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

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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

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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

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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

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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

we carried EST analysis which allowed us to identify different UCP genes and external and internal ND sequences. Expression was studied by semi-quantitative RT-PCR, in etiolated hypocotyls and heterotrophic cultured cells of *Glycine max* L. Merr treated with 1 mM SA for different times. Mitochondria from SA-treated hypocotyls had higher AOX capacity and protein content. Succinate oxidation was not affected while NADH respiration was reduced. A general up-regulation of energy dissipation systems is observed in both hypocotyls and cells. Interestingly, although the different AOX genes are expressed at different levels in both plant materials, as determined by the number of PCR cycles needed for amplification, the kinetics of induction, at the time-points analysed, was conserved between hypocotyls and cells.

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S12.27 Early mitochondrial damage in hippocampus of pilocarpine-treated epileptic rats

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An animal model which has been proven to be appropriate to study human temporal lobe epilepsy (TLE) with Ammon's horn sclerosis (AHS) is the pilocarpine-treated epileptic rat. In this model, the animals are treated systemically with a dose of the muscarinic agonist pilocarpine that induces an acute limbic status. The status epilepticus is terminated after 40 min with diazepam. This acute intoxication is followed by a 'latent' (i.e. seizure free) period lasting usually 1–2 weeks, followed by a chronic epileptic condition with spontaneous seizures, resembling human TLE. From the point of view of hippocampal pathology, pilocarpine-treated rats display changes closely resembling the AHS condition that is seen in the majority of TLE patients. It consists of segmental loss of pyramidal neurons in the CA1, CA3, and CA4 sectors of the Ammon's horn. In the present work we investigated the time course of hippocampal damage after systemic pilocarpine treatment applying metabolite determinations, determinations of hippocampal cell counts and determinations of mitochondrial function and of mtDNA copy numbers. We observed that the pilocarpine-induced status epilepticus is accompanied by early accumulation of lactate and succinate, decline of N-acetyl aspartate and decreased mtDNA copy numbers. These results can be explained as consequence of status epilepticus associated ROS formation.

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S12.28 Rescue of myopathic collagen VI null mice by genetic inactivation of mitochondrial cyclophilin d

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Progress in understanding the pathogenesis of collagen VI-diseases has been made in mice with disrupted *Col6a1* gene, which display an early-onset myopathy due to lack of collagen VI. Mitochondria in skeletal muscle fibers and in myoblasts from *Col6a1*$^{-/-}$ mice depolarize in presence of oligomycin, an anomalous response that is corrected by cyclosporin (CsA). This finding suggests that in collagen VI-myopathies flickering of the permeability transition pore (PTP) is increased and causes depletion of pyridine nucleotides, progressive impairment of respiration, and switch of the F1FO ATP synthase into an ATP hydrolase maintaining the membrane potential at the expense of glycolytic ATP. This interpretation is consistent with the therapeutic effect of treatment of *Col6a1*$^{-/-}$ mice with CsA, which desensitizes the PTP *in vivo*. To further test the role of the PTP in the pathogenesis of collagen VI-myopathies, we have generated *Col6a1*$^{-/-}$*Ppif*$^{-/-}$ mice (*Ppif* is the unique mouse gene encoding for mitochondrial cyclophilin, whose inactivation desensitizes the PTP). We will report the striking rescue of *Col6a1*$^{-/-}$*Ppif*$^{-/-}$ mice from the myopathy despite their total lack of collagen VI.

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S12.29 Mitochondrial respiration in skeletal muscle from Zucker Diabetic Fatty rats

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Mitochondrial dysfunction in skeletal muscle has been suggested to underlie the metabolic aberrations seen in type 2 diabetes mellitus, such as intramyocellular lipid (IMCL) accumulation and a decreased (fat) oxidative capacity. Here we tested this hypothesis by analyzing respiration in isolated mitochondria from 14 week-old Zucker Diabetic Fatty (ZDF) rats vs. healthy controls. At this age, ZDF rats are characterized by elevated IMCL levels and hyperglycemia. Our data show that state 3 respiration fuelled by pyruvate was slightly, but non-significantly, reduced (369.9±15.7 vs. 395.6±9.1 nmol O_2/mg/min; p=0.19; n=6) in mitochondria from ZDF rats as compared to healthy controls. Oligomycin-induced state 4 was significantly reduced in diabetic rats (9.7±0.4 vs. 11.9±0.5 nmol O_2/mg/min; p=0.008; n=8). Surprisingly, diabetic rats displayed an enhanced state 3 respiration fuelled by palmitoyl-CoA plus carnitine (157.0±9.5 vs. 123.4±12.8 nmol O_2/mg/min; p=0.06; n=6), while state 4o respiration remained unchanged (15.4±0.4 nmol and 15.2±1.1 O_2/mg/min (p=0.9; n=6) in ZDF vs. control. Furthermore, mitochondria from ZDF rats displayed a decreased sensitivity to fatty acid (FA)-induced uncoupling as evidenced by an increase in EC_{50} (372±19 vs. 283±16 nM free palmitate, p=0.01; n=4). This difference disappeared in the presence of the adenine nucleotide translocator (ANT) inhibitor carboxyactractyloside (Catr). Whether differences in muscular UCP3- or ANT levels contribute to these observations is currently under investigation. In conclusion, sensitivity for FA-induced uncoupling is reduced in 14-week old ZDF rats, but remarkably, mitochondrial fat oxidative capacity is improved.

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