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3-Hydroxykynurenine(3-HK), kynurenine pathway metabolite, is known as an 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- κ B in 3-HK induced cell death. Our results show that NF- κ B was activated by 3-HK in a dose- and time-dependent manner. Interestingly, inhibition of NF- κ B increased 3-HK-induced apoptotic cell death and enhanced caspase activation evoked by 3-HK. These results indicate NF- κ 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

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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

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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

membrane blebbing, chromatin condensation, and nuclear condensation and fragmentation. Furthermore, SK-N-SN cells were protected from 3HK induced cytotoxicity by prior elevation of HSPs expression. Our results show that the protective effect was abolished by HSP90 anti-sense oligonucleotides while not by HSP27 and HSP70 anti-sense oligonucleotides. Also, our result shows that HSP90 effectively inhibits caspases activities leading to the apoptosis. These results suggest that 3HK induces apoptosis in neuroblastoma SK-N-SN cells and that HSP90 is major contributing protein component of protection against 3HK induced apoptosis.

E135 An Analysis of Homeobox Genes in the Earthworm, *Eisenia andrei* (Annelida: Oligochaeta)

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The oligochaeta, *Eisenia andrei*, was surveyed for homeobox-containing genes using the method of polymerase chain reaction and subsequent sequence determination. Twenty distinct homeobox-containing gene fragments were identified. Ten of fragments are classified with Hox-type homeobox classes and other fragments show orthology with *caudal*, *Prh*, *Xlox*, *Lox* and *engrailed* homeobox classes. Detection of *Abd-B* type candidate genes, *Eahox19* and *Eahox20*, resemble to the result of same oligochaeta, *Stylaria lacustris* (ST-5; Snow and Buss, 1994) as well as leeches (*Lox21*; Irvine and Martindale, 1996). These studies suggest that this type gene exists in annelid lineage. Because of failure to discover of *Abd-B* homolog gene in polychaeta (Irvine et al., 1997), this type gene would have been preserved in

oligochaeta and leeches, whereas it has been lost in polychaeta during separation of these phyla.

E136 Molecular and biochemical characterization of mouse cardiac junctate

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Screening a cDNA library using canine junctin cDNA as a probe yielded mouse heart 3 complete cDNAs homologous to the human junctate known as a endoplasmic/sarcoplasmic reticulum single transmembrane Ca²⁺ binding protein. We named the proteins mouse junctate 1, 2 and 3. Mouse junctate 1, 2 and 3 are composed of 270, 259 and 215 amino acids. The apparent molecular weights (Mr) of mouse junctate in SDS-PAGE were between 40-50 kDa, whereas the reported Mr of the human junctate (299 aa) was 33 kDa. Western blot experiments showed that mouse junctate was expressed in heart, brain, spleen, lung, liver, kidney and stomach, but not in skeletal muscle. It was found that heart and brain tissues express unique isoforms which are different from other tissues. Immunohistochemical studies showed that junctate was localized in the heart tissues such as ventricles, atria and purkinje fibers.

E137 Hox Genes from the Earthworm *Perionyx excavatus* (Annelida, Oligochaeta) : New Sequence Information and Phylogenetic Analysis

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