S12.30 Apoptosis regulation by the mitochondrial chaperone TRAP-1/HSP-75

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TRAP1 is a mitochondrial chaperone also known as heat shock protein 75, which is overexpressed in several tumor cell types. Here we analyze whether mitochondrial TRAP1 elicits cytoprotective functions in a model of human osteosarcoma, SAOS-2 cells, either wild-type or in which TRAP1 expression was knocked down by RNA interference. Cells were exposed to different kinds of pro-apoptotic stimuli: chemotherapeutics, oxidative stress or death ligands, and several apoptotic parameters were measured in order to dissect whether and how TRAP1 impacts on these stress-induced transduction pathways. TRAP1 displays a general antiapoptotic role in all the examined conditions, whereas TRAP1 interference increases cell sensitivity to death. Serine phosphorylation and mitochondrial localization are required for TRAP1 cytoprotective function. In fact, a deletion mutant lacking the mitochondrial import sequence is not phosphorylated and is unable to counteract apoptosis induction in all conditions. Preliminary data show that TRAP1 interacts with Bcl-2 family proteins and is involved in the regulation of the mitochondrial permeability transition pore opening. Altogether, these results suggest that TRAP1 acts as a key anti-apoptotic molecule in mitochondria of neoplastic cells.

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S12.31 Hexokinase II detachment from mitochondria triggers apoptosis through the permeability transition pore independent of voltage-dependent anion channels

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Type II hexokinase (HKII) is overexpressed in the outer mitochondrial membrane of most neoplastic cells. Current work postulates that HKII release from its mitochondrial interactor, the voltage-dependent anion channel, prompts outer mitochondrial membrane permeabilization and the ensuing release of apoptogenic proteins, and that these events are inhibited by growth factors. Here we show that a HKII N-terminal peptide selectively detaches HKII from mitochondria transduces a permeability transition pore opening signal that results in cell death, does not require the voltage-dependent anion channel and is not affected by insulin stimulation. These findings have implications for our understanding of the pathways of outer mitochondrial membrane permeabilization and their inactivation in tumors.

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S12.32 Methylmalonate inhibits succinate-supported oxygen consumption by interfering with mitochondrial dicarboxylate transport: Implications for the methylmalonic acidemia physiophatology

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In the present work, we show that while millimolar concentrations of methylmalonate (MMA) inhibit succinate-supported oxygen consumption by isolated rat brain mitochondria, there is no effect when either a pool of NADH-linked substrates or TMPD/ascorbate were used as electron donors. Interestingly, the inhibitory effect of MMA, but not of malonate, on succinate-supported brain mitochondrial oxygen consumption was minimized when non-selective permeabilization of mitochondrial membranes was induced by alamethicin. In addition, only a slight inhibitory effect of MMA was observed on succinate-supported oxygen consumption by inside-out submitochondrial particles. In agreement with these observations, brain mitochondrial swelling experiments indicate that MMA is an important inhibitor of succinate transport by the dicarboxylate carrier. We conclude that MMA inhibits succinate-supported mitochondrial oxygen consumption by interfering with the uptake of this substrate. Although succinate generated outside the mitochondria is probably not a significant contributor to energy generation, MMA-induced inhibition of substrate transport by the mitochondrial dicarboxylate carrier may have important physiopatological implications, such as: i) inhibition of gluconeogenesis; ii) impairment of neuronal energy metabolism and glutamatergic neurotransmission; and iii) it has also been proposed that MMA may inhibits glutathione transport into mitochondria promoting oxidative stress.

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S12.33 Diabetes-induced up-regulation of uncoupling protein-2 results in increased mitochondrial uncoupling in kidney proximal tubular cells

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We have previously reported increased O_2 consumption unrelated to active transport by kidney proximal tubular cells and up-regulated mitochondrial uncoupling protein (UCP)-2 expression in the diabetic kidney. It is presently unknown if the increased UCP-2 levels in the diabetic kidney results in mitochondrial uncoupling, which we therefore investigated in this study. Increased UCP-2 expression in the diabetic kidneys was confirmed by Western Blot. Isolated diabetic proximal tubular cells had increased total and ouabain-insensitive O_2 consumption compared to controls. Isolated diabetic mitochondria displayed increased glutamate-stimulated O_2 consumption, in the absence of ADP and the ATP synthase blocked by oligomycin, compared to controls. Guanosine diphosphate; an UCP inhibitor, and essentially fatty free bovine serum albumin; removing fatty acids that are essential for the function of UCP, independently prevented the glutamate-stimulated O_2 consumption by the diabetic mitochondria. In conclusion, diabetic rats have increased mitochondrial UCP-2 expression in renal proximal

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