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