with degenerative encephalopathy, and indicated alteration of F_1 biosynthesis [1]. The search in other cases for disease causing gene by expression profiling and homozygosity mapping, identified mutation in *TMEM70* gene encoding a 30 kDa mitochondrial protein of unknown function [2]. Enzyme defect was complemented by *wtTMEM70* and TMEM70 protein turned out to be a novel ancillary factor of ATP synthase biosynthesis, interestingly the first one specific for higher eukaryotes. Homozygous *TMEM70* c.317-2A>G mutation leading to aberrant splicing and loss of the *TMEM70* mRNA was found in 24 patients, one patient was compound heterozygote for c.118_119insGT frame-shift mutation. Since then *TMEM70* mutations were found in other patients [3] thus being the most frequent cause of ATP synthase deficiency. Nevertheless, other cases may exist where additional nuclear genes are involved.

Supported by MZD CR - NS9759

References

[1] De Meirleir L. *et al* (2004) *J. Med. Genet.* **41:** 120–124.
[2] Cizkova A. *et al* (2008) *Nat. Genet.* **40:** 1288–1290.
[3] Honzik T. *et al* (2010) *Arch. Dis. Child.* **95:** 296–301.

doi:10.1016/j.bbabio.2010.04.158

4L.4 Mitochondrial pathways to autism

Luigi Palmieri[1], Federica Lombardi[2], Vito Porcelli[1], Roberto Sacco[2], Pasquale Scarcia[1], Antonio M. Persico[2]
[1]*University of Bari and CNR Institute of Biomembranes and Bioenergetics, Bari, Italy*
[2]*University "Campus Bio-Medico" and I.R.C.C.S. "Fondazione Santa Lucia", Rome, Italy*
E-mail: lpalm@farmbiol.uniba.it

Autism is a severe pervasive developmental disorder characterized by variable degrees of impairment in language, communication and social skills, as well as by repetitive and stereotypic patterns of behaviour. Despite strong familial components, clinical and genetic complexities have posed a major challenge to our understanding of autism pathogenesis. A significant subset of autistic patients display biochemical or neuropathological evidence of mitochondrial dysfunction and/or oxidative stress. However, only in a very few cases abnormal energy metabolism could be linked to a specific genetic defect. Interest in assessing the role of mitochondria in this disorder has been revitalized by the association between autism and variants of the *SLC25A12* gene [1], which encodes the predominant isoform of the mitochondrial aspartate/glutamate carrier (AGC) in brain [2]. Cytosolic Ca^{2+} can rapidly activate AGC transport through four "EF-hand" domains located at its N-terminus, thereby increasing the NADH/NAD ratio in the mitochondrial matrix and consequently boosting electron flow through the respiratory chain and ATP generation by oxidative phosphorylation [3]. Post-mortem studies of temporocortical gray matter from matched patient–control pairs revealed that AGC transport rates were significantly higher in brains from autistic patients [4]. This difference was blunted by Ca^{2+} chelator EGTA and direct fluorimetric measurements confirmed significantly higher Ca^{2+} levels in the patients, compared to their matched controls [4]. Oxidized mitochondrial proteins were markedly increased in the majority of the patients tested. Interestingly, oxidative damage correlated with the reduction of complex I activity indicating that excessive Ca^{2+} levels boost AGC activity in neurons and, to a more variable degree, cause oxidative stress and mitochondrial dysfunction in autistic brains. Furthermore, we identified a protective *SLC25A12* gene variant in a sizable group of unaffected siblings modulating AGC1 mRNA levels and protein activity. Our results suggest that mitochondria may play a critical role in the cascade of signalling events leading to autism and in determining to what extent different prenatal triggers will derange neurodevelopment and yield abnormal postnatal behaviour.

References

[1] Ramoz N. *et al.* (2004) *Am. J. Psychiatry* **161:** 662–669.
[2] Palmieri L. *et al.* (2001) *EMBO J.* **20:** 5060–5069.
[3] Lasorsa F.M. *et al.* (2003) *J. Biol. Chem.* **278:** 38686–38692.
[4] Palmieri L. *et al.* (2010) *Mol. Psychiatry* **15:** 38–52.

doi:10.1016/j.bbabio.2010.04.159

4L.5 Mitochondrial energy, redox dysregulation in human heart failure: Role of post-oxidative enzyme modification

Salvatore Pepe, Freya Sheeran
Murdoch Children's Research Institute, Heart Research, Critical Care & Neurosciences, Department of Cardiology, Royal Children's Hospital, Melbourne, Australia
E-mail: salvatore.pepe@mcri.edu.au

Heart failure is characterized by chronic, progressively worsening, insufficient energy supply and failure of ventricular contraction for the maintenance of blood circulation. We examined the abundance and activity of crucial mitochondrial enzymes as potential contributors to heart failure. Human left ventricular tissue was biopsied from non-failing donor hearts and end-stage failing hearts. Activity of complexes I and IV, the NADH-linked Krebs enzymes isocitrate dehydrogenase and malate dehydrogenase, NADPH transhydrogenase and aconitase was lower in failing hearts, as determined spectrophotometrically, while that of complexes II, III and citrate synthase was unchanged. Specific protein expression of each of these, determined by western blotting did not differ between the non-failing and failing heart groups, implying post-translational protein perturbation. Oxidative modification was explored as an underlying cause of enzyme dysfunction. Of the three oxidative markers measured, total mitochondrial protein carbonylation was greater by 31% in the failing tissues, while levels of 4-hydroxy-2-nonenal and protein nitration did not differ. Isolation of complexes I, IV and V by immunocapture revealed that subunits containing the iron–sulphur or heme redox centers were targets of oxidative modification, which may explain decreased activity in these enzymes. Notably the lower level of mitochondrial activity in heart failure coincided with significantly higher levels of oxidized glutathione, lower glutathione reductase activity, and lower content of total Coenzyme Q_{10}, cardiolipin, total adenine nucleotides, NADH and NADPH. In conclusion, the energy insufficiency of the failing human heart involves impaired activity of key mitochondrial enzyme subunits, at least in part due to oxidative modification. Thus the management of reactive oxygen species which markedly deteriorates concomitant with augmenting contractile failure may be a critical factor contributing to spiralling energy deficiency in the failing human heart.

doi:10.1016/j.bbabio.2010.04.160

4L.6 Oxygen tension significantly affects mitochondrial mass and structure in human fibroblasts

Giancarlo Solaini, Alessandra Baracca, Marianna Del Sole, Giorgio Lenaz, Laura Mediani, Alessandra Russo, Gianluca Sgarbi
Dipartimento di Biochimica "G. Moruzzi", via Irnerio 48 40126 Bologna, Italy
E-mail: giancarlo.solaini@unibo.it

Oxygen homeostasis is essential for normal cellular function; as oxygen level decreases (hypoxia), cells respond by changing their metabolism and by activating hypoxia-inducible factor dependent gene transcription to adapt and survive. Mitochondria sense the

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