Transforming growth factor-β (TGF-β), a hormonally active polypeptide found in normal and transformed tissues, regulates cellular growth and phenotyphic plasticity. We have previously shown that H-ras, but not N-ras, induces invasive phenotype in MCF10A human breast epithelial cells. In this study, we wished to examine the effect of TGF-β on H-ras-induced invasion and motility in MCF10A cells by performing *in vitro* invasion assay and wound migration assay. TGF-β significantly induced invasive phenotype of non-invasive parental MCF10A and N-ras MCF10A cells. Since matrix metalloproteinase (MMP)-2 and MMP-9 play critical roles in cellular invasion, we investigated MMP-2 and MMP-9 activities in TGF-β-treated cells. A prominent upregulation of MMP-2 and a slight increase of MMP-9 were detected upon TGF-β treatment, suggesting that TGF-β-induced invasive phenotype may possibly be mediated by MMP-2 rather than MMP-9. TGF-β enhanced migration of H-ras MCF10A and N-ras MCF10A cells in a dose-dependent manner while it did not affect non-transformed MCF10A cell migration. The data suggest that the stimulatory effect of TGF-β on migration is seen only in cells where the ras signaling pathway is activated but not in the parental MCF10A cells. In order to study the molecular mechanisms under which TGF-β enhances cell migration, activation of ras downstream effector molecules by TGF-β is currently being investigated

[PC1-44] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

cDNA cloning of a membrane-associated, magnesium-dependent 30kDa neutral sphingomyelinase

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A major lipid-signaling pathway in mammalian cells implicated the activation of sphingomyelinase (SMase), which hydrolyses sphingomyeline to generate ceramide and phosphocholine. Sphingomyelinase is divided into many isoform groups dependent on optimal pH, and essential cation especially magnesium in their activation. Such as acidic sphingomyelinase, neutral sphingomyelinase and alkaline sphingomyelinase.
Ceramide is known as a crucial second messenger in cell responses like cell proliferation, cell cycle arrest, cellular senescence, and apoptosis. However there are many reports that ceramide activates CAPP (ceramide-activating protein phosphatese), CAPK (ceramide-activating protein kinase) and phospholipaseA2 etc.
In this study, it was confirmed that the 30 kDa protein has the SMase activity. cDNA encoding the 30 kDa protein was cloned using anti-30 kDa protein antiserum. cDNA sequencing analysis of the DNAs showed that the 30 kDa protein is identified as a well-known protein.
For further study, the protein will be expressed in the Eukaryotic cells and the SMase activity will be measured. And the cellular fuction of this protein will be studied using 2-D elctrophoresis and Maldi-tof.

[PC1-45] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Stability of the current biological drugs(typhoid vaccine)

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This paper presents stability of tyhoid vaccine -attenuuated vaccine(oral) and killed vaccine(vi polysaccharide)- 5 classes with various temperature(3 points; iced temperature, refrigeration temp., indoor temp.). Analytical techniques -vi polysaccharide content, pH, sterility, assay - have been used for the quantity of pharmacologically active chemical entries. From this study, we have found the attenuated vaccine is show iced temp. 258% and indoor temp. 0.02% compare with refrigeration temp. in assay examination and the killed vaccine is not found different.

[PC1-46] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

An *In Vitro* Bioassay for Nerve Growth Factor

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A convenient bioassay of nerve growth factor(NGF) is essential for assessing its potency during the course of product development and quality controls afterwards. We have set up a cell-based bioassay for determining the potency of recombinant NGF using rat pheochromocytoma (PC12) cells. Cell survival was measured by monitoring the reduction of the alamarBlue^{TM} dye by living cells. The assay is simple and dose not require collagen-coated culture plates and shows reproducible dose-response growth responses after 2 or 3 days incubation under serum free conditions. We tested three different types of NGF which were recombinant human β-NGF, WHO reference reagent(93/556) and murine NGF. The effective ranges were 1-100 ng/ml for human β-NGF and 5-500 ng/ml for the others. The curve patterns of first two NGFs were steeper than that of murine NGF, the slops of them dramatically increased around EC_{50}. This cell survival assay determined the NGF potency, indirectly by using its downstream cellular response as an end-point, therefore we have tried a direct method at upstream level by measuring NGF-induced tyrosine kinase receptor TrkA combined with ELISA in terms of receptor phosphorylation using endogenously expressed NGF receptor in PC12 cells. We compared the result with antiphosphotyrosine Western blot analysis and they showed good correlation.

[PC1-47] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Alternation of Adhesion Molecules during Aging and Modulation by Calorie Restriction

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Expressions of adhesion molecules (AMs) are closely related to the formation of early atherosclerosis, an age-dependent process. However, previous research only provided limited and conflicted reports about alternated AMs' expressions during aging and even much less is known about modulation of AMs by calorie restriction (CR), the only established anti-aging experimental paradigm.
In this study, expression of inflammatory AMs: vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin and plate/endothelial cell adhesion molecule-1 (PECAM-1) and non-inflammatory AM: vascular adhesion protein1 (VAP-1) in aorta and kidney were investigated by western blot and immuno-histochemistry stain in Ad libitum (AL) and CR rats. Their mRNA level were detected by RT-PCR. Current data demonstrated that: (1) in the aorta, expression of VCAM-1 significantly increased during aging. (2) in the kidney, expression of VCAM-1, E-selectin, PECAM-1 in kidney increased during aging; VCAM-1, E-selectin expression were down-regulated by CR. (3) RT-PCR data shown increased expression of VACM-1 and PECAM-1 during aging and blunted by CR, while ICAM-1 mRNA level kept no change during aging.
In conclusion, our data demonstrated that most of the inflammatory AMs increased expression during aging and down-regulated by CR. Increased AMs contribute to pathological process of vascular aging.

[PC1-48] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Studies on standardization and characterization of recombinant interferon alfa

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This study was intended to establish test methods equivalent to those of "Interferon alfa-2 concentrated solution" monograph in European Pharmacophoeia(EP). Two recombinant interferon alfa concentrated solutions manufactured in Korea were tested according to the monograph of EP. Tests of identification(biological activity, isoelectric focusing, SDS-PAGE under reducing condition, peptide mapping), related proteins, impurities of molecular masses differing from that of interferon alfa-2 (SDS-PAGE under reducing and non-reducing condition), bacterial endotoxin, protein, potency, host-cell-derived proteins, and host- cell-derived DNA were performed in the laboratories of manufactures and division of biotechnology, KFDA. The results of this study showed that specifications of interferon alfa concentrated solutions manufactured in Korea were within the acceptance criteria of EP. Based on this study, specifications and test methods for interferon alfa concentrated solution can be established according to the monograph of EP suggesting the revision of 「Minimum requirements