Department of Life Science, Kwangju Institute of Science and Technology

During apoptosis, p130Cas is cleaved by caspase-3 in phosphorylation-dependent manner and the 31kDa fragment is generated. The phosphorylated p130Cas by LPA appears to be resistant to cleavage by caspase-3 and the dephosphorylation of p130Cas by CIP, PP1 and LAR enhanced the production of 31kDa fragments. Moreover, double mutations substituting the Glu of S743 and Y751 at a cleavage site was also resistant to caspase-3 cleavage, suggesting that production of the 31kDa fragment is regulated by phosphorylation. The 31kDa fragment, which contains a divergent helix-loop-helix(HLH) motif, is translocated into nucleus during etoposide-induced apoptosis and is likely to interact with E47 via HLH domain. In addition, the overexpression of GFP-fused 31kDa fragment induced the morphological changes characteristic of apoptosis, suggesting that the 31kDa fragment may be translocated into nucleus and thereby regulate the onset of apoptosis through interaction with the transcriptional regulatory HLH proteins, in which the complex may lead to induction of cell death gene associated with the induction of apoptosis.

E112 Role of Cloned ApCAM-Associated Protein (CAMAP) on Long-Term Facilitation in Aplysia

Chae-Seok Lim*, Jin-A Lee, Jeong-Yeon Kim and Bong-Kiun Kaang
Institute of Molecular Biology and Genetics, School of Biological Sciences, Seoul National University

The growth of synaptic connections that accompanies 5-HT-induced long-term facilitation (LTF) in *Aplysia* is associated with the internalization of apCAM at the surface membrane of the sensory neuron. In this study, to gain more insight into apCAM function, we searched for binding proteins of apCAM by the yeast two-hybrid method by using as bait the cytoplasmic tail of apCAM. Sequence analysis revealed that one cDNA clone, we obtained, has some homology to a microtubule associated protein. This apCAM-associated protein (CAMAP) was coimmunoprecipitated with apCAM from transfected HEK293T cells. Confocal microscopic analysis also showed that CAMAP colocalized with apCAM at the cytoplasmic face of plasma membrane in cultured *Aplysia* neurons. In sensori-motor coculture, overexpression of CAMAP in *Aplysia* sensory neurons induced LTF by application of a single pulse of 5-HT that normally induced only short-term facilitation (STF). In addition, microinjection of antisense CAMAP into the sensory neurons completely blocked the LTF induced by 5-HT, without affecting STF. These results suggest that CAMAP plays a crucial role in LTF presumably by interacting with apCAM to induce internalization and synaptic growth associated with LTF in *Aplysia*.

E113 Presence of Specific Receptor for Dendroaspis Natriuretic Peptide in the Freshwater Turtle Kidney

Sung Zoo Kim*, Yoon Ah Kim, Soo Mi Kim, Suhn Hee Kim, and Kyung Woo Cho
Department of Physiology, Chonbuk National University Medical School

Dendroaspis natriuretic peptide (DNP), a 38-amino acid residue peptide, was isolated from the venom of the green mamba snake (*Dendroaspis angusticeps*), and shared functionally important sequence homologies with ANP, BNP and CNP. Although it has been designated as a new member of natriuretic peptide family, the natriuretic peptide receptor for DNP is still not clear. The present study was undertaken to investigate the presence of DNP-dependent receptor in the kidney of freshwater turtle. By quantitative in vitro receptor autoradiography, specific ^{125}I-DNP binding sites were localized in glomerulus and renal tubules. In the presence of excess

concentration of unlabeled DNP, the dense glomerular and renal tubular bindings were completely displaced. The maximal binding densities of ^{125}I-DNP in glomerulus was higher rather than those in renal tubules. Various natriuretic peptides competed with the bindings of ^{125}I-DNP to the glomerulus and renal tubules. These results indicate that specific receptor for DNP is localized in the kidney, and DNP may be a regulator of renal functions in the freshwater turtle.

E114 Src Kinase and PKC Are Involved in CD99 Type II-Mediated Signaling Pathway Which Leads to Promotion of Cell Motility and MMP-9 Secretion in Human Breast Carcinoma Cells

Hyuk-Jun Lee[1*], Eunsook Kim[2], Jang-Hee Hahn[2] and Hansoo Lee[1]
[1]Division of Life Sciences, [2]Lab. of Anatomy, Kangwon National University

We have shown previously that expression of a splice variant of CD99 membrane protein (CD99 type II) increases cell motility and matrix metalloprotease-9 (MMP-9) secretion in MDA-MB-231 human breast carcinoma cells. When various inhibitors for signal transduction mediators were tested for their effects on motility and MMP-9 activity of CD99 type II-transfected breast carcinoma cells. PP1, a src kinase-specific inhibitor, exhibited a significant inhibition on motility of CD99 type II-expressing cells. Among src transfectants, dominant-negative src- transfected cells were 80-90% less motile than mock transfectant cells, while v-src- and c-src-transfected cells exhibited motility levels at or slightly above the mock transfectants. Meanwhile, MMP-9 activity in a culture of CD99 type II-expressing cells was completely inhibited by PKC-specific inhibitors, GF109203X and myristoylated PKC peptide, whereas PMA, a PKC activator, increased MMP-9 activity in cells devoid of CD99 type II expression to a similar level of that in CD99 type II-expressing cells. Our data strongly suggest that CD99 type II promotes motility and MMP-9 secretion of human breast carcinoma cells through the activation of src kinase and PKC, respectively.

E115 Biochemical and Biological Analyses of a Novel GTP-Binding Protein Interacting with NF2 Tumor Suppressor

Bokeun Jee[1], Jin Q Cheng[2] and Hansoo Lee[1*]
[1]Division of Life Sciences, Kangwon National University; [2]Dept. of Pathology, H. Lee Moffitt Cancer Center, Tampa, FL, USA

In attempt to understand the molecular mechanism allowing the neurofibromatosis-2 (NF2) gene product to function as a tumor suppressor, we have previously identified a novel GTP-binding protein, named NGB (NF2-associated GTP-binding protein) that specifically associates with NF2 protein *in vitro* and *in vivo*. GTP binding region of NGB was shown to be highly homologous to Ras and Rho small G-protein family members. In this study, we have found that NGB has a intrinsic GTPase activity as well as GTP-specific binding affinity. Although both biochemical activities of NGB protein were not affected by the NF2 binding, degradation of NF2 protein was strongly protected by NGB. Overexpression of NGB in JS-1 rat schwannoma cells significantly inhibited cell growth *in vitro* and *in vivo*, and brought about changes in cell-cell adhesion and actin cytoskeleton structure. The cell growth-inhibiting activity of NGB was shown to be partially mediated by NF2 protein. These data indicate that NGB protein in association with NF2 tumor suppressor plays an important role in controlling cell proliferation.