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Abstracts

S4 Mitochondrial Medicine

Lectures

4L.1 Genotype–phenotype correlation in Leber hereditary optic neuropathy

Katarzyna Tońska[1], Agata Kodroń[1], Ewa Bartnik[1,2]
[1]*Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland*
[2]*Institute of Biochemistry and Biophysics, Polich Academy of Sciences, Poland*
E-mail: ebartnik@igib.uw.edu.pl

Leber hereditary optic neuropathy (LHON), acute or subacute vision loss due to retinal ganglion cell death which in the long run leads to optic nerve atrophy is one of the most widely studied maternally inherited diseases caused by mutations in mitochondrial DNA. Although three common mutations, 11778G>A, 14484T>C or 3460G>A are responsible for over 90% of cases and affect genes encoding complex I subunits of the respiratory chain, their influence on bioenergetic properties of the cell is marginal and cannot fully explain the pathology of the disease. The following chain of events was proposed, based on biochemical and anatomical properties of retinal ganglion cells whose axons form the optic nerve: mitochondrial DNA mutations increase reactive oxygen species production in these sensitive cells, leading to caspase independent apoptosis. As LHON is characterized by low penetrance and sex bias (men are affected about 5 times more frequently than women) the participation of the other factors — genetic and environmental — besides mtDNA mutations was studied. Mitochondrial haplogroups and smoking are some of the factors involved in the complex etiology of this disease. The results of mutation and haplogroup distribution in Polish LHON patients is presented as well as molecular study of lymphoblasts with two mtDNA mutations 3460G>A and 11778G>A.

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4L.2 Defects in mitochondrial oxidative phosphorylation: Role of supercomplexes

Charles L. Hoppel, Mariana Rosca
Center for Mitochondrial Diseases and Departments of Medicine and Pharmacology, Case Western Reserve University, Cleveland, Ohio, USA
E-mail: charles.hoppel@case.edu

Mitochondrial dysfunction is a major contributor in heart failure (HF). In moderately severe coronary microembolization-induced HF in dogs we reported a decrease in oxidative phosphorylation in cardiac mitochondria due to the decrease in the amount of the supercomplex consisting of complex I/complex III dimer/complex IV. We concluded that the mitochondrial defect in HF lies in the organization of respirasomes. We asked whether this defect in the supramolecular assembly is due to changes in the phospholipids of the mitochondrial inner membrane or modifications of the subunits of the electron transport chain complexes. The contents of the main phospholipid species, including cardiolipin, as well as the molecular species of cardiolipin, were unchanged in cardiac mitochondria in HF. In heart mitochondria isolated from HF complex IV not incorporated into the respirasomes contained an increased content of threonine phosphorylation. This suggested that cyclic AMP may have a role. In saponin-permeabilized heart muscle fibers, cAMP caused a decrease in oxidative phosphorylation, at least at the level of complex IV. We suggest that phosphorylation of specific complex IV subunits either limits the incorporation of complex IV in supercomplexes or decreases supercomplex stability.

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4L.3 Genetic disorders of mitochondrial ATP synthase

Josef Houštěk[1], Stanislav Kmoch[2], Johannes A. Mayr[3], Wolfgang Sperl[3], Jiří Zeman[4]
[1]*Institute of Physiology, Academy of Science of the Czech Republic, Prague, Czech Republic*
[2]*Institute of Inherited Metabolic Disorders, Czech Republic*
[3]*Paracelsus Medical University, Salzburg, Austria*
[4]*Department of Pediatrics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic*
E-mail: houstek@biomed.cas.cz

Mitochondrial diseases due to inborn defects of ATP synthase result from mutations in both mitochondrial and nuclear genes. Maternally transmitted dysfunction of ATP synthase is mainly caused by mtDNA missense mutations of varying pathogenicity in the *ATP6* gene. They alter the function of F_O proton channel, result in loss of ATP-synthetic activity and manifest as NARP, MILS or striatal necrosis syndromes. Rarely also *ATP8* mutation or altered splicing of *ATP8–ATP6* transcript is found. A distinct group of inborn defects is represented by ATP synthase deficiency due to nuclear genome mutations. Biochemically, it is characterized by selective inhibition of enzyme biogenesis and patients show generalized ≥70% decrease in the content of ATP synthase. Insufficient ATP synthase phosphorylating capacity results in impaired energy provision and increased ROS production due to elevated level of mitochondrial membrane potential. ATP synthase deficiency appears to be rather frequent, most cases present with neonatal onset, hypotonia, lactic acidosis, hyperammonemia, cardiomyopathy and 3-methylglutaconic aciduria; about half of them die within few months or years. Up to now pathogenic mutations have been found in two genes, both coding for ancillary factors of ATP synthase biogenesis. The first genetic defect was described in *ATPAF2* gene for assembly factor of F_1 subunit a, in a case

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.

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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.

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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.

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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