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Molecular fate of thyroglobulin (Tg) is controlled by oligomerization, and deoligomerization. Here, we investigated the protein disulfide isomerase (PDI) and/or peroxidase-induced oligomerization of unfolded thyroglobulins, which were prepared from the treatment of bovine thyroglobulin with heat, urea or thiol/urea, on the basis of SDS-PAGE analyses. In addition, the enzymatic oligomerization was compared with non-enzymatic oligomerization. The thermally-induced oligomerzation of Tg, dependent on GSH redox state, was affected by ionic strength or detergent. Meanwhile, the PDI-catalyzed oligomerization, time-dependent, was the most remarkable with unfolded/reduced Tg, prepared from the treatment with urea/DTT, while thermally-unfolded Tg was less sensitive. Similarly, the oligomerization of unfolded/reduced Tg was also mediated by peroxidase/hydrogen peroxide. However, PDI showed no remarkable effect on the peroxidase-mediated oligomerization of unfolded or unfolded/reduced Tg. Additionally, the reductive deoligomerization of oligomeric Tg was exerted by PDI in excessively reducing state. Based on these results, it is suggested that PDI catalyzes oligomerization of thyroglobulin through the formation of disulfide bond, and its deoligomerization in the molecular fate, and this process may require a specific molecular form of thryglobulin, optimally unfolded/reduced, in proper redox state.

[PC1-43] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Influence of Aging on MAPKs Activity in Rat Kidney

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Mitogen-activated protein kinases (MAPK), which include the extracellular signal-related kinases (ERK), the c-Jun N-terminal kinases (JNK), and the p38 MAPK, are important regulatory proteins by which a wide variety of extracellular signals are transduced into intracellular sites. Recent studies report that reactive oxygen species (ROS) regulate mitogenic signal transduction in various cell types. In the present study, we investigated the effects of aging and calorie restriction (CR) on ROS generation and MAPK activity in rats using kidney isolated from Fischer 344 rats, ages 6, 12, 18, and 24 months fed ad libitum (AL) and CR diets. Results showed that total ROS generation increased with age in the AL rats, while little change occurred in CR rats, even in senescent, 24-month-old rats. Results also showed that the aging process strongly enhanced all three of the MAPK activities studied, ERK, JNK, and p38 MAPK, in a fashion parallel to increased ROS generation. Conversely, we observed CR to markedly suppress the age-related activation of MAPKs. Based on these data, we concluded that an age-related increase in MAPK activity is associated with increased ROS and that CR modulates the MAPK activity.

[PC1-44] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Collaborative study for establishment of KFDA standard for human recombinant Interferon alpha-2a for potency

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The complexity of human recombinant interferon alpha made by different manufacturers can show various biological activities and raise a number of scientific issues with regard to biological standardization. Therefore, individual standards were required for biological standardization and potency determinations of individual interferon alpha subtypes. To establish KFDA standard for human recombinant interferon alpha-2a, KFDA organized a collaborative study. KFDA and other 4 manufacturers participated in this study. Participants were asked to assay the candidate standard in their own bioassay, which was an antiviral assay using MDBK/VSV system, in terms of the 2nd international standard for recombinant interferon alpha-2a (NIBSC 95/650). For each assay, all raw data were analysed by their own analytical methods and the same data were analysed by methods of parallel line analysis with assessment of regression, linearity and

parallelism of the log dose-response lines. It was found that there was no significant difference in the potency between the two analytical methods. The weighted geometric mean of each of the laboratory geometric mean (95% confidence interval) was 3,370,219 IU (3,265,821~3,477,954) by the participants' own methods and 3,289,187 IU (3,202,000~3,330,752) by parallel line analysis.

[PC1-45] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Effect of lawsone methyl ether on the release of glycosylphospatidylinositol-anchored renal dipeptidase and alkaline phosphatase

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Detergent-insoluble microdomains (rafts) may play crucial roles in many cellular functions such as membrane trafficking, cell signaling and human diseases. The variant surface glycoproteins are localized in microdomains and anchored to the cell surface via a glycosylphosphatidylinositol (GPI) anchor. Renal proximal tubules (PTs) were used to compare the release between GPI-anchored renal dipeptidase (RDPase, EC 3.4.13.19) related with cellular signaling pathway and GPI-anchored alkaline phosphatase (APase EC 3.1.3.1) of ubiquitous location. Cell viability was evaluated using a MTT assay and was shown to be intact. The RDPase was released by endogenous GPI-specific phospholipase C (GPI-PLC). Although APase is also a target of GPI-PLC, it showed different activity as compared with RDPase when released from renal PTs in the presence of lawsone methyl ether (LME), a naphtoquinone compound isolated from a Tai medicinal plant. Incubating PTs with various concentration of LME, RDPase activity was increased to 300% (500μM LME) of the control whereas APase activity was not affected significantly. Such increase was also confirmed as a function of time. The results suggest that the release mechanism of RDPase was different from that of APase.

[PC1-46] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Retrovirus-mediated Gene Delivery of TIMP-2 Inhibits Invasion, Migration and Angiogenesis

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The matrix metalloproteases (MMPs) play important roles in invasion, metastasis and angiogenesis in various cell types. An endogenous inhibitor of MMP, tissue inhibitor of metalloprotease-2 (TIMP-2), has high specificity for MMP-2. An imbalance between MMP-2 and TIMP-2 causes the degradation of the extracellular matrix associated with pathological events including invasion, metastasis and angiogenesis. Since TIMPs are secreted molecules, they have the potential to be used for gene therapy of certain tumors. In the present study, we have studied the retrovirus-mediated delivery of TIMP-2 in H-ras MCF10A cells in which MMP-2 was shown to be responsible for the H-ras-induced invasive phenotype. Recombinant retrovirus containing TIMP-2 gene was used to infect PG13 cells (packaging cell line). When the H-ras MCF10A cells were treated with the conditioned media of PG13/TIMP-2, a dose-dependent inhibition of MMP-2 secretion was observed by gelatin zymography. TIMP-2 overexpression mediated by retrovirus significantly reduced the invasiveness and migration of H-ras MCF10A cells in a dose-dependent manner. In addition, retroviral delivery of TIMP-2 efficiently inhibited angiogenesis of HUVEC cells in a dose-dependent manner as evidenced by in vitro tube formation assay. Taken together, we show that the down-regulation of MMP-2 by TIMP-2 overexpression inhibits invasive and migrative properties of H-ras MCF10A cells and angiogenesis of HUVEC cells. Our data showing efficient inhibition of cancer progression by retrovirus-mediated delivery of TIMP-2 suggest a possible application for gene therapy to prevent and treat cancer.

[PC1-47] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]