S12.30 Apoptosis regulation by the mitochondrial chaperone TRAP1/HSP-75

Andrea Sciacovelli,1,2, Serenah Calise,1, Rossella Sirignano,1, Federica Chiara1, Boris Pantic,3, Andrea Rasola,1, Paolo Bernardi1,3,1Franca Esposito1
1Istituto CNR di Neuroscienze e Dipartimento di Scienze Biomediche, Università di Padova, Padova, Italy
2Dipartimento di Biochimica e Biotecnologie Mediche, Università di Napoli Federico II, Napoli, Italy
E-mail: marco.sciacovelli@unipd.it

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.

doi:10.1016/j.bbabio.2008.05.324

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

Andrea Rasola1, Federica Chiara2, Diego Castellar2, Oriano Marin2, Valeria Petronill2, William S. Brusilow2, Magdalena Juhasztova3, Steven J. Sollott2, Michael Forte3, Paolo Bernardi4
1CNR Institute of Neuroscience and Department of Biomedical Sciences, University of Padova, Padova, Italy
2Department of Biological Chemistry and Venetian Institute of Molecular Medicine, University of Padova, Padova, Italy
3Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, Michigan, USA
4Laboratory of Cardiovascular Science, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, USA

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.

doi:10.1016/j.bbabio.2008.05.325

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

Sandra R. Mirandola1, Daniela R. Mello2, Patricia F. Schuck2, Gustavo C. Ferreira2, Moacir Wajner3, Roger F. Castilho4
1Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Brazil
2Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil
E-mail: srm@fcm.unicamp.br

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 physiopathological 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.

doi:10.1016/j.bbabio.2008.05.326

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

Malou Friederich1, Angelica Fasching2, Peter Hansell2, Lina Nordquist2, Frederik Palm2,3
1Department of Medical Cell Biology, Division of Integrative Physiology, Uppsala University, Uppsala, Sweden
2Department of Medicine, Division of Nephrology and Hypertension, Georgetown University Medical Center, Washington, DC, USA
E-mail: Malou.Friederich@mcb.uu.se

We have previously reported increased O2 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 O2 consumption compared to controls. Isolated diabetic mitochondria displayed increased glutamate-stimulated O2 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 O2 consumption by the diabetic mitochondria. In conclusion, diabetic rats have increased mitochondrial UCP-2 expression in renal proximal...