the rat embryo show that βPix-d isoform is expressed mainly in the central nervous system. In contrast to other βPix isoforms, βPix-mediated membrane ruffles are not detected and the cellular localization of βPix-d is mainly in nucleus in NIH3T3 fibroblast. NLS sequences in GIT1 binding domain of βPix-d are critical for nuclear localization of βPix-d. These finding imply that βPix-d might have novel function in nucleus.

E130 Calcineurin-Dependent Dephosphorylation of Ryanodine Receptor Down-Regulates Activity of the Ca2+ Release Channel in Skeletal Muscle

Dong Wook Shin* and Do Han Kim
Department of Life Science, Kwangju Institute of Science and Technology

Calcineurin is a Ca^{2+} and calmodulin-dependent protein phosphatase with diverse cellular functions. Here we examined the physical and functional interactions between calcineurin and RyR/Ca^{2+} release channel in skeletal C2C12 myotubes. Co-immunoprecipitation experiments revealed that the association between RyR and calcineurin exhibits a strong Ca^{2+} dependence. This association involves a Ca^{2+} dependent interaction between calcineurin and FK506-binding protein (FKBP12), an accessory subunit of RyR. Pretreatment with cyclosporin A (CsA), an inhibitor of calcineurin, enhanced the caffeine-induced Ca2+ release (CICR) in C2C12 cells. Overexpression of a constitutively active form of calcineurin in C2C12 cells, △CnA(deletion of 391-521 a. a), resulted in a decrease in CICR. This decrease in CICR activity was partially recovered by pretreatment with CsA. Furthermore, overexpression of an endogenous calcineurin inhibitor (cain) or an inactive form of calcineurin (△CnA(H101Q)) resulted in upregulation of CICR. Taken together, our data suggest that. calcineurin-mediated dephosphorylation of RyR through FKBP12 may play an important role in the Ca^{2+} signaling of muscle contraction and relaxation.

E131 The Physiological Role of Asp-Rich Region of Calsequestrin in the Regulation of Ca^{2+} Homeostasis of Skeletal Muscle

Dong Wook Shin*, Jae Man Lee and Do Han Kim
Department of Life Science Kwangju Institute of Science and Technology

Calsequestrin (CSQ) is a high capacity Ca^{2+} binding protein in the junctional sarcoplasmic reticulum (SR) of striated muscles, and has been shown to regulate the RyR / Ca^{2+} release channel through triadin and junctin. We previously reported that *asp*-rich region (352-367 a.a) of CSQ binds to triadin as well as Ca^{2+} (Shin, D et al., 2000). Here, we investigated the physiological role of this region on the channel activity of RyR by measuring cytoplasmic Ca^{2+} concentration using C2C12 skeletal myotubes. Overexpression of wt CSQ in C2C12 cells enhanced caffeine-induced Ca^{2+} release, whereas overexpression of *asp*-rich region deleted CSQ (△*asp*-CSQ; deletion of 352-367 a.a) reduced the caffeine-induced Ca^{2+} release. In addition, overexpression of △*asp*-CSQ recovered the peak amplitude of depolarization-induced Ca^{2+} release which was down-regulated by overexpressed wt CSQ. Furthermore, overexpression of △*asp*-CSQ restored thapsigargin-induced Ca^{2+} and Mn^{2+} influxes which was markedly diminished in wt CSQ-overexpressed myotubes. Taken together, these findings suggest that the *asp*-rich region is essential for function of CSQ and Ca^{2+} homeostasis of skeletal muscle.

E132 NF-kB Attenuates 3-Hydroxykynurenine-Induced Neuronal Cell Death

Hyun Jung Lee[1], Myoung Woo Lee[2]*, Hee Sun Chae[1], Jae Hyung Bach[1], Myung Ju Shin[2] and Soon Cheol Park[2]
[1]Department of Anatomy, College of Medicine and [2]Department of Life Science, College of Natural

Science, Chung-Ang University, Seoul, Korea

3-Hydroxykynurenine(3-HK), kynurenine pathway metabolite, is known as a endogenous neurotoxin and increasing evidence is that 3-HK may be associated with several neurodegenerative disorder. To investigate molecular mechanism of 3-HK, we examined 3-HK effect on SK-N-SH cells. Our results show that 3-HK induced apoptotic cell death and evoked generation of reactive oxygen species (ROS). Also, inhibition of ROS using antioxidant attenuates 3-HK induced neuronal cell death. These results suggest that 3-HK induces neuronal apoptosis which mediated by generation of ROS. Furthermore, we investigated roles of NF-*k*B in 3-HK induced cell death. Our results show that NF-kB was activated by 3-HK in a dose- and time-dependent manner. Interestingly, inhibition of NF-*k*B increased 3-HK-induced apoptotic cell death and enhanced caspase activation evoked by 3-HK. These results indicate NF-*k*B activation plays a protective role in 3-HK induced apoptosis and acts an upstream regulators of caspase.

E133 Involvement of Reactive Oxygen Species (ROS) and p38 Mitogen-Activated Protein (MAP) Kinase in TRAIL-Induced Apoptosis

Myoung Woo Lee[1*], Hee Sun Chae[2], Jae Hyung Bach[2], Hyun Jung Lee[2], Sung Jin Cho[1] and Soon Cheol Park[1]
[1]Department of Life Science, College of Natural Science and [2]Department of Anatomy, College of Medicine, Chung-Ang University, Seoul, Korea

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) serves as an extracellular signal triggering apoptosis in tumor cells. However, the molecular mechanisms leading to the apoptosis are largely unknown. To characterize the molecular events involved in TRAIL-induced apoptosis, we examined the association of reactive oxygen species (ROS), p38 mitogen-activated protein (MAP) kinase and caspases in human adenocarcinoma Hela cells. Here we show that TRAIL strongly accumulated ROS and activated p38 MAP kinase, followed by activation of caspases, leading to apoptosis. The administration with antioxidants, either GSH or estrogen, prevented ROS generation, p38 MAP kinase and caspases activation, eventually attenuated apoptosis. Also, p38 MAP kinase inhibitor SB203580 prevented apoptosis through the reduction of caspase activation, although TRAIL-induced ROS generation was not reduced. Furthermore, pan-caspase inhibitor zVAD-fmk perfectly blocked the apoptosis, while it did not affect on ROS generation and p38 MAP kinase activation. Therefore, our results suggest that TRAIL-induced apoptosis is mediated by ROS-activated p38 MAP kinase followed by caspases activation in Hela cells.

E134 The Protective Role of Heat Shock Protein 90 against 3-Hydroxykynurenine Induced Neuronal Apoptosis

Myoung Woo Lee[1*], Jae Hyung Bach[2], Hee Sun Chae[2], Hyun Jung Lee[2], Myung Sik Lee[1] and Soon Cheol Park[1]
[1]Department of Life Science, College of Natural Science and [2]Department of Anatomy, College of Medicine, Chung-Ang University, Seoul, Korea

3-Hydroxykynurenine (3HK), an endogenous metabolite of tryptophan in the kynurenine pathway, is a potential neurotoxin in several neurodegenerative disorders. Stabilizing protein structure, heat shock proteins (HSPs) have diverse roles as molecular chaperones to mediate stress tolerance. In present study, we investigated the possible protective role of HSPs against 3HK induced neuronal cell death. Here we report that 3HK induced in a dose- and time- dependent manner neuronal cell death in neuroblastoma SK-N-SH cells. The cell death showed characteristic apoptotic features such as cell shrinkage, plasma