regulation of cell death. We have explored the effect of IF_1 expression on apoptotic cell death. HeLa cells in which IF_1 was overexpressed ($+IF_1$) or suppressed using siRNA ($-IF_1$) were exposed to staurosporine (STS, 1 μM) or etoposide (Eto, 100 μM) for up to14 h. STS- or Eto-induced cell death was significantly reduced in $+IF_1$ (by ~30%) and increased in $-IF_1$ cells. In $+IF_1$ cells, caspase activation and annexin V binding were reduced and mitochondrial morphology was better preserved. Cytochrome *c* release, measured using the redistribution of cyt-GFP, was also significantly delayed in $+IF_1$ cells. Following STS treatment, $\Delta\psi_m$ measured using TMRM, collapsed relatively rapidly in $+IF_1$ cells, while it was maintained for up to 2 h in $-IF_1$ cells. IF_1 may protect cells from apoptotic cell death by regulating changes in $\Delta\psi_m$ and ATP levels, or by regulating mitochondrial structure and limiting cyt *c* release. Thus, IF_1 upregulation may predispose tissues to tumour growth by suppressing apoptotic responses following moderate injury and may also promote resistance to anti-cancer therapies.

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S12.38 Nutrient modulation of mitochondrial function, oxidative stress and cell cycle in human colon cancer

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Mitochondria are intimately involved in the life and death of the cell, capable of integrating pro- and anti-apoptotic signals and committing the cancer cell to apoptosis. Moreover, these organelles are the main source of intracellular reactive oxygen species. Therefore, the aim of this study was to investigate the effects of dietary antioxidants and glucose deprivation on mitochondrial function, cell cycle and oxidative stress in cell lines corresponding to different stages of human colon cancer. Tocopheryl acetate, resveratrol and vitamin C caused apoptosis in a cell line derived from metastatic tumour (SW-620); however, only resveratrol increased the apoptosis in a cell line derived from primary colorectal adenocarcinoma (HT-29). Additionally, vitamin C exhibited opposite effects on cell proliferation between the studied stages. Basal differences in cytochrome *c* oxidase, lactate dehydrogenase activity and H_2O_2 production were suppressed by glucose deprivation. Glucose-deprived HT-29 cells showed an upward in oxygen consumption coupled to a decrease in pro-oxidant production and lipid peroxidation. In conclusion, antioxidant compounds might modulate cell cycle in human colon cancer cells and oxidative stress could be one of the underlying mechanisms responsible for the observed phenotypic variations between its stages.

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S12.39 Natural sunlight damage to human skin mitochondria

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The aim of this in vitro study was to assess mitochondrial damage expressed in human skin cells exposed to simulated sunlight from a Q-Sun solar simulator. Excessive or continuous exposure to ultraviolet radiation contained in sunlight can result in the initiation and promotion of skin cancers, with many of the Irish population possessing particularly sensitive skin types. Non-tumour skin keratinocytes (HaCaT) and amelanotic tumour keratinocytes (C32) were exposed to different lengths of simulated sunlight and examined for mitochondrial damage. Effects on cell proliferation, mitochondrial mass and metabolism were assessed through a range of colorimetric assays. Mitochondrial DNA was assessed for induction of deletions, genome frequency and comparison of PCR efficiency of a 16Kbp product (almost the entire genome) versus a short conserved region. Results demonstrate that exposure of human skin cells *in vitro* to simulated sunlight causes mitochondrial DNA damage and influences the regulation of mitochondrial genome copy number. A substantial increase in mitochondrial activity was observed in non-tumour cells 4 h post exposure to simulated sunlight. The $mtDNA^{4977}$, though detected, did not increase in frequency with sunlight exposure. The $mtDNA^{3895}$ deletion was observed to be induced substantially in the amelanotic tumour cells. The frequency of deletions identified in this study may provide a potential biomarker for cumulative sunlight exposure in human skin.

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S12.40 Response to metabolic stress in cybrids obtained from patients with Leber's hereditary optic neuropathy

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Leber's hereditary optic neuropathy (LHON), the first maternally inherited disease to be associated with point mutations in mtDNA, is the second prevalent mitochondrial disorder. LHON is characterized by selective loss of ganglion cells in the retina leading to central vision loss and optic atrophy. ROS overproduction has been reported in cells harbouring mtDNA pathogenic mutations. 11778/ND4, 3460/ND1, and 14484/ND6 are the three most frequent LHON pathogenic mtDNA point mutations affecting complex I, and result in decreased ATP synthesis and increased oxidative stress. We studied ROS production and GSH level in ND4, ND1 and ND6 cybrid cellular model. Cybrids were obtained by fusing a rho° cell line completely devoid of mtDNA with cytoplasts derived by enucleated cells from LHON or healthy patients. ROS production and GSH content were measured in basal condition and in experimental stress induced by glucose-deprivation galactose-replacement. Basal ROS production measured by flow cytometry was modestly more elevated in cybrids harbouring the three LHON mutations than in healthy cells. GSH content in all cybrids in basal condition were not different. LHON mutated cybrids showed decreased growth and larger increase ROS and GSSG production compared with control cybrids. The response to stress was slight different among the three mtDNA point mutations. These results indicate that this cybrid cell model is a useful tool to explain the pathogenic mechanism of LHON, and may provide convenient system to test novel therapy strategies.

