oxovalerate aldolase and 4-oxalocrotonate decarboxylase were determined. The 4-hydroxy-2-oxovalerate aldolase gene (tomF) was consisted of 1047 bases. Amino acid sequence of the tomF gene product exhibited 84% identity with those of 4-hydroxy-2-oxovalerate aldolase from Comamonas testosteroni and P. putida. The 4-oxalocrotonate decarboxylase gene (tomD) was consisted of 687 bases. Amino acid sequence of the tomD gene product exhibited 75% identity with that of 4-oxalocrotonate decarboxylase from P. putida.

[PC1-25] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Activation of $p21^{WAF1/Cip1}$ transcription through Sp1 sites by histone deacetylase inhibitor apicidin: Involvement of protein kinase C

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We previously reported that apicidin, a novel histone deacetylase (HDAC) inhibitor, inhibited the proliferation of tumor cells via induction of $p21^{WAF1/Cip1}$. In this study, we determined the molecular mechanisms by which apicidin induced the $p21^{WAF1/Cip1}$ gene expression in HeLa cells. Apicidin induced $p21^{WAF1/Cip1}$ mRNA independent of the de novo protein synthesis and activated the $p21^{WAF1/Cip1}$ promoter through Sp1-3 site located at -, 82 and -, 77 relative to the transcription start site. Calphostin C, a protein kinase C (PKC) inhibitor, significantly attenuated the activation of $p21^{WAF1/Cip1}$ promoter via Sp1 sites, which was accompanied by a marked suppression of $p21^{WAF1/Cip1}$ mRNA and protein expression induced by apicidin. Consistent with the transcriptional activation of $p21^{WAF1/Cip1}$ promoter by apicidin, apicidin treatment led to the translocation of PKCε from cytosolic to particulate fraction, which was reversed by pretreatment with calphostin C, indicating the involvement of PKC in the transcriptional activation of $p21^{WAF1/Cip1}$ via Sp1 sites by apicidin. However, the PKC-mediated transcriptional activation of $p21^{WAF1/Cip1}$ by apicidin appears to be independent of the histone hyperacetylation, since apicidin-induced histone hyperacetylation was not affected by calphostin C. Furthermore, a PKC activator, PDBu alone induced the transcriptional activation of $p21^{WAF1/Cip1}$ promoter, $p21^{WAF1/Cip1}$ mRNA and protein expression, without induction of the histone hyperacetylation, suggesting that the transcriptional activation of $p21^{WAF1/Cip1}$ by apicidin might have been mediated by a mechanism other than chromatin remodeling through the histone hyperacetylation. Taken together, these results suggest that the PKC signaling pathway plays a pivotal role in the transcriptional activation of the $p21^{WAF1/Cip1}$ gene by apicidin.

[PC1-26] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

2D/MALDI-TOF MS Analysis of Age-dependent Rat Proteome in Rat Liver Mitochondria

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Mitochondria is called power plant of the cell because they product biological energy, ATP, using electron transport system and proton pump. This system is very toxic to living cells because these systems generate the superoxide anion radical and hydroxyl radical, which induce apoptosis via

unstablilizing permeability of mitochondria membrane and releasing cytochrome C into cytosol that activate caspase 3 and 9. At the sample 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. Howerver, 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 H_2O_2 or ascorbate, while oxidants such as peroxynitrite or HOCl had no remarkable effect. Inclusion of Cu^{2+} 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 acorbate/Cu^{2+} system was much more effective than the ascorbate/Fe^{2+} 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 Cu^{2+} exerted no remarkable enhancement of cumene hydroperoxide-induced