S12.18 A genetic system to study mitochondrial DNA mutations and their propagation in mice

James B. Stewart[a], Christoph Freyer[a], Joanna L. Elson[b], Anna Wredenberg[a], Zekiye Cansu[a], Aleksandra Trifunovic[a], Nils-Göran Larsson[a]
[a]*Division of Metabolic Diseases, Karolinska Institute, Sweden*
[b]*Mitochondrial Research Group, University of Newcastle upon Tyne, UK*
E-mail: jim.stewart@ki.se

The number of mutations in mitochondrial DNA (mtDNA) thought to cause metabolic disorders in patients is growing. At present, we lack the technology to introduce these mutations into the mtDNA of model organisms for detailed study. In this study, random mtDNA mutations were generated using the transgenic mtDNA mutator mouse, which expresses an engineered mitochondrial polymerase defective in its proofreading function. Female lines were then established to transmit and segregate the resulting mutations. We observed the selective loss of nonsense mutations in the protein coding genes, consistent with strong purifying selection by the female germline. The selection was evident two generations after the founder mtDNA mutator mouse, eliminating these nonsense mutations before they were detectable by Sanger sequencing methods. Curiously, the tRNA and rRNA genes do not show the same rapid selection against mutations despite strong evolutionary sequence conservation. Despite this purifying selection, putative deleterious mutations were identified and propagated in the mouse lines. Also, mutations analogous to human pathogenic mtDNA mutations were identified. These results will aid in the generation of more accurate models of inheritance of disease-causing mtDNA mutations and allow for the generation of laboratory mouse models of mtDNA mutations known in human disease.

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S12.19 Adaptation of breast cancer cells to glucose deprivation: Increase in capacity and affinity of the oxphos system

Katarína Smolková[a,b], Nadège Bellance[a,b], Giovanni Bénard[a,b], Thierry Letellier[a,b], Erich Gnaiger[c], Lydie Hlavatá[d], Petr Jezek[d], Rodrigue Rossignol[a,b]
[a]*INSERM U688, Bordeaux, France*
[b]*Université Bordeaux 2, Bordeaux, France*
[c]*Innsbruck Medical University, Austria*
[d]*Czech Academy of Sciences, Prague, Republic Czech*
E-mail: smolkova@biomed.cas.cz

The aim of this study was to investigate the adaptation of cancer cells to low glucose conditions, as regard to their mitochondrial features. To mimick glucose deprivation we used the glutamine/galactose medium. We chose a model of human breast carcinoma (HTB-126) and control (HTB-125) cultured cells, as nearly 40% of breast malignancies exhibit hypoxic tumour regions with low oxygen concentrations (pO_2<2 mmHg) and poor glucose delivery. In these cells we measured the sensitivity of mitochondria towards a decrease in oxygen concentration by high resolution respirometry. This can be quantified by the p50 value, *i.e* the oxygen concentration at half-maximal respiration in intact cells. In cancer cells, glucose deprivation lead to a change in this apparent affinity for oxygen, as p50 values decreased from 0.62±0.13 μM (glucose) to 0.46±0.11 μM O_2 (galactose). There was also a 2 to 3-fold increase in routine respiration in glucose versus galactose medium, both in control and cancer cells, respectively. Mitochondrial network morphology also presented typical adaptations. These results suggest that in absence of glucose, as can occur in solid tumors, mitochondria are enhanced to produce the vital ATP.

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S12.20 Delayed assembly kinetics of respiratory chain complex I in cybrids harbouring primary LHON mutations

Rosa Pello[a], Valerio Carelli[b], Andrea Martinuzzi[c], Leo Nijtmans[d], Miguel A. Martín[a], Jan Smeitink[d], Joaquín Arenas[a], Cristina Ugalde[a]
[a]*Hospital Universitario 12 de Octubre, Madrid, Spain*
[b]*Università di Bologna, Italy*
[c]*E. Medea Scientific Institute, Italy*
[d]*Nijmegen Centre for Mitochondrial Disorders, The Netherlands*
E-mail: cugalde@h12o.es

