Altered mitochondrial functions in blood malignancies have been recently described and their involvement in tumorigenesis suggested. In this study a functional and molecular characterization of acute megakaryoblastic leukaemic (AMegL) cells is reported. It is shown by respirometry on intact AMegL cells a higher endogenous rate of oxygen consumption as compared with normal CD34+ HSPCs. This activity was related to ROS generation and linked to dysfunction of complex I. Altered biogenesis/assembly of the respiratory chain complexes was also detected by 2D BN-SDS PAGE. MtDNA-Sequencing revealed, along with a number of diffused polymorphisms, two missense homoplasmic mutations in the ND1 gene of complex I. Of these one (G3316A → A4T) has been described in NIDD whereas the other (A3418G → N38D) has never been reported before and occurs in a highly conserved TM-helices-connecting loop where other mutations have been shown to be causally linked to LHON. Based on structural model of the mutant ND1, a ROS-generating mechanism in complex I is suggested and its possible role in the AMegL tumorigenic progression discussed.

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S12.15 Mt-DNA and *PINK1* mutations in early onset parkinsonism: A family case report

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Various genes have been identified for monogenic disorders resembling Parkinson disease and their products are all associated with mitochondria and have been implicated in cellular protection against oxidative damage. In the present study we analysed fibroblasts from a patient carrying the homozygous mutation p. W437X in the PTEN-induced kinase1 (PINK1), which manifested a very early onset parkinsonism. Patient's fibroblasts did not show variation in the mtDNA copy number or in the expression of the OXPHOS complexes. Sequence analysis of the patient's mtDNA presented two new missense mutations in the ND5 (m.12397A>G, p.T21A) and ND6 (m. 14319T>C, p.N119D) genes. Both mutations were homoplasmic in the patient and patient's mother. Patient's fibroblasts resulted in enhanced constitutive production of ROS abrogated by inhibition of Complex I. Moreover enzyme kinetic analysis of the NADH:ubiquinone oxidoreductase showed changes in the substrates affinity. To our knowledge, this is the first report showing co-segregation of mutations in a PD-related nuclear gene and mtDNA. This finding highlights the hitherto unappreciated impact of coexisting mtDNA mutations in determining the development, clinical course and heterogeneity of the hereditary cases of PD.

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S12.16 Mitochondrial dysfunction in neuroblastoma cells infected with sindbis virus

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In this study we demonstrate for the first time that Sindbis Virus (SV) infection induce important alterations in the respiratory parameters of neuroblastoma cells, Neuro2A. Oxygen consumption was measured in intact cells using high-resolution respirometry (OROBOROS 2K). Our results showed that infected cells present a 45% decrease in basal respiration ($n=5$; $P<0.05$) and a 38% decrease in FCCP-induced maximum respiration ($n=5$; $P<0.05$) when compared to mock-infected cells. Additionally, SV-infected cells show a significant decrease ($P<0.05$) in oligomycin-independent respiration (mean±SE; $n=5$; 18.63±1.32 for SV-infected and 32.38±5.57 for mock-infected cells) and a significantly increase ($P<0.05$) in respiratory control ratio [(RCR) mean±SE; $n=5$; 2.02±0.06 for SV-infected and 1.68±0.13 for mock-infected cells]. The decrease in oligomycin-inhibited respiration and the increase in RCR suggest mitochondrial coupling and a decrease in proton leak induced by SV-infection possibly as a compensatory mechanism for the decrease in basal and maximum respiration. Since we also found that SV-infection significantly increase by two-fold hexokinase K_m for glucose, the mitochondrial coupling found in infected cells may also be important to compensate a possible decrease in glycolytic flux. We propose that bioenergetics alterations of Neuro2A cells are early signs of cell death and may be involved in the pathophysiology of encephalitis observed in SV-infection.

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S12.17 Effect of hemorrhagic shock on mitochondrial functions in rats

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Inadequate delivery of oxygen during severe hemorrhage and trauma (HTS) is supposed to impair cellular functions causing organ failure. Inducible NO synthase (iNOS) and heme oxygenase (HO-1) are known to influence the outcome of HTS. Our aim was to investigate the effect of HTS on the functional activity of mitochondria in liver, heart, and kidney. Anesthetized rats were subjected to HTS (laparotomy, bleeding and resuscitation) followed by a 2 h observation period. The mitochondrial function (MF) was estimated by means of respirometry. Respiration in state 2 and 3, respiratory control, effect of cytochrome *c* and CCCP on the respiration rate were determined. MF was unchanged in the heart, tended to decrease in the liver (State 3), and was significantly decreased in kidney (state 3 and respiratory control) of HTS vs. sham animals. In all organs MF negatively correlated with the mRNA of inducible NO synthase (iNOS) and in kidney additionally with the mRNA of heme oxygenase (HO-1), suggesting modulation by NO/CO. Our data show that HTS impairs MF in kidney, but not in liver and heart. Our data suggest that iNOS and HO-1 may modulate MF in HTS.

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