2006년 한국노화학회 추계 학술대회

일 시 : 2006년 12월 15일(금) 09:00~
장 소 : 한림대학교 일송생명과학연구소 일송생활 일송연수센터 및 문화홀
주 관 : 한국노화학회
한림대학교 일송생명과학연구소
주 최 : 보건복지부지정 노화기전연구 및 노화억제물질개발센터
발표연자 : Naoki Maruyama, M.D., Ph.D.
(Tokyo Metropolitan Institute of Gerontology)
Toshihiko Aosaki, M.D., Ph.D.
(Tokyo Metropolitan Institute of Gerontology)
Weiming Xia, Ph.D.(Harvard Medical School)
진병관 교수( 아주대학교 의과대학)
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초록열람 : 한국노화학회 홈페이지와 본 학회지(제16권 4호)에 게재.
(발표 초록 7편 / 포스터 초록 73편)
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<th>Time</th>
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<td>09:30-10:30</td>
<td>Registration</td>
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<td>10:30-10:35</td>
<td>Opening Remarks</td>
<td>Dr. Yong-Sun Kim</td>
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<td><em>Director, Ilsong Institute of Life Science, Hallym University, Korea</em></td>
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<td><em>President, The Korean Society for Gerontology, Korea</em></td>
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<td>10:35-10:40</td>
<td>Welcome Address</td>
<td>Dr. Sang-Hoon Bae</td>
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<td><em>President, Hallym University Medical Center, Korea</em></td>
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<td>10:40-11:20</td>
<td>Lecture(1) Molecular Dysfunction of Neuromuscular Junction: A Gateway to Aging Research</td>
<td>Dr. Jae-Bong Park</td>
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<td>11:20-12:00</td>
<td>Lecture(2) Long-term Changes in Regional Profiles of Prefrontal Gene Expression in an Animal Model of Stress-induced Hypercortisolism</td>
<td>Dr. Eun-Kyoung Choi</td>
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<td>Lecture(3) Antiparkinsonian Effects of Dextromethorphan Analogs</td>
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<td>Lecture(4) Cannabinoid Receptors Contribute to Survival of Nigral Dopaminergic Neurons by Inhibiting Microglial Activation in the MPTP Model of Parkinson's Disease</td>
<td>Dr. Young-Ho Koh</td>
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<td>15:20-16:00</td>
<td>Lecture(5) A Physiological Approach to DRPLA, a Type of Triplet Repeat Disease</td>
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<td>16:00-16:40</td>
<td>Lecture(6) Protein Handling Problems in Neurodegenerative Diseases: Dysfunction of Ubiquitin Proteasome System in Huntington's Disease</td>
<td>Dr. Yong-Ho Koh</td>
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<td>16:40-17:20</td>
<td>Lecture(7) In Memory of Pre-senile Neurodegeneration: Presenilin, Enhancer, and Inhibitor</td>
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<td>Ceremony of Awarding the Best Ilsong Poster</td>
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Molecular Dysfunction of Neuromuscular Junction: A Gateway to Aging Research

Naoki Maruyama, M.D., Ph.D.
Tokyo Metropolitan Institute of Gerontology, Japan

Elderly people are suffering from various muscular problems. Most of those problems have been regarded as results of dysfunctions in nervous, joint, or muscle systems. To participate in the scientific competition for decreasing those problems we focused on the neuromuscular junction. Recent molecular studies revealed several molecules involved in the junction. However, those functions have not been clarified yet. To generate the muscle power the synaptic clustering requires various signal transductions triggered by agrin released from motoneurons. The triggering by agrin induces activation of muscle-specific kinase (MuSK). Therefore, the suppression of MuSK activation induces muscle weakness. We generated antibody against the ectodomain of MuSK in rabbits. Those rabbits developed flaccid weakness within 3 weeks after the last injection. This finding shows the myasthenia gravis is inducible by autoantibody against MuSK. The muscle in diseased rabbits showed no clustering of acetylcholine receptors. The signal transduction by MuSK is disturbed in this experimental model. The myasthenia gravis patients with anti-MuSK antibodies tend to develop several facial weakness and bulbar symptoms, including dysphagia, dysarthria, and respiratory crisis with some atrophy of facial muscles. These findings lead us to overcome various muscular problems including sarcopenia in elderly people. The analysis of neuromuscular junction will be a gateway to aging research.


