tubular cells, which results in mitochondrial uncoupling and increased O_2 consumption. This mechanism may be protective against diabetes-induced oxidative stress, but will increase O_2 usage. The subsequently reduced O_2 availability may contribute to diabetes-induced progressive kidney damage.

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S12.34 The alternative oxidase as a tool to study mitochondrial function and to correct mitochondrial pathologies

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Alterations in mitochondrial function are related with different diseases (e.g. Parkinson or diabetes). It is not clear if such alterations are caused by the increase in mitochondrial Reactive Oxygen Production (mtROS) generation, deficits in ATP synthesis or both. This lack of knowledge make difficult to produce more efficient treatments. In order to clarify the mechanism involved in different kind of mitochondrial pathologies we have introduced the alternative oxidase (AOX) gene of *Ciona intestinalis* in the genome of Drosophila melanogaster. AOX expression in Drosophila decreases mtROS generation and partially by-pass the blockage of respiration elicited by inhibitors of both complexes III (antymicin A) and IV (KCN). Thus, AOX flies have new physiological properties as for example resistance in vivo to respiratory inhibitors and increase survival at low temperatures (4 °C). Moreover, AOX is able to correct mitochondrial alterations related with increases in oxidative stress. Mutations in DJ-1 gene provoke a Parkinson-like phenotype in Drosophila (e.g. alterations in locomotive function). Thus, DJ-1 mutant flies produce more mtROS without major alteration in the mitochondrial oxygen consumption. AOX expression in DJ-1 mutants flies either decreases mtROS generation to normal levels or rescue the alteration in locomotive function. Our results indicate that AOX is a potent model to study the molecular mechanism of mitochondrial pathologies.

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S12.35 Mitochondria as regulators of apoptosis through the redox state of cytochrome *c*

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When in cytosol cytochrome *c* triggers caspase activation by apoptosome, and oxidized cytochrome *c* is more effective in this process than reduced form of the enzyme. We investigated which cellular activities can reduce cytochrome *c* and by doing so may regulate apoptosis. When added to the cytosols from control or staurosporine-treated, apoptotic cells cytochrome *c* was gradually reduced whereas in homogenates of apoptotic cells it was rapidly oxidised by mitochondrial cytochrome oxidase (COX). The cytochrome *c* reducing activity of cell homogenates (but not cytosols) was enhanced in the presence of NADH. NADH-dependent reduction of cytochrome *c* in homogenates was significantly inhibited by DIDS or by removal of mitochondria indicating that this activity may be related to the mitochondrial porin/VDAC. Isolated heart mitochondria exhibited high rates of DIDS-inhibitable NADH-cytochrome *c* reductase activity. In liver mitochondria, DIDS only partially inhibited NADH-cytochrome *c*-reductase suggesting that more than one enzyme may be responsible for this activity. To test whether inhibition of COX or enhancement of cytosolic NADH can increase reduction of cytochrome *c* in cells and rescue them from apoptosis we incubated cells with staurosporine in the presence of azide and lactate. Such treatment decreased the rate of staurosporine-induced caspase activation by 40%, indicating that increasing the level of NADH may inhibit or delay caspase activation by mechanisms that may involve reduction of cytochrome *c* in the cytosol. Altogether our data suggest that mitochondria can regulate caspase activation by reducing or oxidizing cytochrome *c* released into cytosol after induction of apoptosis.

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S12.36 Oxidative stress in hypercholesterolemic LDL receptor knockout mice: Role of mitochondrial NADP-linked substrates and intracellular calcium levels

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Recently, we demonstrated that hypercholesterolemic LDL receptor knockout (LDLr k/o) mice present increased mitochondrial and cellular ROS production and a lower antioxidant capacity probably due to a large consumption of reducing equivalents from NADPH to sustain high rates of lipogenesis. Here we show that when k/o mice were treated with citrate containing drinking water during one week, the rates of oxygen consumption supported by endogenous NAD(P)-linked substrates, ROS production and NADPH oxidation by liver mitochondria were partially restored. We also observed that spleen mononuclear cells isolated from the k/o mice present cytosolic free Ca^{2+} concentrations and ROS production 2–3 times higher than the controls. To ascertain the role of Ca^{2+} in the k/o mice lymphocyte ROS production, we treated the k/o mice with verapamil, an L-type Ca^{2+} channel antagonist. The increase of ROS generation and Ca^{2+} concentration were partially inhibited in spleen mononuclear cells, but no effect was verified in liver mitochondrial ROS production and NADPH oxidation rates. These data demonstrate that the oxidative stress in spleen and liver of LDLr k/o mice results from distinct mechanisms. While liver mitochondria are deficient in NADPH-linked substrates, spleen lymphocytes are activated by high intracellular Ca^{2+} concentrations.

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S12.37 The inhibitory protein $1F_1$ regulates cellular sensitivity to staurosporine-induced cell death

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IF_1 inhibits the reverse activity of the ATP synthase, limiting mitochondrial ATP consumption during pathological states (e.g. ischaemia). IF_1 expression has also been reported to be upregulated in neoplastic cell lines. Thus, IF_1 may play a fundamental role in the

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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