Leber's hereditary optic neuropathy (LHON) constitutes the most frequent mitochondrial disorder. Over 90% of LHON cases are due to three point mutations in the mitochondrial *ND1*, *ND4* and *ND6* complex I genes. However, the functional effects of these mutations on complex I remain unsolved. By using blue native gel electrophoresis, we have studied complex I assembly in cybrids harbouring the three most common LHON mutations under different mitochondrial haplotypes. No decrease in complex I activity or in the steady-state levels of respiratory chain complexes was detected in the mutant cybrids. However, an accumulation of low molecular weight subcomplexes suggested an assembly or stability defect in the mutants. To check the assembly kinetics of respiratory chain complexes, cells were incubated for six days in the presence of a reversible inhibitor of mitochondrial translation, doxycicline. After this time the drug was released from the medium, which let assembly resume. Our results show delayed kinetics of complex I assembly and late recovery of complex I activity in all LHON cybrids compared to controls. Differences amongst cybrids carrying the same mutation under different haplogroups were observed. These results will provide essential information about the nature of complex I deficiencies and will enhance our understanding of LHON disease mechanisms.

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S12.21 Respiratory control and mitochondrial defects in the failing human heart

H. Lemieux[a], S. Semsroth[b], E. Gnaiger[a]
[a]*Daniel Swarovski Research Laboratory, Medical University Innsbruck, Austria*
[b]*Department of Cardiac Surgery, Medical University Innsbruck, Austria*
E-mail: helene.lemieux@oroboros.at

Heart failure (HF) is a consequence of progressive deterioration of cardiac function. Little information is available on mitochondrial dysfunction in failing human hearts. We evaluated cardiac mitochondrial respiration in small biopsies from patients ranging from healthy donors to advanced stage of HF (dilated cardiomyopathy, during transplantation). Coupled OXPHOS capacity and uncoupled respiration (capacity of the electron transport system; ETS) were measured by high-resolution respirometry in permeabilized fibres (left and right ventricle, atrial appendage). ADP-stimulated flux through Complex I was only 0.4 of uncoupled respiration, indicating a strong limitation by the phosphorylation system. ETS capacity was higher with NADH-related substrates and succinate, supporting convergent electron input through Complexes I and II simultaneously into the Q-cycle. The additive effect of succinate was higher in patients with advanced stage of HF compared to donor hearts. HF mitochondria were tightly coupled, as evaluated by respiratory control in the absence of ADP. Fibres from atrial appendage showed lower respiratory fluxes with all substrate combinations compared to left and right ventricle. Respiratory control patterns (flux ratios for specific substrates relative to ETS capacity with convergent electron) were similar in the three tissues, but significantly different compared to the rodent heart. This study provides a basis for characterization of

HF and evaluation of mitochondrial ischemia–reperfusion injury in the human heart.

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S12.23 Mitochondrial OXPHOS system is enhanced in human lung cancer

Nadège Bellance[a,b], Giovanni Bénard[a,b], Katarina Smolkova[a,b], Hugues Bégueret[c], Thierry Letellier[a,b], Michel Merle[b,d], Jean-Marc Baste[c], Patrick Moreau[b,e], Rodrigue Rossignol[a,b]
[a]*INSERM U688, Bordeaux 33076, France*
[b]*Université Victor Segalen Bordeaux 2, Bordeaux 33076, France*
[c]*CHU Haut Lévèque, Pessac 33604, France*
[d]*UMR 5536 CNRS, Bordeaux 33076, France*
[e]*UMR 5200 CNRS, Bordeaux 33076, France*
E-mail: nadege.bellance@etud.u-bordeaux2.fr

The typical metabolic remodeling of most cancer cells includes the enhancement of glycolysis, but a class of tumors present more with an improved oxidative phosphorylation system. Little is known about the determinants of these extreme opposite situations, and the role played by mitochondria in tumorogenesis. Here, we approached this problem by determining the relative contribution of glycolysis and oxidative phosphorylation (OXPHOS) in lung cancer. We chose to study broncho-pulmonary tumors since they are the first cause of cancer for men in France, with a poor prognosis, aiming to identify therapeutic strategies at the mitochondrial level. We analyzed several mitochondrial features on normal and malignant lung surgical pieces, as well as corresponding cellular models. We measured cellular respiration, coupling degree, ATP synthesis, OXPHOS complexes activity, and PDH activity as well as protein expression levels. We also looked at mitochondrial membrane composition and overall structure of the organellar network by fluorescence microscopy. Lastly, we performed a quantitative analysis of energy metabolites by NMR, and the follow-up of cell proliferation in glucose versus galactose medium. We conclude that lung cancer belong to the OXPHOS class, with a predominant participation of mitochondria to the synthesis of vital ATP. Our results also evidence interesting differences in mitochondrial membrane composition between cancer and normal tissues.

