119 (4): 511–13. https://doi.org/10.1161/CIRCRESAHA.116.309394.

[4] Sullivan, Lucas B., Dan Y. Gui, Aaron M. Hosios, Lauren N. Bush, Elizaveta Freinkman, and Matthew G. Vander Heiden. 2015. "Supporting Aspartate Biosynthesis Is an Essential Function of Respiration in Proliferating Cells." *Cell* 162 (3): 552–63. https://doi.org/10.1016/j.cell.2015.07.017.