result of a point mutation (T to C) at nucleotide position 8851 of the mitochondrial DNA (mtDNA). This mutation changes a highly conserved tryptophane residue to arginine at amino acid position 109 of the subunit *a* of mitochondrial F_1F_O-ATP synthase, a complex which provides most of the cellular ATP in humans. Nothing is known on the consequences of the T8851C mutation on the mitochondrial ATP synthase. To gain insight into the primary pathogenic mechanisms induced by T8851C, we have investigated the consequences of this mutation on the ATP synthase of yeast where the protein homologous to subunit *a* (referred to as Atp6p) is also encoded by the mtDNA. The modified yeast exhibited a very slow growth phenotype on non-fermentable carbon sources, both at 28 °C (the optimal temperature for growing yeast) and at 36 °C. In *vitro*, mitochondria from T8851C yeast grown at 28 °C showed a 60% deficit in ATP production, while those prepared from the mutant grown at 36 °C had an ATP synthesis activity below 5% that of the wild type. The mutated F_1F_O complex was correctly assembled, at both temperatures, and had a very poor ATPase activity (10% that of the wild type), both in mitochondria and after purification. Electron microscopy revealed that many of the mitochondrial matrices in T8851C yeast grown at 36 °C exhibited septae made of apposed inner mitochondrial membranes. Another anomaly was an increased mitophagic activity, presumably in response to the T8851C-induced damaging of mitochondria. Thus, in addition to a bioenergetic deficit, alterations in mitochondrial dynamics and homeostasis may also participate in the pathogenic mechanism induced by T8851C.

doi:10.1016/j.bbabio.2010.04.169

4P.8 Iron deficiency in children with mitochondrial disease

Young-Mock Lee, Hae Eun Kwon, Junh Hun Lee, Hoon Chul Kang, Joon Soo Lee, Heung Dong Kim
Department of Pediatrics, Gangnam Severance Hospital, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea
E-mail: ymleemd@yuhs.ac

Mitochondrial disease is an energy metabolic disorder with various organ involvements. Iron is widely known to be one of the most important nutriments required for normal brain development and several essential metabolic functions. We retrospectively studied the laboratory data on iron deficiency (ID) in 69 children with mitochondrial respiratory chain complex (MRC) defects by biochemical enzyme assay using muscle tissue. We analyzed the differences between groups of mitochondrial disease based on the presence of ID. ID has higher prevalence in children with mitochondrial disease than in the normal population. There were 6 (9%) patients with low hemoglobin, 12 (17%) with low serum ferritin, and 22 (32%) with low transferrin saturation levels among children with MRC defects. In comparisons between the ID and the non-ID group of MRC-defect patients, the frequency of MRC I defect was significantly higher in the ID group while that of MRC IV defect was higher in the non-ID group. Abnormal brain magnetic resonance imaging (MRI) findings were more frequently detected in the ID group. The incidence of failure to thrive and gastrointestinal symptoms were significantly higher in the ID group. Early diagnosis and proper treatment of ID are recommended. Especially in cases with risk factors such as failure to thrive or gastrointestinal manifestation, active evaluation of ID should be encouraged.

doi:10.1016/j.bbabio.2010.04.170

4P.9 The new molecular p.M177T identified in two unrelated patients with clinical features of SCO2-dependent cytochrome *c* oxidase deficiency

Sylwia Łuczak, Dorota Piekutowska-Abramczuk, Paweł Kowalski, Elżbieta Ciara, Dorota Jurkiewicz, Maria Borucka-Mankiewicz, Anna Tańska, Magdalena Pelc, Joanna Trubicka, Małgorzata Krajewska-Walasek, Ewa Pronicka
The Children's Memorial Health Institute, Warsaw, Poland
E-mail: s.luczak@czd.pl