Long-term Changes in Regional Profiles of Prefrontal Gene Expression in an Animal Model of Stress-induced Hypercortisolism

Song Her, Ph.D.
Korea Basic Science Institute, Korea

Because of the neuropathogenic potential of hypercortisolism in major depression, it is of clinical importance to know how the stress hormone cortisol affects gene expression in brain regions involved in depressive disorders. Here we used a model of hypercortisolism to examine profiles of prefrontal gene expression in monkeys exposed to social separations or a matched control. Three prefrontal regions were interrogated with Affymetrix GeneChips. Twelve weeks after exposure to six intermittent social separations, 2-4-fold more genes than expected by chance were differentially expressed in ventromedial prefrontal cortex compared to non-separated controls. Most of these genes were downregulated (79%), including ubiquitin conjugation enzymes and ligases reproducibly identified with different computational protocols and statistical tests. The recently reported SSAT candidate gene for suicidal depression also gained partial support. Social separations did not affect gene expression beyond that expected by chance in prefrontal white matter or dorsolateral prefrontal cortex. All but 3 of 32 comparisons chosen for verification by quantitative real-time polymerase chain reaction (qPCR) showed differences that were consistent with the microarray-predicted direction. Furthermore, qPCR and microarray data were highly correlated. These results demonstrate selective effects on gene expression in a brain region previously implicated in depression by anatomical, histological, and functional neuroimaging studies. Comparisons between transcriptional profiles of ventromedial prefrontal cortex described here for monkeys and those now needed for humans with a history of major depression may help to distinguish the molecular biology of depression from adaptations secondary to various confounding conditions in human postmortem brain research.

Key words - Major Depression, Cortisol, Oligonucleotide Microarray, Squirrel Monkey, Ventromedial Prefrontal Cortex
Antiparkinsonian Effects of Dextromethorphan Analogs

Eun-Joo Shin, Ph.D., Hyoung-Chun Kim, Ph.D.*

Neuropsychopharmacology and Toxicology Program, College of Pharmacy, Kangwon National University, Korea

We investigated the neuroprotective property of analogs of dextromethorphan (DM) in lipopolysaccharide (LPS) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models to identify neuroprotective drugs for Parkinson's disease (PD). In vivo studies showed that daily injections with DM analogs protected dopamine (DA) neurons in substantia nigra pars compacta and restored DA levels in striatum using two different models for PD. Among the 5 analogs studies, 3-hydroxymorphinan (3-HM), a metabolite of DM, was the most potent, which restored excellent correlation between potency for preventing toxin-induced decrease in motor activities and neuroprotective effects among the DM analogs studied, of which 3-HM was the most potent one in attenuating the behavioral damage. In vivo studies revealed two glia-dependent mechanism for the neuroprotection by 3-HM. First, astroglia mediated 3-HM-induced neurotrophic effect by increasing the gene expression of neurotrophic factors, which was associated with the increased acetylation of histone H3. Second, microglia participated in 3-HM-mediated neuroprotection by reducing MPTP-elicited reactive microgliosis as evidenced by the decreased production of reactive oxygen species. In summary, we show the potent neuroprotection by 3-HM may be a novel therapy for PD [Supported by a grant (M103KV010013-06K22010310) from the Brain research Center from 21st Century Frontier Research Program funded by the Ministry of Science and Technology, by a grant of the Korea Health 21 R and D Project (A020007), Ministry of Health and welfare, Republic of Korea, and by BK 21 Project, Korea Research Foundation.]

Cannabinoid Receptors Contribute To Survival Of Nigral Dopaminergic Neurons By Inhibiting Microglial Activation In The Mptp Model Of Parkinson’s Disease

Young C. Chung, Eun S. Chung, Byung K. Jin, Ph.D.

Brain Disease Research Center, Neuroscience Graduate Program, Ajou University School of Medicine, Korea

