(S9) Mitochondrial dynamics symposium lecture abstracts

S9/1 Correlated light and electron microscopy illuminates the role of mitochondrial inner membrane remodelling during apoptosis

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In addition to their role in providing ATP for cellular functions via oxidative phosphorylation, mitochondria also play a critical role in initiating and/or regulating apoptosis through the release of proteins such as cytochrome *c* from intermembrane and intracristal compartments. The mechanism by which these proteins are able to cross the outer mitochondrial membrane has been a subject of controversy. This paper will review some recent results that demonstrate that inner mitochondrial membrane remodeling does occur during apoptosis in HeLa cells but does not appear to be a requirement for release of cytochrome *c* from intracristal compartments. Inner membrane remodeling does appear to be related to fragmentation of the mitochondrial matrix, and the form of the remodeling suggests a topological mechanism for inner membrane fission and fusion.

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S9/2 Cause and consequence of altered mitochondrial dynamics in mitochondrial complex I deficiency

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We aim to understand the interconnection between mitochondrial dynamics and function at the molecular and life cell level. As a model we use cells derived from healthy subjects and mitochondrial disease patients with inherited deficiency of mitochondrial complex I (CI). We recently combined confocal, fluorescence correlation and video microscopy of chemical and genetically-encoded reporter molecules with image analysis and native electrophoresis to establish the quantitative relationship between CI assembly/activity, mitochondrial shape, reactive oxygen species (ROS), mitochondrial membrane potential ($\Delta\psi$), redox environment, NADH levels, and Ca^{2+}/ATP homeostasis. It was found that cells with CI deficiency displayed aberrant cytosolic and mitochondrial Ca^{2+}/ATP homeostasis. Mitochondria were fragmented in patient cells with a very low CI activity (class I) whereas in patient cells with a moderate reduction in CI activity mitochondrial morphology was normal (class II). Western blot analysis revealed that these distinct morphological phenotypes were associated with altered expression of mitochondrial fission and fusion proteins. Although all patient cells displayed a reduced amount/activity of CI and increased ROS and NADH levels, these changes were significantly more pronounced in class I cells. We conclude that increased ROS production, when not appropriately counterbalanced by the cell antioxidant defense systems, induces mitochondrial fragmentation. In line with this hypothesis we observed that application of exogenous antioxidants improved mitochondrial structure and function.

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S9/3 Extending the Opa1-dependent cristae remodelling pathway in cell life and death

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Mitochondria are complex organelles whose shape is continuously regulated by a growing set of "mitochondria-shaping" proteins. During apoptosis, mitochondria undergo dramatic changes in their morphology as well as ultrastructure (the so called "cristae remodelling") in order to release cytochrome *c* which is stored in the cristae compartment. In the last years we found that cristae remodelling is controlled by Opa1. Opa1 is a dynamin related proteins of the inner mitochondrial membrane that has genetically distinguishable functions in mitochondrial fusion and apoptosis. In order to keep in check remodelling of the cristae and apoptosis, it forms oligomers of unknown composition, requiring the inner membrane rhomboid protease Parl. We are now investigating the proteomic composition of these oligomers in normal and apoptotic mitochondria. Several hits indicate an interaction between Opa1 and chaperones, substantiated by the ability of Opa1 to regulate stress responses in the intermembrane space during heat shock. Of note, Opa1 regulates also physiological changes in the shape of the cristae, such as during trophoblast syncitialization. In conclusion, Opa1 is centrally positioned to regulate mitochondrial shape and intermembrane space functions during life, stress and death of the cell.

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(S9) Mitochondrial dynamics symposium abstracts (poster and raised abstracts)

S9.4 Altered mitochondrial dynamics caused by loss of PTEN-induced kinase 1 function, associated with recessive parkinsonism, are reversed by downregulation of Dynamin-related protein 1

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PTEN-induced novel kinase 1 (PINK1) mutations are associated with recessive parkinsonism. Because PINK1 is a mitochondrial kinase, loss of function mutations suggests that signaling molecules may be important in neuronal survival. Recent data suggests that mammalian PINK1 protects against mitochondrial dysfunction and oxidative stress. We aimed to determine the role of PINK1 in mitochondrial dynamics. Using fluorescence recovery after photobleaching (FRAP), we show that manipulation of PINK1 alters mitochondrial morphology. Expression of wild-type PINK1, but not recessive mutant or kinase dead versions, protects against rotenone-induced mitochondrial fission. Conversely, PINK1 shRNA mitochondrial fragmentation is enhanced by this complex I inhibitor and overexpression of Dynamin-related protein 1 (Drp1) but not by exogenous Fis1. Drp1 RNAi shows elongation of the mitochondrial network in our PINK1 deficient cells, rescuing the fragmented phenotype. Currently, we are investigating PINK1 mediated phosphorylation of Drp1 and its influence on Drp1 GTPase activity. Downregulation of fission by PINK1-dependent signaling could be a likely mechanism for promoting neuroprotection.

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