Cytochrome *c* oxidase (COX, CIV) is one of the complexes of the OXPHOS system located in the inner mitochondrial membrane and catalyzing the last step of electron's transfer from cytochrome *c* to molecular oxygen. It is composed of 13 subunits encoded by mitochondrial and nuclear DNA. A correct assembly and function of COX require a substantial number of the nuclear, ancillary proteins, including SCO2, which is involved in the transport and incorporation of the cooper ions to the CuA enzymatic site on COXII subunit. Human *SCO2* gene is located on the chromosome 22q13, and contains two exons. Only the 801 bp fragment of exon 2 undergoes translation into 266 amino acid protein. Mutations in the *SCO2* gene lead to serious damage of the protein resulting in severe COX deficiency observed mainly in muscle, heart and brain. The common substitution, g.1541G>A (p.E140K) was identified at least on one allele in all so far reported patients with COX deficiency. In the group of 23 Polish patients, the common substitution was found on 84% of the studied alleles. The clinical features of the disease associated with SCO2 deficiency include early onset, fatal hypertrophic cardiomyopathy with respiratory insufficiency, encephalopathy, hypotonia and metabolic acidosis. The aim of this study was to characterize the molecular background of the disease in three patients from two unrelated families with clinically and biochemically recognized cytochrome *c* oxidase deficiency. Here we present patients with the same genotype, comprising the common mutation, g.1541G>A and a new, not described in the literature, molecular variant g.1653T>C. The new variant affects the highly conserved methionine at 177 position of the SCO2 protein (p.M177T) and was not found on 600 control alleles. Additionally, g.1653T>C substitution was predicted by SIFT BLink programme as a pathogenic mutation. Our findings indicate that the compound heterozygous genotype, p.M177T/p.E140K, is responsible for clinical manifestation of destroyed SCO2 protein.

The study was partly supported by the CMHI project no. S11/2009 and Polish Ministry of Science Project no. PB 0890/P05/2005/29.

doi:10.1016/j.bbabio.2010.04.171

4P.10 Impaired mitochondrial energetic in patients harbouring *SURF1* mutations is caused by uncoupling of cytochrome *c* oxidase

Petr Pecina, Alena Pecinova, Nikola Kovarova, Martin Kalous, Ivan Miksik, Josef Houstek
Institute of Physiology and Center for Applied Genomics, AS CR,v.v.i., Prague, Czech Republic
E-mail: petr.pecina@gmail.com

Leigh syndrome is most frequently caused by mutations of *SURF1* gene, which encodes cytochrome *c* oxidase (COX) specific assembly factor. Our previous studies suggested that fibroblasts from patients harbouring *SURF1* mutations accumulate incomplete forms of COX lacking several small nuclear-encoded subunits with decreased H^+/e stoichiometry. In experiments aimed at detailed characterization of the mitochondrial energetics, we observed 30–50% decreased respiratory capacity available for ATP synthesis (RCR_P). When using COX-specific substrates ascorbate + TMPD, the RCR_P was 7-fold lower than in controls, suggestive of deficient proton pumping of COX in patient

fibroblasts. The impairment of proton-translocation activity of COX was directly confirmed by mitochondrial membrane potential measurements using TPP^+ electrode. While proton pumping at complexes I and III in patient fibroblasts was similar to controls, ascorbate + TMPD substrates were unable to support generation of proton gradient. Consequently, mitochondrial membrane potential as estimated by JC-1 staining was lower in intact patient fibroblasts, leading to extremely decreased rates of mitochondrial ATP production to 25% of control values. Such drop in energy provision ultimately resulted in two-fold decrease of ATP/ADP ratio in patient cells grown in galactose medium, when most of ATP must be synthesized by mitochondria. In contrast to profound impairment of mitochondrial energetics, no changes in the production of reactive oxygen species (ROS) or antioxidant defences could be found in patient fibroblasts. This is perhaps due to decreased mitochondrial membrane potential, which may serve as a paradoxical ROS-preventing mechanism. We conclude that unlike to mitochondrial disorders caused by dysfunction of ATPase or complex I, the pathogenic mechanism of COX deficiencies seems to have only single component — impaired mitochondrial energy provision.

This work was supported by grants from Grant Agency of the Czech Republic (303/07/781) and Ministry of Education (1M6837805002, AV0Z 50110509) of the Czech Republic.

doi:10.1016/j.bbabio.2010.04.172

4P.11 Increased oxidative stress in fibroblasts from patients with ATP synthase deficiency

Alena Pecinova, Tomas Mracek, Petr Pecina, Pavel Jesina, Martin Kalous, Josef Houstek
Institute of Physiology and Center for Applied Genomics, AS CR, v.v.i., Prague, Czech Republic
E-mail: alena.pecinova@gmail.com

Genetic defects in enzymes of oxidative phosphorylation cause a broad spectrum of mitochondrial encephalomyopathies. Apart from diminished ATP production per se, elevated oxidative stress is implicated in pathogenic mechanism of mitochondrial diseases. In our work we used fibroblasts from patients with isolated deficiency of ATP synthase caused by mutation in *TMEM70* gene to study consequences on mitochondrial function, *in vivo* ROS production and levels of cellular ROS scavengers. With the aim to elucidate how the low ATP synthase content affects mitochondrial energy provision, we have investigated fibroblasts from patients with ATP synthase content decreased to <30% of the control. Measurements of cellular respiration showed insufficient ATP synthase capacity for basal respiration and mitochondrial ATP synthesis was decreased to 26–33%.Cytofluorometric analysis using TMRM revealed increased mitochondrial membrane potential ($\Delta\psi_m$) at state 3-ADP in patient cells. Consequently, viability of patient fibroblasts was more sensitive to ATP synthase inhibitors oligomycin or aurovertin. Analysis of ROS production by CM-H_2DCFDA demonstrated increase in ROS production and decrease of MnSOD activity in two patients, while level of main cellular ROS scavenger glutathione was only mildly decreased compared to control. In the third patient ROS production was not changed but MnSOD activity was dramatically increased and glutathione level decreased. Our results indicate two-component pathological mechanisms in ATP synthase deficient patient cells — impairment of ATP provision and oxidative stress.

