oxygen level fall, change their morphology, dynamics, and function, and play a major role in activating the cellular adapting mechanisms [for recent reviews see 1, 2]. Although these mechanisms are extremely important in pathophysiology, many of their aspects at the molecular level are still elusive. In addition, most studies concerning hypoxia have been carried out exposing to low oxygen levels the experimental models (animals, organs, or cells) for several hours only. Since our research group has a long experience in studying the bioenergetics of hypoxia-associated pathophysiological states, including aging [3, 4], heart and liver ischemia [5], cancer [6], and Alzheimer Disease [7], we evaluated the hypothesis that the mass, organization and function of mitochondria might be impaired when cells are exposed to prolonged hypoxia under various metabolic conditions. Therefore, as a first approach, we analyzed oxygen dependence of mitochondrial mass and function in human fibroblasts following 72 h exposition to variable oxygen pressure and energy substrates. In presence of glucose as the main fuel, the oligomycin-sensitive ATP synthesis rate of cells exposed to 1% O_2 resulted greatly decreased with respect to controls exposed to air (21% O_2) or to oxygen levels (4–6% O_2) corresponding to those present in the extracellular liquid in humans. Structural analysis of the 1% O_2 exposed fibroblasts indicated a more fragmented state and a decreased mass of the mitochondria than controls (i.e. exposed to 21% O_2); the latter was confirmed by assaying the citrate synthase activity of the cells exposed to different oxygen tensions. These results will be discussed in relation with supramolecular organization of the oxphos complexes in the mitochondrial inner membrane.

References

[1] Semenza GL (2009) *Physiology (Bethesda)* **24:** 97–106.
[2] Solaini G. *et al.* (2010) *Biochim. Biophys. Acta.* Feb 11. [Electronic publication ahead of print].
[3] Barogi S *et al.* (1995) *Mech. Ageing Dev.* **84:** 139–150.
[4] Lenaz G *et al.* (2006) *Antioxid. Redox Signal.* **8:** 417–437.
[5] Solaini G. Harris D.A. (2005) *Biochem. J.* **390:** 377–394.
[6] Baracca A. *et al.* (1797) *Biochim. Biophys. Acta.* **2010:** 314–323.
[7] Bosetti F. *et al.* (2002) *Neurobiol. Aging.* **23:** 371–376.

doi:10.1016/j.bbabio.2010.04.161

4L.7 Cytochrome *c* oxidase biogenesis, its disorders in childhood

Jiri Zeman, Lukas Stiburek
Department of Pediatrics, First Faculty of Medicine, Charles University, Prague, Czech Republic
E-mail: jzem@lf1.cuni.cz

Eukaryotic cytochrome *c* oxidase (CcO) is a hetero-oligomeric, heme–copper oxidase complex composed of both mitochondrially and nuclear-encoded subunits. CcO biogenesis is a complicated process that requires numerous specific assembly factors including translational activators, translocases, molecular chaperones, copper metallochaperones, heme *a* biosynthetic enzymes. We present the results of clinical, biochemical and molecular analyses in 107 CcO deficient children from our department. Methods: The activities of respiratory chain complexes were measured spectrophotometrically. The amount and protein composition were studied by BN-PAGE western blotting. DNA sequencing and PCR-RFLP were used for molecular analyses. Results: Encephalopathy was present in 90% of children, Leigh syndrome in 20%, and cardiomyopathy in 23%. Isolated CcO deficiency was found in 51 children and CcO deficiency combined with deficiency of other complexes was found in 56. In children with isolated CcO deficiency, SURF1 mutations were found in 15/51 children, SCO2 mutations in 12/51, and SCO1 mutation in one. Mutations c.845_846delCT in SURF1 and g.1541G>A in SCO2 were prevalent. At the biochemical level, SCO1, SCO2 and SURF1 deficiency resulted in tissue specific pattern of CcO assembly impairment that was not paralleled by corresponding reduction in the particular proteins' levels. Moderate to profound decrease of cellular copper was observed in muscle biopsies. MtDNA mutations were found in 7 patients with combined CcO deficiency. Conclusion: CcO deficiencies represent a heterogeneous group of diseases. Isolated CcO deficiency resulting from mutations in CcO assembly factors Surf1 and Sco2 represents the most frequently recognized causes of CcO defects in childhood. Owing to their incidence, absence of therapy and serious social–economical consequences, elucidation of the molecular mechanisms is essential for diagnostics, prevention and development of future therapeutic protocols. The reduced cellular copper levels of SCO1, SCO2 and SURF1 samples may indicate additional role of these CcO assembly proteins in copper homeostasis maintenance. The particular tissue-specific impact of SCO1, SCO2 and SURF1 deficiency suggests once again highly tissue-specific nature of respiratory chain biogenesis.

Supported by MSM 0021620806 and IGA MZ 10581/3.

References

[1] Stiburek L *et al.* (2009) *Am. J. Physiol. Cell Physiol.* **296:** C1218–226
[2] Vesela K *et al.* (2008) *APMIS* **116:** 41–49
[3] Pejcnochova M *et al.* (2010) *Mitochondrion.*

doi:10.1016/j.bbabio.2010.04.162

Posters

4P.1 Detection of single large-scale mitochondrial DNA deletions by MLPA technique

D. Piekutowska-Abramczuk[1], A. Tanska[1], P. Kowalski[1], K. Tonska[2], E. Ciara[1], D. Jurkiewicz[1], M. Borucka-Mankiewicz[1], S. Luczak[1], M. Pelc[1], J. Sykut-Cegielska[3], M. Krajewska-Walasek[1], E. Bartnik[2], E. Pronicka[1]
[1]*Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland*
[2]*The Institute of Genetics and Biotechnology, University of Warsaw, Poland*
[3]*Department of Metabolic Diseases, Endocrinology and Diabetology, The Children's Memorial Health Institute, Warsaw, Poland*
E-mail: d.abramczuk@czd.pl

Large-scale rearrangements consist of single partial mtDNA deletions or, more rarely, partial duplications. They are heteroplasmic since they coexist with variable amounts of wild-type mtDNA. Over 150 different mtDNA deletions have been associated with known sporadic deletion syndromes: Kearns–Sayre Syndrome (KSS), progressive external ophtalmoplegia (PEO), and Pearson Syndrome (PS), although they may occasionally be identified in patients with other mitochondrial cytopathies (e.g. MELAS). The most common deletion responsible for almost 30% of deletion syndromes, contains 4977 bp and is located between nucleotides m.8469 and m.13147. Characteristic clinical features associated with large-scale mtDNA deletions include: progressive external ophtalmoplegia, generalized muscle weakness with difficulties in swallowing and articulation, short stature, deafness, conduct disturbances, delayed puberty, and endocrine dysfunction. The aim of the study was to characterize the role of the large-scale mtDNA deletions in the pathogenesis of mitochondrial disease in selected patients. Fifteen patients with mitochondrial cytopathies (including 5 KSS cases), and seven controls (5 healthy subjects, and 2 patients with known m.3243A>G mutation) were enrolled into our study. Blood samples and muscle biopsies were used as DNA source in molecular analyses. MLPA (Multiplex Ligation-dependent Probe Amplification) technique was applied in the detection of deletions. SALSA MLPA KIT P125 Mitochondria (MRC-Holland) containing 31 probes for different mtDNA sequences, and 1 mutation-specific probe for the frequent point substitution m.3243A>G (MELAS) was used. Two various deletions spanning regions: m.9169_14174 (ATP6, MTCOIII, MTND3–MTND6