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S12.41 Altered mitochondrial respiration and energy metabolism in brain cells from transgenic Alzheimer's disease mice

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Mitochondrial dysfunction is recognized as earliest event in Alzheimer's disease. To better understand the direct impact of Aβ/tau interplay, Alzheimer's proteins, on mitochondria, we are currently investigating the brains of double (APP (KM670/671NL)/PS2 (N141I)), triple (APP (KM670/671NL)/PS2 (N141I)/Tau (P301L)) and single Tau (P301L) transgenic mice at the age of 2, 4, 7–8, 12 and 16 months. In triple transgenic mice, mitochondrial respiration is reduced compared to double transgenic mice and to single transgenic tau mice at the age of 12 months. In fact, activities of mitochondrial complexes I and IV were decreased as well as membrane potential and ATP levels. On the contrary, ROS production was increased. These effects seem to be age dependent and correlate with the corresponding Aβ and tau histopathologies. Based on these preliminary findings, we conclude that tau and Aβ have synergistic effects on mitochondria. Supported by SNF grant 310000–108223 and Eli Lilly International Foundation grant to AE.

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S12.42 The frataxin-interacting protein GRP75 chaperone plays an essential role in mitochondrial Fe/S cluster biogenesis

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The purpose of the study is to identify the pathophysiological consequences of depletion of the mitochondrial protein frataxin. The neurodegenerative disorder Friedreich's ataxia is caused by mutations in frataxin, a mitochondrial protein whose function remains controversial. Our previous work showed that frataxin interacts with GRP75, a homolog of the yeast ssq1 chaperone that integrates iron–sulfur clusters into imported mitochondrial proteins (Shan et al; 2007). Although ssq1′s function has been well characterized in the yeast, the role of GRP75 in Fe–S cluster biogenesis in mammals has never been evaluated. Interactions between frataxin and GRP75 were confirmed by co-immunoprecipitation and GST-pulldown analysis in mammalian cells. GRP75 strongly binds mitochondrial ISCU and mitochondrial Nfs1, two main components of the mitochondrial Iron Sulfur Cluster Assembly machine. Only weak interactions were observed between GRP75 and extramitochondrial ISCU, and no interaction was found between GRP75 and extramitochondrial Nfs1. Endogenous immunoprecipitation analysis confirmed the interaction of GRP75 with mitochondrial ISCU, Nfs1 and Nfu. Upon GRP75 depletion by siRNA in HeLa cells, the amount of the ISCU, Nfu and mitochondrial aconitase protein and aconitase activity declined. GRP75 depletion also increased transferrin receptor levels and cellular iron content, i.e. a phenocopy of frataxin knockdown. These data suggest that the frataxin partner GRP75 functions specifically in mitochondrial iron–sulfur biogenesis, and multiple consequences of GRP75 deficiency are duplicated by frataxin deficiency.

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S12.43 Gene expression profiling of liver and skeletal muscle in newborn mice exposed to an in utero low protein diet with or without taurine

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Fetal nutritional deprivation is associated with increased risk of dysregulation of glucose metabolism in adult life, however the exact mechanism for this is unknown. Neonatal administration of taurine has a beneficial effect on glucose homeostasis in mice in adult life. The aim of the study was to examine if maternal taurine supplementation had an effect upon gene expression patterns in liver and skeletal muscle in newborn mice subjected to a maternal low protein (LP) diet. LP offspring had decreased birth weight, liver mass and muscle mass compared to normal protein (NP) offspring, with taurine supplementation partially rescuing this effect. Changes in mitochondrial genes were found to be overrepresented in both liver and skeletal muscle. LP offspring had a significant change in 451 genes in liver and 330 genes in skeletal muscle compared with NP offspring. Taurine had a rescuing effect on 164 genes (36%) in the liver and 223 genes (68%) in the muscle. In conclusion, maternal taurine supplementation partially rescued changes in body mass and gene expression patterns in liver and skeletal muscle of newborn mice subjected to a maternal LP diet.

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S12.44 Mitochondrial function in lamb as a consequence of maternal caloric restriction during pregnancy and high-fat-high-carbohydrate diet post partum

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The aim of this study was to examine effects of foetal programming upon adult life. We examined muscle biopsies from lambs of ewes that were exposed to a low calorie (LC) diet containing only 50% of their normal calorie intake for 6 weeks prior to giving birth. Subsequently half of the lambs were exposed to a high-fat high-carbohydrate (HFHC) diet before they were sacrificed at the age of 6 month. The HFHC diet induced a 50% increase in mtDNA while mitochondrial $VO_{2\,max}$ was decreased, especially in the LC groups. The most pronounced change, however, was a two-fold change in respiratory coupling ratio (RCR) in the group receiving HFHC post partum, independent of the feeding of the mothers. UCP3 mRNA levels were decreased in all groups compared to control. PGC-1α mRNA levels were increased in the LC group independent of HFHC. In conclusion, the increased mitochondrial coupling induced by HFHC feeding will contribute to an increased ROS load and thereby offer a possible mechanism of how such combined effects of intrauterine and postnatal nutritional conditions may damage mitochondria and suggest a mechanism that further down the road may lead to metabolic disorders and type 2 diabetes.

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