involved in ATX-mediated tumor cell motility stimulation.

[PA1-63] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Delayed ERK Activation by Ceramide Reduces Melanin Synthesis in Human Melanocytes

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We investigated the effects of sphingolipid metabolites and the possible signaling pathways involved in human melanocytes. Our data shows that C2-ceramide inhibits cell growth in a dose-dependent manner, whereas SPP has no effect. Moreover, we observed that the melanin content of the cells was significantly decreased by C2-ceramide. The pigmentation-inhibiting effect of C2-ceramide at 1-10 μM was much stronger than that of kojic acid, tested at 1-100 μM. The tyrosinase activity of cell extracts was reduced by C2-ceramide treatment. However, in the cell-free system, C2-ceramide could not suppress tyrosinase, whereas kojic acid directly inhibited tyrosinase. These results suggest that C2-ceramide decreases the pigmentation of melanocytes indirectly regulating tyrosinase. Furthermore, we found that C2-ceramide inhibited the production of microphthalmia-associated transcription factor (MITF), which is required for tyrosinase expression. To identify the signaling pathway of ceramide, we studied the ability of C2-ceramide to influence extracellular signal-regulated protein kinase (ERK) and Akt/protein kinase B (PKB) activation. C2-ceramide induced a delayed activation of ERK (>1 h) and a much later activation of Akt/PKB (>3 h) in human melanocytes. In addition, the specific inhibition of the ERK and the Akt signaling pathways by PD98059 and LY294002, respectively, increased melanin synthesis. Thus, it seems that sustained ERK and Akt activation may lead to the suppression of cell growth and melanogenesis.

[PA1-64] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Intracellular signaling pathway associated with sphingosylphosphorylcholine-induced contraction in feline ileal smooth muscle cells.

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It is proposed that SPC-induced contraction mechanism involves a certain G protein, phospholipase and kinases-dependent pathway. G protein subtypes of Gi1, Gi3 and Go are immunoblotted in feline ileum. To investigate which subtype mediates the contraction, permeabilized cells were used. Gi3 antibody-treated cells showed the decrease of contraction induced by SPC. In addition, incubation of [^{35}S]GTPγS with membrane fraction increased its binding to Gi3 subtype after SPC treatment, suggesting that the signaling pathways invoked by SPC were mediated by Gi3 protein. After treatment of PLC inhibitor neomycin, the contraction was reduced. Of existing PLC subtypes, PLCγ1 antibodies decreased SPC-induced response when treated to permeabilized cells. MEK inhibitor PD98059 and PKC inhibitor chelerythrine blocked the contraction significantly, but p38 MAP kinase inhibitor SB202190 did not. However, co-treatment of PD98059 and chelerythrine showed no significant difference. SPC-induced contraction was inhibited with the incubation of PKCε antibodies to penetrate permeabilized cells. Phosphorylation and activity of p44/42 MAP kinase and was increased by SPC treatment, which was reversed by pretreatment of inhibitors of signaling molecules that decreased SPC-induced contraction previously. Thus, in feline ileal smooth muscle cells, the mechanism of contraction by SPC involves Gi3 protein, followed by the activation of PLCγ1, PKCε, and p44/42 MAP kinase.

[PA1-65] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Autotaxin increase the motility is breast cancer cells

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Autotaxin(ATX) is an exo-nucleotide pyrophosphate and phosphodiesterase (NPP) which stimulates tumor cell motility at low nanomolar concentration. We compared the expression of ATX in normal breast and tumor tissues and linked the differential expression of ATX with the invasiveness of breast cancer cells. Cellular ATX mRNA expression was more than 3times higher in breast tumor cells. Breast cancer cells with high invasive and/or metastatic capacity such as MDA-MB 435S and MDA-157 showed relatively higher ATX expression. Overproduction of ATX in MCF7 cells incerased the cell migration response to ATX as a chemoattractant. Takem together, these data suggest that cellular expression of ATX is correlated with growth and invasiveness of breast tumor cells.

[PA1-66] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Homo-or hetero-dimerization of muscarinic receptor subtypes are not mediated by protein-protein interaction through intracellular and C-terminal regions.

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A number of cell surface receptors including G-protein coupled receptors (GPCRs) mediate their actions via dimerization which alters the ligand-binding, signaling and properties of the receptors. GPCR dimerization have shown to occur by various mechanisms such as disulfide linkage, transmembrane domain-involved (noncovalent) interaction or direct interaction through C-terminal tails. Muscarinic receptors which are members of GPCRs may also be arranged in dimeric or oligomeric complexes but their mechanisms are not clear. Here we examined whether muscarinic receptors form homo-oligomer or heteromer by direct protein-protein interactions among the same subtypes or different subtypes using yeast two hybrid system. Each cytoplasmic loop and C-terminal cytoplasmic tail of human muscarinic (hm) receptor subtypes, hm1, hm2 and hm3, were cloned into vectors of two hybrid system and examined direct protein-protein interactions between cytoplasmic domains. We also cloned extracellular loops and N-terminal to reveal the interactions between extracellular loops. No detectable interactions were observed in all Hm/Hm receptor sets tested. These results indicate that the hm1, hm2 and hm3 receptors do not interact directly through hydrophilic intracellular and C- terminal tail domains to form dimer or oligomer. N-terminal of Hm2 also showed no interaction with any extracellular domain. Our study raises the possibility that interactions for dimerization of muscarinic receptors may occur indirectly or require proper conformation or subunit formation.

[PA1-67] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Natural resistance-associated macrophage protein 2(DMT1/DCT1) expression is associated with pH-dependent Lead uptake in astrocytes.

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Nramp2, also known as DMT1 and DCT1, is a 12-transmembrane(TM) domain protein responsible for dietary iron uptake as well as metal ions such as lead, manganese, zinc, copper, nickel, cadmium, and cobalt. In duodenal cells iron transport by Nramp2 occurs by a proton-dependent mechanism. Metal ion overload is toxic and leads to neurodegenerative diseases in CNS. Astrocytes may play a role in the scavenger of toxic divalent heavy metal ions in CNS. The process of lead uptake by Nramp2 was not reported in astrocytes.