Oral Presentations

[OA-1] [04/19/2002 (Fri) 14:00 - 14:10 / Hall A]

Characterization of a zebrafish (Danio rerio) sphingosine 1-phosphate receptor expressed *in the embryonic brain.*

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Sphingosine 1-phosphate (S1P), a metabolite of sphingosine, is one of the biologically active lysophospholipids that evokes a variety of cellular responses, including cell proliferation, anti-apoptosis, and neurite retraction. The actions of S1P are mediated via its specific interaction with cell-surface receptors, at least five G protein-coupled Edg receptors. We cloned zebrafish edg1 and expressed it in RH7777 cells. In these cultures, S1P inhibited forskolin-driven rises in cAMP and this response was eliminated by pretreatment of the cultures with pertussis toxin. In RH7777 membranes, S1P stimulated GTPgamma[(35)S] binding 2-3 fold. Zebrafish edg1 is expressed in embryonic brain, particularly ventral diencephalon, optic stalks, and anterior hindbrain. Our findings suggest that nonmammalian vertebrates use S1P to signal during embryogenesis and that the properties of Edg1 receptor have been conserved for 400 million years.

[OA-2] [04/19/2002 (Fri) 14:10 - 14:20 / Hall A]

Effect of Recombinant Human FSH on Ovulation, Pregnancy and In Vitro Fertilization in Androgen-Sterilized Mice

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The present study was performed to examine the effect of a new rhFSH, PG-0801, on oocyte quality, ovulation and in vitro fertilization (IVF) in androgen-sterilized mice. Experimental sterility was produced by a single subcutaneous injection of testosterone propionate (TP, 1 mg/head) into 5 day old female mice. Ovulation was induced in 10 to 13-week old TP-injected mice by a subcutaneous injection of rhFSH (1, 5 10 IU/head) followed forty-eight hours later by a second injection of rhFSH (1, 5 or 10 IU/head). For comparison, subcutaneous PMSG (5 IU/head) was used for folliculogenesis and hCG (5 IU/head) for ovulation, and these were administered using the same protocol. Seventeen to twenty hours after the second injection, eggs were harvested from the oviducts and counted. To determine the functional activity of the eggs, IVF was performed by adding sperms ($2x10^5$/ml to $2x10^6$/ml) and the fertilization rate was determined. In addition, the pregnancy rate and fetal development were examined on days 15-17 of gestation. The number of oocytes recovered from the rhFSH/rhFSH group increased dose-dependently and was slightly higher than that of the PMSG/hCG group. The pregnancy rates for the group receiving 1, 5, and 10 IU of rhFSH/rhFSH were 50%, 66.7%, and 75%, respectively, which were significantly higher than that of the control (untreated) group (0%). The numbers of viable fetuses in the 1, 5, and 10 IU/head of the rhFSH/rhFSH group (8.0 ±1.50, 8.9 ±1.02, and 8.9 ±1.12 fetuses/dam, respectively) were comparable to that of the 5 IU/head PMSG/hCG group (9.4 ±0.94). Mice receiving rhFSH/rhFSH and PMSG/hCG showed similar fertilization rates (around 65%) via the IVF procedure. These results demonstrate that a new rhFSH, PG-0801, may be useful for the induction of ovulation in functionally infertile patients and for the superovulation in ovulatory patients participating in an assisted reproductive technology (ART) programs.

[OA-3] [04/19/2002 (Fri) 14:20 - 14:30 / Hall A]

Unusual Induction of Cyclooxygenase-2 in H-Ras-Transformed Human Breast Epithelial Cells Undergoing Apoptotic Death

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Cyclooxygenase-2 (COX-2) is an inducible enzyme expressed in response to a variety of proinflammatory. The presence of oncogenic *ras* has been associated with sustained induction of COX-2, which confers resistance to apoptosis. Contrary to the above notion, we found that MCF10A-*ras* cells treated with an anti-tumor agent, ET-18-O-CH3, underwent apoptosis as revealed by proteolytic cleavage of poly(ADP-ribose) polymerase, pro-caspase 3 activity, and TUNEL staining, while the same treatment caused an increased expression of COX-2 as well as the elevated production of prostaglandin E_2(PGE_2). The apoptotic effect of ET-18-O-CH3 involved intracellular accumulation of reactive oxygen species. Treatment of MCF10A-*ras* cells with the selective COX-2 inhibitor celecoxib (50 μM) attenuated ET-18-O-CH3-induced apoptosis as well as COX-2 expression and production of PGE_2, suggesting that unusual expression of COX-2 by ET-18-O-CH3 is causatively implicated in the induction of apoptosis. ET-18-O-CH3 inhibited activation of both Akt/protein kinase B and transcription factor NF-κB that are involved in cell survival pathways. ET-18-O-CH3 also inhibited activation of ERK1/2 and p38. ET-18-O-CH3-induced inactivation of these protein kinases and NF-κB was attenuated by ce ecoxib. Taken together, the above findings suggest that COX-2 up-regulation does not necessarily confer the resistance to apoptosis in *ras*-transformed cells, but may rather sensitize these cells to apoptotic death. This work was supported by the grant (01-PJ1-PG1-01CH05-0001) from the Ministry of Health and Welfare, Republic of Korea.

[OB-1] [04/19/2002 (Fri) 14:30 - 14:40 / Hall A]

Cyclin-Dependent Protein Kinases Play an Essential Role in Apoptotic Progression

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In our earlier report, we have shown that activation of cyclin A-cdk2 activity is an essential event in apoptotic progression of SK-HEP1 cells induced by treatment with ginsenoside-Rh2 (G-Rh2). In the present study, we provide evidence that abnormal activation of cyclin-dependent protein kinases activities are commonly occurring in different cell types induced by various apoptosis inducing systems, including Etoposide, Paclitaxel, and TRAIL. Both Cdk2 and Cdc2 kinase activities were dramatically up-regulated in apoptotic cells induced by treatment with Paclitaxel or TRAIL. By contrast, Cdk2 but not Cdc2 kinase activity was remarkably up-regulated in Etoposide-induced apoptosis. Forced down-regulation of cdk2 activity by ectopic overexpression of p21WAF1/CIP1, a potent inhibitory protein of Cdk2, or that of the dominant negative version of Cdk2 (Cdk2-dn) efficiently and equally blocked the apoptosis progression of the cells induced by three different apoptosis inducers. Overexpression of cyclin A in the cells resulted in a dramatic up-regulation of cyclin A-Cdk2 activity and accordingly, enhanced apoptosis in same system. Ectopic overexpression of dominant negative version of Cdc2 also successfully suppressed the TRAIL- or pacletaxel-induced apoptosis. From these data, we propose that activation of cdk2 and/or cdc2 is a general prerequisite event in apoptotic progression that undergoes mediating through either mitochondria and death-receptor pathways.

[OC-1] [04/19/2002 (Fri) 14:40 - 14:50 / Hall A]

Celecoxib Down-regulates Phorbol Ester-induced Expression of Cyclooxygenase-2 through Inhibition of AP-1 and C/EBP Transcripton Factors in Mouse Skin In Vivo

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Cyclooxygenase (COX) catalyzes the rate-limiting step in the formation of prostaglandins from arachidonic