The present study examined whether cannabinoid receptor 1 (CB1) could contribute to prevent the loss of dopaminergic neurons in the substantia nigra (SN) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease (PD). Tyrosine hydroxylase (TH) immunocytochemistry showed a significant loss of dopaminergic neurons and microglial activation, visualized by immunocytochemical staining in the SN. Western blot analysis and double-label immunohistochemistry show that the upregulation of membrane (gp91phox) and cytosolic (p47phox and p67phox) components, translocation of cytosolic proteins (p47phox and p67phox) to the membrane, and gp91phox, p67phox and p47phox expression of NADPH oxidase in microglia in the SN in vivo, indicating the activation of NADPH oxidase. Additional reactive oxygen species (ROS) production, assessed by hydroethidine histochemistry, were observed in the SN in which degeneration of dopaminergic neurons occurred. However, treatment with cannabinoids (WIN55212-2 and HU210) increased survival of nigral dopaminergic neurons in MPTP-treated mice. Inhibition of NADPH oxidase in activated microglia and improvement of motor behavior were also detected. However, all of these effects were reversed by treatment with CB1 antagonist AM251, indicating involvement of CB1 receptors. These results suggest that cannabinoids system may be beneficial for the treatment of neurodegenerative diseases, such as PD, that are associated with microglial activation.

A Physiological Approach to Drpla, a Type of Triplet Repeat Disease

Toshihiko Aosaki, M.D., Ph.D.

Tokyo Metropolitan Institute of Gerontology, Japan

We analyzed an animal model of a neurodegenerative disease, dentatorubral-pallidoluysian atrophy (DRPLA). The transgenic mice harbor a single copy of the full-length human mutant DRPLA gene with 129 CAG repeats. The generated Q129 mice exhibited devastating neurological phenotypes similar to those of juvenile-onset DRPLA characterized by cerebellarataxia, myoclonus and epilepsy. Furthermore, age-dependent and region-specific electrophysiological abnormalities were revealed including synaptic dysfunctions in the globus pallidus and cerebellum, and the downregulation of AMPA and GABAA receptor functions in...
hippocampal CA1 neurons. Neuropathological studies revealed progressive brain atrophy without obvious neuronal loss and neuronal
intranuclear accumulation (NIA) of mutant proteins starting at postnatal day 4 with the regional specificity vulnerable to DRPLA.
The expression profile revealed age-dependent transcriptional dysregulations including those of CREB-dependent genes, which may
underlie neuronal dysfunctions in polyglutamine diseases.

Protein Handling Problems in Neurodegenerative Diseases: Dysfunction
of Ubiquitin Proteasome System in Huntington's Disease

Hyemyung Seo, Ph.D.
Department of Molecular and Life Science, Hanyang University, Korea

A key to understanding the progressive pathologies of neurodegenerative diseases appears to be the intra- and extra cellular
metabolism and handling of the abnormal proteins such as huntingtin for Huntington’s disease. Intraneuronal nuclear protein
aggregates of mutant huntingtin are present in HD brains, suggesting a dysfunction of the ubiquitin proteasome system (UPS). In
our recent studies, we found inhibition of UPS function in both early (0 –1, with no or little neuronal loss) and late (3– 4, with
more severe neuronal loss) stage HD patients’ cerebellum, cortex, substantia nigra and caudate-pyramidal brain regions. Late HD
stage increases in ubiquitin levels were unique to caudate-pyramidal. HD patients’ skin fibroblasts also had UPS inhibition similar
to brain despite increases in proteasome b subunit expression. In HD skin fibroblasts or striatal HD-model neurons, we genetically
engineered and overexpressed proteasome activators involved either in the ubiquinated or the non-ubiquitinated protein degradation
pathways. Following compromise of the UPS, overexpression of the proteasome activator subunit PA28g but not subunit S5a
recovered proteasome function and also improved cell viability in both HD model cells. These results demonstrate that specific
activation of the UPS could be a potential intervention in HD to provide neuroprotection and enhanced cell function.

In Memory of Pre-senile Neurodegeneration: Presenilin, Enhancer, and Inhibitor

Weiming Xia, Ph.D.
Center for Neurologic Diseases, Harvard Medical School and Brigham and Women’s Hospital, USA

