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Macromolecular reorganization of mitochondria during aging

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As the powerhouse of the eukaryotic cell, the mitochondrion plays a major role in age-related processes. The respiratory chain constantly produces deleterious reactive oxygen species (ROS), which cause accumulating damage and ultimately leads to cell death. We employ electron cryo-tomography on whole mitochondria isolated from the model organism *Podospora anserina* to investigate the macromolecular reorganization of the ATP synthase and respiratory chain complexes during aging. In mitochondria from juvenile *Podospora* cells, the mitochondrial ATP synthase forms extended dimer ribbons located along highly curved ridges of mitochondrial cristae, whereas respiratory complexes are located in the flat membrane regions flanking them. During aging, we observe a dramatic change in the mitochondrial morphology, which is accompanied by a retraction of the cristae, fragmentation of the mitochondrial matrix, disassembly of ATP synthase dimers and reorganization of respiratory chain complexes. Sub-tomogram averaging reveals the appearance of a unique additional protein density at the ATP synthase dimer interface, prior to disassembly of the complex. Initial mass spectrometry experiments indicate the association of a 113 kDa protein at a specific time point during aging. The identity of this protein remains elusive, yet it is conceivable that it plays a role in the disassembly of the ATP synthase dimer. Disassembly of the dimer ribbons in turn induces aging related mitochondrial fragmentation processes and consequently cell death.

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2,3-Dehydrosilybin mimics the effect of postconditioning in rat neonatal cardiomyocytes

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Aims: Ischemic postconditioning and remote conditioning are potentially useful tools for protecting ischemic myocardium. There is comprehensive experimental and clinical evidence that either exogenous supplementation of natural antioxidants or augmentation of endogenous antioxidants attenuates myocardial infarction. Because we recently described the interaction of 2,3-dehydrosilybin (DHSB), a flavonolignan component of *Silybum marianum*, with cardiomyocyte mitochondria, our hypothesis said that the compound could attenuate cardiomyocyte damage following hypoxia/reoxygenation modulating mitochondrial bioenergetics.

Methods and results: After 5–6 days of cell culture in normoxic conditions the rat neonatal cardiomyocytes were divided into three groups: control group (incubation for 9 h at normoxic conditions), hypoxia-reoxygenation group (incubation in a hypoxic incubator, atmosphere of 95% N_2 and 5% CO_2 for 3 h followed by 10 min of DHSB and 6 h of reoxygenation) and postconditioning group (cardiomyocytes were postconditioned after the 3 h index hypoxia by 3 cycles of 5 min of reoxygenation and 5 min of hypoxia followed by 6 h of normoxia). Cell viability assessed by propidium iodide staining was decreased after hypoxia reoxygenation while this effect was diminished following postconditioning or DHSB treatment. The same apparent cell damage was shown by increased levels of lactate-dehydrogenase (LDH) after reoxygenation whereas postconditioning and DHSB significantly reduced LDH leakage. Both postconditioning and DHSB treatment at the onset of reoxygenation reduced H_2O_2 production as well as the generation of ROS in the H/Re group as evidenced by a fluorescence indicator. DHSB treatment was associated with a decrease in protein carbonyls detected by probe fluorescein-5-thiosemicarbazide.

Conclusion: Our data suggest that DHSB treatment reduces reoxygenation-induced injury in cardiomyocytes by limiting ROS generation and the consequent cell damage as evidenced by protein carbonyl levels. We conclude that DHSB mimics the effect of postconditioning.

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Characterization of a novel TFAM transcript, which lacks the exons 4 and 5, in human skin fibroblasts

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Photoaging of the skin is tightly associated with increased levels of the mitochondrial common deletion (CD) and disturbances of the mitochondrial function [1]. The mitochondrial transcription factor A (TFAM) has been shown to be a multifunctional mitochondrial protein. It is involved in transcription, mtDNA copy number control, mtDNA stabilization and also in mtDNA repair. Its overexpression has been shown to be cell protective in general [2]. However, in the case of d-galactosidase-induced aging in rats, TFAM overexpression is associated with a deficiency in base excision repair and elevated levels of the CD [3]. Several alternative TFAM transcripts can be found in humans. One isoform (d5), which lacks exon 5, has been found to influence mitochondrial transcription [4].

In this study we analyzed the TFAM isoforms in human skin fibroblasts and their influence on mitochondrial transcription, mtDNA copy number and CD levels by quantitative real-time PCR. We also were interested if the overexpression of the TFAM isoforms would change the resistance of cells to solar irradiation.

We could isolate the d5 isoform and a novel TFAM isoform, which lacks the exons 4 and 5 (d4,5), from human primary skin fibroblasts. The exons 4 and 5 absence truncates both high mobility group (HMG) boxes and leads to a fusion of the truncated HMG1 and HMG2 boxes in the putative protein.