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S12.24 Mutations in UCP2 in congenital hyperinsulinism reveal a role in human beta cell disease

M. Mar González-Barroso[b], Irina Giurgea[c], Frédéric Bouillaud[a], Andrea Anedda[b], Pascale de Lonlay[c], Daniel Ricquier[a]
[a]*Université Paris Descartes, CNRS UPR 9078 BIOTRAM, Paris, France*
[b]*Centro de Investigaciones Biológicas, CSIC, Madrid, Spain*
[c]*INSERM U781 and Departments of Pediatrics, Hôpital Necker-Enfants Malades, Paris, France*
E-mail: margb@cib.csic.es

Congenital hyperinsulinism (CHI) is a genetic disorder characterised by severe hypoglycaemia caused by disproportionate insulin secretion. The most common mechanism underlying CHI is dysfunction of the pancreatic ATP-sensitive potassium channel (K^+_{ATP}). Although mutations in other genes have been described, the pathogenesis and genetic origins of this disease remain unexplained in more than half of all patients. Uncoupling protein 2 (UCP2) knockout mice exhibit hypoglycaemia because of increased insulin secretion, which supports a role for UCP2 in the regulation of insulin secretion. However, its contribution to the development of human beta cell disease has not yet been investigated. The aim of this study was to explore whether UCP2 is involved in human CHI. Ten CHI children, without detectable mutations in other known CHI-causing genes, were studied. Parental-inherited heterozygous UCP2 variants encoding amino-acid changes were found in two unrelated CHI childs. Functional assays were carried out in yeasts and in insulin-secreting cells revealing that the two UCP2 mutants have an impaired activity. Our results demonstrate, for the first time, a role for UCP2 in the regulation of insulin secretion and glucose metabolism in humans and a link between UCP2 mutations and human disease.

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S12.25 Low level radiation and bystander factor(s) damage to mitochondria

Sharon M. Nugent[a], Carmel E. Mothersill[b], Colin Seymour[b], Brendan McClean[c], Fiona M. Lyng[a], James E.J. Murphy[a]
[a]*Radiation and Environmental Science Centre, DIT, Dublin, Ireland*
[b]*McMaster University, Hamilton, Canada*
[c]*St. Luke's Hospital, Rathgar, Dublin, Ireland*
E-mail: james.murphy@dit.ie

This study aimed to further characterise the, mitochondrial response to both direct irradiation and bystander factor(s). The 'bystander effect' describes radiation-like damage in unirradiated cells in the vicinity of irradiated cells. Cells were exposed to either γ radiation or growth medium taken from irradiated cells. Analysis was performed on mitochondrial DNA 4 to 96 h post exposure and included analysis of common deletion and point mutations, mitochondrial genome copy number, oxygen consumption rates and mitochondrial mass. A novel deletion was observed in HPV-G cells exposed to radiation and bystander factor(s). Point mutation analysis identified point mutations, in a non-consistent manner, in only the D-loop region and only in cells exposed to 5 Gy direct radiation. CHO-K1 cells showed a significant, though transient, reduced oxygen consumption rates. The latter apparent recovery was likely due to the substantial increase in mitochondrial mass observed in these. HPV-G cells showed a sustained increase in oxygen consumption rates post ICCM exposure and a transient increase 4 h post exposure to 5 Gy direct irradiation. Significant increases were observed in mitochondrial mass per exposed HPV-G cells. Findings are indicative of a stress response to mitochondrial dysfunction and DNA damage that increases the number of mitochondria per cell.

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S12.26 Effect of salicylic acid on the expression of mitochondrial energy dissipation systems in soybean

Ana Rita Matos, Ana Teresa Mendes, Miriam Klausberger, Jorge Faria, João Daniel Arrabaça
Centro de Engenharia Biológica, Faculdade de Ciências da Universidade de Lisboa, Portugal
E-mail: darrabaca@fc.ul.pt

Salicylic acid (SA) is a phenolic compound involved in plant stress responses. SA application generates reactive oxygen species (ROS) and induces alternative oxidase (AOX) expression. The aim of this work was the study of the effects of SA on the expression of AOX and other mitochondrial energy dissipation systems present in plants eg the rotenone-insensitive NADH dehydrogenases (ND) and uncoupling proteins (UCP). The three AOX genes present in soybean were previously identified as well as two incomplete UCP sequences. Here