

have already existed at the time of surgery. Therefore, understanding how metastasis is regulated at the molecular level is required to devise new modalities of gastric cancer therapy. In present study, we measured the invasive potentials of various Korean-derived gastric cancer cell lines and subsequently performed subtractive suppression hybridization to identify metastasis-related genes by comparing differential gene expression profiles between highly invasive SNU-638 and poorly invasive SNU-484 gastric cancer cell lines. Twenty-two cDNAs were identified as overexpressed genes in SNU-638 cells confirmed by Northern blot analysis. Among them, a splicing variant of aspartyl beta hydroxylase (Humbug) was also identified as a gene overexpressed in metastatic SNU-638 cells. Humbug encodes a protein identical to aspartyl-beta hydroxylase through the NH₂-terminal half of the protein, but completely lacks the catalytic domain of aspartyl-beta hydroxylase. Therefore, we further investigated the possible involvement of this gene in metastatic progression of cancer cells. Expression analysis showed that the level of Humbug mRNA was well correlated with invasive and metastatic potential in various gastric cancer cell lines. Moreover, gastric tumor tissue exhibited much higher Humbug mRNA expression than the normal counterparts. Transfection of Humbug cDNA into poorly invasive Az-521 cells resulted in the increase of its migratory and invasive potentials. These results imply that Humbug could be an overexpressed gene during metastatic progression of human gastric cancer cells, and promote tumor cell invasion and metastasis.

[PC1-40] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Modulation of Redox-sensitive Transcription Factor, AP-1 by Aging and Calorie Restriction

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Oxidative stress is claimed to be the major cause of aging and many age-related diseases. Recent data strongly suggested that the life-prolonging calorie restriction (CR) might retard aging by its anti-oxidative action on the regulation of the intracellular redox status. Currently, there is little information concerning the influences of age and CR on the redox-sensitive transcription factor, activator protein-1 (AP-1). In this present study, we investigated whether age affects the regulation of AP-1, and how the age effect is modulated by CR. The kidney isolated from Fischer 344 rats at 6, 12, 18, and 24 months of age fed ad libitum (AL) and CR rats were used. Results showed that AP-1 binding activity markedly increased with age in parallel with increased ROS generation, and CR suppressed the activation at the level of 6 months old. Recently, accumulating evidence indicate that mitogen-activated protein kinase (MAPK) cascade can contribute to AP-1-dependent transcription. Results showed that the aging process strongly enhanced all three MAPKs activities, while CR markedly suppressed the age-related activation of MAPK. It is known that thioredoxin (Trx), which is mainly in the cytoplasm, quickly translocates into the nucleus and activates AP-1 transcriptional activity by direct association with an intranuclear redox factor, Ref-1. We present evidence that the increased AP-1 activity during aging is correlates with increased nuclear protein levels of Trx and Ref-1. Based on these data, we concluded that the age-related increases in redox-sensitive AP-1 binding activity are associated with increased ROS, and CR modulates the AP-1 activation by suppressing oxidative stress. This molecular insight provides a better understanding of the regulation of cellular events leading to age-associated pathogenic process and furthermore reveals pertinent clues on possible therapeutic intervention.

[PC1-41] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Homology modeling of human TCTP using three different computer programs

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The translationally controlled tumor-associated proteins (TCTPs) are a highly conserved and abundantly expressed family of eukaryotic proteins that are implicated in both cell growth and the human acute

allergic response but whose intracellular biochemical function has remained elusive. Recently the solution structure of TCTP from *Schizosaccharomyces pombe* has been reported. A sequence alignment with human and *S. pombe* TCTPs shows approximately 49% identity and 65% similarity. We tried homology modeling of human TCTP using the 3D structure of *S. pombe* TCTP as template by three different computer programs COMPOSER, SWISS-MODEL, and GENO3D. Three different models were obtained and their structures were superimposed to display the similarities of the structures. The results present distinct similarity in α -helix region whereas the numbers and positions of β -strands vary depending on the program. The refinements of the models are in development taking the function of the protein into consideration to end up with a final conformation.

[PC1-42] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Measurement of Estrogen Receptor Binding Activities of Estradiol and Endocrine Disrupters by Homogeneous Fluorescence Polarization Assay

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A simple homogeneous fluorescence polarization receptor assay (FPRA) was developed to measure the receptor binding activities of various endocrine disrupting chemicals (EDCs) using ethylenediamine fluorescein thiocarbonyl (EDF)-labeled estrogen and estrogen receptor. EDF-estrogen tracer was synthesized from 6-ketoestradiol-6-(*o*-carboxymethyl)oxime and EDF using EDC coupling reaction, and cytosolic estrogen receptors were purified from rat uterine.

Calibration curve of FPRA was established using 17β -estradiol ($K_d = 1.1 \times 10^{-9}$ M) which can bind with estrogen receptor in the range between 100 nM and 1 mM at the optimized condition. After receptor binding activities of FPRA were characterized with the estrogenic chemicals (diethylstilbesterol and tamoxifen) and androgenic chemicals (methyltestosterone and flutamide), each EDC was compared for the binding activity with estradiol. The detail relative binding activities of various EDCs will be discussed. This homogeneous FPRA system takes 20 minutes for 10 samples using photo check mode of a TDx analyzer. It needs no separation step between free tracer and receptor-bound tracer. Therefore, the system has high potential to test estrogen receptor binding activities for various endocrine disrupters.

[PC1-43] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Platelet Activating Factor Acetylhydrolase Activity in CSF of Children with Acute Systemic or Neurological illness

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Platelet activating factor acetylhydrolase (PAF-AH) is the enzyme that hydrolyzes inflammatory mediator PAF and phospholipids containing oxidized truncated fatty acids. It is distributed widely in tissues and plasma and is thought to be a defense mechanism of protecting the host against the toxic effects from PAF and other biologically active oxidized phospholipids. Higher levels of PAF-AH have been found in plasma and other body fluids in a variety of different diseases, but few studies have been conducted to measure the level of PAF-AH in CSF. Therefore, we measured the PAF-AH activity in cerebrospinal fluid (CSF) to determine if it is involved in CSF defense mechanism against the injury from a variety of neurological conditions including meningitis, seizures, receiving intrathecal chemotherapy etc. A total 85 patients (55 males and 30 females, mean age 3.8 years) were involved in the study. Subjects studied were 7 patients with meningitis, 24 with acute febrile illness, 4 with CNS inflammatory diseases, 24 with