

unstabilizing permeability of mitochondria membrane and releasing cytochrome C into cytosol that activate caspase 3 and 9. At the same time, proteins are damaged, which is accumulated by oxidative stress in aging process. That is one of the most possible factors responsible for the functional destruction in aged tissues. Using two-dimensional electrophoresis and MALDI-TOF MS, we investigated mitochondrial proteome in young (13 months of age) and old (31 months of age) rats for establishing the proteome map and profile of age-dependent proteins in mitochondria. At the same time, we studied LPS effects on mitochondrial proteome in young and old rats to mimic the inflammation in aging process. About ninety spots were detected by silver and colloidal Coomassie blue stain as a result of image analysis, we observed age-dependent expression patterns that were increased or decreased. These results suggested that age-related changes of mitochondrial proteome are responsible for functional loss of organ in aging process.

[PC1-27] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Screening Age-related Genes by cDNA-RDA

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Aging was accompanied by changes in gene expression associated with increased inflammation, cellular stress, and fibrosis, and reduced capacity for apoptosis, xenobiotic metabolism, normal cell-cycling, and DNA replication. These changes are associated with an increase risk of morbidity, mortality and disability in old age, but the molecular mechanisms by which this occurs are not fully understood. One of the major difficulties in the study of aging is the lack of biomarkers of aging. Therefore, we performed representational difference analysis (RDA) of cDNA for screening of genes of biomarker in aging rat kidney. To identify genes up-regulated in the aging process, we used a polymerase chain reaction (PCR)-based subtraction method, that is, representational difference analysis of cDNA (cDNA-RDA). Two genes that were age-dependent and differentially-expressed in the kidney were identified. We confirmed by semi-quantitative RT-PCR that these genes showed reproducible age-dependent expression. These results lead to a better understanding of the molecular mechanisms of aging and possibility of candidates of aging biomarkers.

[PC1-28] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Oxidative inactivation of paraoxonase from human plasma

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Paraoxonase (PON), a serum enzyme associated with high density lipoprotein (HDL), is known to protect low density lipoprotein (LDL) from lipid peroxidation involving copper ion. However, PON activity was observed to decrease during LDL oxidation. Here, we attempted to elucidate the possible mechanism for the inactivation of PON. PON was purified from human plasma, and subjected to various oxidant systems. PON activity, based on the hydrolysis of phenyl acetate, decreased slightly after the exposure to H₂O₂ or ascorbate, while oxidants such as peroxyxynitrite or HOCl had no remarkable effect. Inclusion of Cu²⁺ ion in the incubation with ascorbate (0.3~ 1 mM) led to a rapid decrease of activity in a time- and concentration-dependent manner. In comparison, the ascorbate/Cu²⁺ system was much more effective than the ascorbate/Fe²⁺ system in inactivating PON. A further study indicates that general hydroxyl radical scavengers such as mannitol or benzoate failed to prevent the PON inactivation. Separately, when PON was subjected to alkylhydroperoxide, it was found that cumene hydroperoxide inactivated PON in a time-dependent manner, in contrast to t-butylhydroperoxide showing no effect. However, the inclusion of Cu²⁺ exerted no remarkable enhancement of cumene hydroperoxide-induced

inactivation of PON. Based on these results, it is proposed that the PON inactivation during LDL oxidation may be ascribed mainly to the Cu^{2+} -catalyzed oxidation. This work was financially supported by research grant (1-209-003-2) from the Korea Science and Engineering Foundation, Korea.

[PC1-29] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Protein disulfide isomerase-catalyzed multimerization of partially reduced bovine thyroglobulin

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Multimerization of thyroglobulin (Tg) is known to be a way to store Tg at high concentrations in the lumen of the thyroid follicles. Multimerization of bovine thyroglobulin has been intermolecular reactions through the oxidative processes, such as disulfide and dityrosine formation, as well as isopeptide formation. It is known that the disulfide formation is more responsible for Tg multimerization than the other reactions. Here, we investigated the protein disulfide isomerase (PDI) or peroxidase-induced multimerization of normal bovine Tg or partially denatured thyroglobulins, which were prepared from the treatment with thiol, urea or thiol/urea, on the basis of SDS-PAGE analyses. In addition, the enzymatic multimerization was compared with non-enzymatic multimerization, thiol-mediated or thermal. The thiol-induced multimerization of normal Tg was dependent on GSH concentration, in the presence of low GSH concentrations, the dimeric form, 660 kD, was predominant, while multimeric forms were the major in incubations with higher GSH concentration. In addition, the multimerization of Tg under thermal denaturation at 60 °C also required glutathione. Meanwhile, the multimerization of partially reduced Tg was accelerated by PDI in a time-dependent manner up to 30 min at 38 °C. Similarly, the multimerization of partially reduced Tg was also mediated by peroxidase. However, PDI showed no remarkable effect on the peroxidase-mediated or nonenzymatic multimerization. Accordingly, the PDI-mediated multimerization of thyroglobulin through the disulfide linkage may require a specific molecular form of thyroglobulin, optimally reduced and denatured. This work was supported by Korean Research Foundation Grant (KRF-2000-F00302).

[PC1-30] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

BNip3 expression in hypoxia-induced neuronal apoptosis

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Hypoxia/ischemia is one of the most common causes of neuronal injury and implicated in the pathogenesis of degenerative diseases, including dementia. It has been reported that hypoxia/ischemia induces acute neuronal necrosis or delayed cell death, depending on severity and duration of hypoxic injury. Hypoxia-induced delayed neuronal death (apoptosis) plays critical roles in the process of ischemia progression. Even though putative molecules mediating hypoxic injury, including p53 and Bax, were suspected, the precise molecular mechanisms are still unknown. Recently, it was shown that BNip3, a member of pro-apoptotic Bcl-2 family proteins, was transcriptionally activated by hypoxic injury, suggesting certain roles in neuronal apoptosis. The object of this study is to clarify the functions of BNip3 in the apoptotic process induced by hypoxia and we show evidences that BNip3 is one of the strong candidate molecules mediating hypoxic injury.

[PC1-31] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

The release of GPI-anchored proteins from the renal proximal tubules by Bacillus