The toxic amyloid b protein (Ab) is the major component of plaques in the brains of Alzheimer’s disease patients. It is derived
from amyloid precursor protein (APP) under multiple proteolytic cleavages catalyzed by the - and -secretases. The -secretase
complex is composed of Presenilin, Presenilin Enhancer, nicastrin, and Aph-1. The same protease complex also cleaves Notch to
generate Notch intracellular domain which is critical for proper neuronal development. Our previous studies have demonstrated a
requirement of two critical aspartate residuesin Presenilin 1 (D257 and D385) for -secretase activity. Mouse embryonic fibroblasts
lacking Presenilin 1 or CHO cells expressing D257A or D385A mutant Presenilin 1 failed to generate Ab. In zebrafish, we knocked
down the expression of individual -secretase components to understand their functions in vivo. The expression of endogenous
Presenilin Enhancer Pen-2 was blocked by injecting the morpholino (MO) against Pen-2. In addition, we simultaneously knocked
down the endogenous Pen-2 and expressed the truncated Pen-2 proteins lacking either the cytosolic or the C-terminal domain. We
found that the Pen-2 cytosolic loop is essential for protecting embryos from caspase-dependent apoptosis caused by the reduction
of Pen-2. The C-terminal 12 amino acids of Pen-2 were dispensable and could not rescue the Pen-2 knockdown-induced apoptotic
phenotype. When we treated zebrafish with a well characterized -secretase inhibitor, DAPT, we did not observe enhanced apoptosis
in embryos similar to those Pen-2 knockdown embryos. Treating zebrafish with DAPT or knocking down -secretase components
reduced Notch signaling, as demonstrated by reduced expression of the Notch target gene her6. However, those phenotypes found
in Pen-2 knockdown embryos were not simply caused by defective Notch signaling. For example, we found that knockdown of
Pen-2 led to a reduction of islet-1 positive neurons, but the neuronal loss is not due to a lack of neuronal precursor cells or cell
proliferation. Instead, the loss of islet-1 or acetylated tubulin positive neurons in Pen-2 knockdown embryos could be partially rescued
by knockdown of p53. In conclusion, the unique combination of cultured cell and zebrafish systems provides excellent tools to dissect
key pathways downstream of the -secretase cleavage of multiple substrates; many of them are critically involved in the pathogenesis
of a number of neurodegenerative diseases including Alzheimer’s disease.
Abstracts Of Poster Presentation

[P-1]
Apoptotic Signal Regulation in Human Melanoma Cell Terminal Differentiation

Gyoung-Mi Kim, and Dong-Chul Kang
Ilsong Institute of Life Science, Hallym University, Anyang, Korea

HO-1 human melanoma cells undergo apoptosis followed terminal differentiation by combined treatment of IFN-β and MEZ, but the exact mechanism of apoptosis is not known. We examined the effects of IFN-β/MEZ/IFN-β + MEZ treatments on the apoptosis of HO-1 melanoma cells. IFN-β treatments activated caspase-2, 8 and increased the protein level of Bax, Bak, tBID and PKR and decreased the protein level of Bcl-2. MEZ treatments activated caspase-2, 8, 9, 3 and increased the protein level of Bax, Bak, tBID and decreased the protein level of Bcl-2. But the PARP degradation was detected only in IFN-β + MEZ treatments. IFN-β/MEZ/IFN-β + MEZ treatments did not affect to the mRNA level of X-linked inhibitor of apoptosis (XIAP). But the mRNA level of XAF1, XIAP-associated factor 1, was increased by IFN-β treatments. In order to investigate the pathway of apoptosis by IFN-β + MEZ treatments, we tested the effects of SB203580, 2-aminopurine, caspase inhibitors, IL-1β antibody and mda-7 (IL-24) antibody but all reagents had no definite effects on the apoptosis of HO-1 melanoma cells by IFN-β + MEZ treatments. Maybe another mechanisms are related to the HO-1 melanoma cells terminal differentiation by IFN-β + MEZ treatments. [KRF-2004-202-C00421]

Key words - Interferon-β, Mezerein, Apoptosis, Caspase

[P-2]
Purkinje Cell Loss Induced by Autophagic Cell Death in Ngsk Prnp-deficient Mice

Hae-Young Shin¹, Hyun-Pil Lee¹, Yong-Cheol Jun¹, RI Carp², and Yong-Sun Kim¹3,*
¹Ilsong Institute of Life Science, Hallym University, Anyang, Korea
²Department of Virology, New York state Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA.
³Department of Microbiology, College of Medicine, Hallym University, Chuncheon, Korea

