[PC1-35] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

The GPI-specific PLC that releases renal dipeptidase from the GPI-anchor is present in detergent-insoluble GPI-rich membrane domains

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The activity of a glycosylphosphatidylinositol (GPI)-PLC that releases renal dipeptidase (RDPase, EC3.4.13.19) from the GPI-anchor was not modulated with the agents affecting the signal transduction via intracellular PI-PLC; carbachol (receptor agonist), AlF_4^- (G protein like activator) and 8-Br-cGMP (cGMP analogue) as well as U73122 (PI-PLC inhibitor), neomycin (PLC inhibitor), phorbol 12-myristate 13-acetate (protein kinase C activator) and staurosporin (protein kinase C inhibitor). These suggest that the GPI-PLC is distinct from intracellular PI-PLC. In contrast, the rapid release of RDPase with insulin, but no effect with epidermal growth factor, results from the hydrolysis of GPI-anchored RDPase by GPI-PLC forming the inositol 1,2-cyclic monophosphate cross reacting determinant. The GPI-PLC was inhibited with $HgCl_2$ and activated with $[Ca^{2+}]_i$ as was demonstrated in trypanosomal GPI-PLC. The monoclonal antibody raised against X domain of phospholipase Cδ4 reduced the GPI-PLC activity at the surface of proximal tubules indicating the cross-immunoreactivity and identified a single polypeptide of 54 kDa in the detergent (Triton X-100) insoluble GPI-rich membrane microdomain (DIG). These results strongly suggest that a GPI-PLC, RDPase-releasing-activity from the proximal tubules which is activated with insulin, is distinct from an intracellular PI-PLC, and that it is present in the DIG with RDPase.

[PC1-36] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Induction of inducible nitric oxide synthase by ceramide in human colon carcinoma HT-29 cell.

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Ceramide generated from sphingomyelin by hydrolysis has been shown to be a novel lipid second messenger for cellular functions ranging from proliferation and differentiation to growth arrest and apoptosis in various cell systems. To investigate the possible role of the sphingomyelin signaling pathway on nitric oxide (NO) production and expression of inducible nitric oxide synthase (iNOS), we studied the effect of synthetic ceramide (C6-ceramide) in human colon carcinoma HT29 cells. Ceramide increased NO synthesis in a concentration-dependent manner. N^G-methyl-L-arginine (NMA), a competitive inhibitor of iNOS blocked ceramide-dependent NO production. However, N^G-methyl-D-arginine (NMDA), an inactive form of NMA, did not show any significant change. Immunoblot analyses showed the expression of iNOS protein was stimulated by ceramide. Treatment of cells with bacterial sphingomyelinase (SMase) also enhanced iNOS expression. However, dihydroceramide, a biologically inactive ceramide, had no effect on iNOS induction. NMA suppressed induction of iNOS expression by ceramide. These results suggest that ceramide may modulate the cellular functions via increasing NO production and iNOS expression in human colon cancer cells.

[PC1-37] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Roles of ERK1/2 and p38 MAPK Signaling Pathways in Phorbol Ester -induced

Expression of Cyclooxygenase-2 via NF-κB in Cultured Human Breast Epithelial Cells

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The inducible form of cyclooxygenase-2 (COX-2) has been often observed in various types of cancerous and transformed cells. In this study, we examined molecular mechanism underlying regulation of COX-2 expression through of the eukaryotic transcription factor NF-κB. Cyclooxygenase expression induced by 12-O-tetradecanoylphorbol-13-actate (TPA) in human breast epithelial cells (MCF10A) were inhibited by specific mitogen-activated protein kinase (MAPK) inhibitors, such as SB 203580 (p38 MAPK inhibitor) and PD 98059 (ERK inhibitor) and also by dominant negative (DN) MAP kinase expressing vectors (pCMV5-p38 MAPK DN mutant or pCEP4-pERK2 DN mutant vector). In the luciferase reporter gene assay, NF-κB transcriptional activities were suppressed by dominant negative MAPKs mutant vectors. In anther study, we assessed the COX-2 expression and NF-κB activation in activated H-ras oncogene transformed MCF10A cells, but we did not found any distinct differences between MCF10-H-ras and its parental cell line in terms of COX-2 and NF-κB activation.
These result suggest that ras activation alone is not sufficient to induce COX-2 expression in human breast epithelial cells and activation of other pathways including p38 MAPK may be required for up-regulation of COX-2 in these cells.

[PC1-38] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Synthesis and Characterization of DDT Immunogens

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For development of the immunodetection method of DDT family (4,4'-dichlorodipheny-2,2,2-trichloroethane, a persistent and broad toxic organochlorine pesticide), various DDT derivatives were synthesized and characterized for the use of immunogen and the coating ligand of the antibody evaluation. The appropriate lengths of linkers were introduced to investigate more efficient DDT derivatives. Carboxylic acid group was chosen as a functional group for coupling with carrier protein at the terminal position of linker. DDA was readily obtained from the oxidation reation of DDT. DDHP having three carbon linker was directly prepared from the reaction of glutaric anhydride with 4-chlorophenylmagnesium bromide, subsequently chlorination of hydroxyl group at C-1 position gave DDCP. Other derivatives with long chain linker and amide group in linker were prepared through similar reaction. The detail synthetic method for DDA, DDAAP, DDHP DDCP, DDHH, DDCH, DDHHAP, DDCHAP will be discussed.
Among these hapten derivatives, DDA, DDHP and DDCP were conjugated with keyhole limpet hemocyanin for the use of immunogen to produce antibodies. The BSA conjugates of these derivatives were prepared as a coating ligand for the antibody screening. Several monoclonal antibody clones were screened using these probes.

[PC1-39] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Effects of endocrine disruptors in whole-organ culture of mouse mammary glands

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