This work was supported by grants from Ministry of Health (NS9759-4/2008) and Ministry of Education (1M6837805002, AV0Z 50110509) of the Czech Republic.

doi:10.1016/j.bbabio.2010.04.173

4P.12 *POLG* mutations lead to decreased mitochondrial DNA repopulation rates after EtBr-induced depletion in fibroblasts

Susanne Schoeler, Miriam Baron, Wolfram S. Kunz
Division of Neurochemistry, Dept. Epileptology and Life&Brain Center, University Bonn, Germany
E-mail: Susanne.Schoeler@gmx.net

Mutations in nuclear genes encoding proteins that are involved in mitochondrial DNA (mtDNA) maintenance, e.g. *POLG, TK2*, are associated with various neurodegenerative disorders [1]. All pathogenic mutations in these nuclear genes lead to mtDNA depletion and secondary mtDNA mutations, which cause dysfunction of the oxidative phosphorylation and lead to disease phenotype. Until now it is a major challenge to demonstrate the direct functional consequences of those mutations. To address the issue, whether *POLG* or *TK2* mutations lead to impaired mtDNA maintenance, a kinetic assay for mtDNA replication in primary human fibroblasts was performed. Different fibroblast cell lines were depleted of their mtDNA by treatment with ethidium bromide (EtBr) and the rates of mtDNA repopulation were determined. Here we demonstrate that the rate of mtDNA depletion, induced by EtBr, showed no significant difference between patients and controls. In contrast, the restoration of mtDNA levels is significantly delayed in fibroblasts from patients with *POLG* mutations, while TK2 mutations have no effect on mtDNA repopulation rates. These findings provide the first *in vivo* evidence that pathogenic *POLG* mutations directly influence the mtDNA maintenance in human cells. Furthermore, these results are in line with *in vitro* data showing reduced catalytic activity and processivity for several pathogenic *POLG* alleles [2–5].

References

[1] DiMauro S. et al. (2005) *Ann. Med.* **37:** 222-232.
[2] Chan S.S. *et al.* (2006) *Hum. Mol. Genet.* **15:** 3473-3483.
[3] Graziewicz M.A. (2004) *Nat. Struct. Mol. Biol.* **11:** 770-776.
[4] Chan S.S. *et al* (2005) *J. Biol. Chem.* **280:** 31341-31346.
[5] Longley M.J. *et al.* (2005) *Gene.* 125-131.

doi:10.1016/j.bbabio.2010.04.174

4P.13 Impact of diabetes-associated lipoproteins on oxygen consumption, enzymatic activities of mitochondrial respiratory chain complexes

Subir Roy Chowdhury, Ganesh Sangle, Xueping Xue, Garry Shen
University of Manitoba, Department of Internal Medicine, Canada
E-mail: gshen@ms.umanitoba.ca

Diabetes is a mitochondrial disease. Atherosclerotic coronary artery disease (CAD) is the leading cause of mortality in diabetic patients. Mitochondrial dysfunction and increased production of reactive oxygen species (ROS) are associated with diabetes and CAD. Elevated levels of glycated low density lipoproteins (glyLDL) and oxidized LDL (oxLDL) were detected in patients with diabetes. Our previous studies demonstrated that oxLDL and glyLDL increased the generation of ROS and altered the activities of antioxidant enzymes in vascular endothelial cells (EC). The present study examined the effects of glyLDL and oxLDL on oxygen consumption in mitochondria and the activities of key enzymes in mitochondrial electron transport chain (ETC) in cultured porcine aortic EC. The results demonstrated that glyLDL or oxLDL significantly impaired oxygen consumption in Complex I, II/III and IV of mitochondrial ETC in EC compared to LDL or vehicle control detected using oxygraphy. Incubation with glyLDL or oxLDL significantly reduced mitochondrial membrane potential, the levels of NAD^+/NADH ratio, and the activities of mitochondrial ETC enzymes (NADH–ubiquinone dehydrogenase, succinate cytochrome *c*