examined a total of 92 patients, of which, 30 (chronic periodontitis [CP] group) were selected for the above analysis. The institutional ethics committee approved this study and informed written consent was obtained from each participant. All clinical parameter scores of the tissue sampling areas in the CP group were significantly higher (<0.001) than in the control group. Complete mtDNA sequencing revealed a total of 264 variations in the patients, including 16 novel mutations, of which 3 were missense mutations (A4234T, A7796G, and G8115R). We also observed significant change in the membrane potential and protein levels of NADH dehydrogenase, Cytochrome *c* oxidase and HSP 60 in the patients.

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S12.11 Differential inhibition of energy-producing pathways of hepg2 cells by 3-bromopyruvate

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It was studied the effects of the alkylating agent 3-bromopyruvate (3-BrPA) on ATP producing pathways of HepG2 cells. 3-BrPA decreases HepG2 viability (10% with 10–100 μM; 25% with 300–1000 μM at 60 min; 60% with 10–100 μM; 75% with 300–1000 μM at 180 min). 150 μM 3-BrPA did not affect hexokinase-II activity, but incubation with 150 μM for 30 min decreased in 60% lactate production. Cells were incubated with 3-BrPA as above in glucose medium (GM-cells) or glucose-free medium (GFM-cells) and the basal respiration was 10,5 ± 0,62 (GM-cells) or 15.3 ± 0.96 (GFM-cells) nmol $O_2/5 \times 10^6$ cell × min^{-1}. A decrease of 22% or 50% of basal respiration by 3-BrPA was detected in GM or GFM cells, respectively. Proton leak was increased only in GM-cells (3.3 ± 0.45 to 5.1 ± 0.5). Maximum respiration was decreased only in GFM-cells (17.3 ± 1.1 to 9.5 ± 1.3). 3-BrPA decreased respiratory control ratio either in GM or GFM cells. In digitonin-permeabilized cells, complex I supported respiration was decreased by 50% in GFM-cells and complex-II supported respiration was inhibited by 50% in both media. Our results suggest that glycolysis and specific sites of mitochondria play a role in 3-BrPA-induced HepG2 death. The toxic effects of 3BrPA depend on the oxidizable substrates supplied to cells.

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S12.12 Restriction of glucose metabolism induces a metabolic switch to oxidative metabolism and drastic alteration of gene expression in glioblastoma cell line LN18

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The aim of this study was to determine the cellular effect of restricting glucose metabolism using 2-deoxyglucose in LN18 cells on oxygen consumption rate (OCR) and glycolysis rate (ECAR) as well as alterations in gene expression. The cellular OCR and ECAR were determined using XF24 Analyzer which measures the two parameters simultaneously in microplates. Gene expression was analyzed using Affimatrix human genome arrays. The normalized OCR of 2-deoxyglucose treated LN18 cells was increased while ECAR was decreased compared to control after 48 h treatment. This metabolic shift towards OXPHOS was confirmed by an observed increase in the sensitivity of cellular ATP levels to oligomycin as well as increased mitochondrial respiration capacity. Apoptosis induced by staurosporine was increased in 2-deoxyglucose treated cells. Gene expression analysis revealed a striking alteration in global gene expression as the result of restricted glucose supply. OXPHOS, pentose phosphate pathway, IGF-1 signaling, PI3K/AKT signaling, cell cycle check point, apoptosis signaling and oxidative stress pathways were among 2710 genes showed significantly altered expression. Our data demonstrated that restricting glucose drove LN-18 cells to a more oxidative state accompanied by growth suppression and drastically altered gene expression in pathways that converge at cellular energy metabolism and cell proliferation as well as death.

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S12.13 Hepatitis C virus proteins cause calcium-mediated mitochondrial dysfunction and hif-linked bioenergetic compensatory adaptation

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Hepatitis C virus (HCV) infection induces a state of oxidative stress that is more pronounced than that in many other inflammatory diseases. In this study we used well-characterized cell lines inducibly expressing the entire HCV open-reading frame to investigate the impact of viral protein on cell bioenergetics. It was shown that HCV protein expression has a profound effect on mitochondrial oxidative metabolism, with specific inhibition of complex I and $F_1F_oF_1F_0$-ATPase activities, depression of mtΔΔ and oxidative phosphorylation coupling efficiency, increased production of reactive oxygen and nitrogen species. Importantly, all these effects were causally related to mitochondrial calcium overload, as inhibition of mitochondrial calcium uptake completely reversed the observed bioenergetic alterations. Noteworthy, in spite of the oxidative phosphorylation impairment, survival of HCV proteins-expressing cells was assured by an adaptive up-regulation of glycolytic enzymes. This was linked to normoxic stabilization of the hypoxia-inducible factor (HIF 1α). Overall, the results presented show that expression of HCV proteins causes deregulation of ER-mitochondrial calcium homeostasis occurring upstream of further mitochondrial dysfunction. The expected bioenergetic unbalance is however compensated by a HIF-dependent transcriptional mechanism. These observations provide new insights into the pathogenesis of hepatitis C and may offer new opportunities for therapeutic intervention.

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S12.14 Novel Mt-DNA missense mutation in ND1 (A3418G →N38D) associated with mitochondrial dysfunction in megakaryoblastic leukaemic cells

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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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