The functional loss of PrP and the ectopic expression of a PrP-like protein, doppel (PrPPLP/Dpl), induce the neurodegeneration and ataxia observed in Ngsk Prnp-deficient mice. This experimentally produced strain, one of several Prnp-deficient mouse models, shows late-onset ataxia and Purkinje cell degeneration and as a result have a deficit in motor coordination. However, the mechanism of Purkinje cell degeneration is not fully understood in Ngsk Prnp-deficient mice at this time. To elucidate the cellular mechanism involved in Purkinje cell degeneration, we used light and electron microscopy to analyze Purkinje cells in Ngsk mice at different ages. Degenerated Purkinje cells were increased in the cerebellum of Ngsk Prnp-deficient mice compared with age-matched control mice. Interestingly, degenerating Purkinje cells were detected in 3-month-old Ngsk Prnp-deficient mice, which contained autophagic vacuoles of various sizes and at various stages. Autophagic vacuoles contained cytoplasmic components, intracellular organelles and residues. Chromatin condensation and nuclear fragmentation, the characteristics of apoptosis, were not observed. These results suggest that Purkinje cell degeneration may be induced by autophagic cell death in the Ngsk Prnp-deficient mouse strain.

Key words - Ngsk Prnp-deficient mice, Ataxia, Purkinje cell, Autophagic vacuole, Autophagic cell death

노화학회지 제16권 제4호 2006년
Providing *In Vitro* Models to Study Endogenous Retrovirus, Murine Leukemia Virus (MULV), Derived From Senescence-accelerated Mice (SAMP8) and Senescence-resistant Mice (SAMR1)

Hong-Seok Choi¹², Bae-Hyun Kim³, Yong-Sun Kim³, and Kyunghoon Kim¹

¹Division of Life Science, Kangwon National University, Chuncheon, Korea,
²Ilsong Institute of Life Science and Department of Microbiology, College of Medicine, Hallym University, Chuncheon, Korea

Senescence-accelerated mouse (SAM), senescence-prone (SAMP) and senescence-resistant (SAMR), strains were originated from ancestral AKR/J mice strains. SAMP8 strain was provided as a murine aging model because SAMP8 has shown marked impairment of learning and memory while SAMR1 has shown normal aging phenotype. Accelerated aging phenotype of SAMP8 was postulated to be resulted from endogenous retroviruses, murine leukemia viruses (MuLVs), however the reason is not clarified yet. To investigate the effect of MuLVs in SAMP8 mice, we have established hippocampal and cortex neuronal cell lines from SAMP8 and SAMR1 mice. We have used Western blot analysis, immunocytochemistry, RT-PCR, and UV plaque assay to characterize the cell lines. The morphology of the cell lines and the cell growth rate/doubling time were determined by inverted microscope and hemacytometer, respectively. So far, we have confirmed the immortalization of SAMR1 cell lines which did not express MuLV gene by RT-PCR using SV40 primers. Although we have not fully characterized SAMR1 and SAMP8 cell lines, the establishment and characterization of these cell lines will be a very useful *in vitro* model for the study of endogenous retrovirus.

**Key words** - Senescence-prone mice (SAMP8), Senescence-resistant mice (SAMR1), Murine leukemia viruses (MuLV)

Seizure Activity Affects Neuroglial Kv1 Channel Immunoreactivities in The Gerbil Hippocampus

Duk-Soo Kim, Ji-Eun Kim, Sung-Eun Kwak, Moo Ho Won, and Tae-Cheon Kang*

Department of Anatomy, College of Medicine, Hallym University, Chuncheon, Korea

In order to confirm the species-specific distribution of voltage-gated K⁺ (Kv) channels and the definitive relationship between their immunoreactivities and seizure activity, we investigated Kv1 channel immunoreactivities in the hippocampus of seizure resistant (SR) and seizure sensitive (SS) gerbils. There was distinct difference of the Kv1 channel subtypes immunoreactivity in the hippocampus in both SR and SS gerbils. Kv1.1, Kv1.2, Kv1.3, Kv1.4, and Kv1.6 immunoreactivities in the SS gerbil hippocampus were lower than those in the SR gerbil hippocampus. However, Kv1 immunoreactivities were obviously present in astrocytes within the stratum radiatum of the CA1 region of pre-seizure SS gerbil hippocampus. Following seizure-onset, Kv1 immunoreactivities (except Kv1.5) were markedly elevated, whereas their immunoreactivities were down-regulated in astrocytes. Therefore, the present study demonstrates that reduced Kv1 immunoreactivities in hippocampal neurons may be closely related to seizure activity, and furthermore suggests that elevated Kv1 immunoreactivity in astrocytes in the epileptic hippocampus may reflect K⁺ buffering system impairments.

**Key words** - Kv1 channel, Hippocampus, Immunohistochemistry, Epilepsy, Gerbil
Reduced Parvalbumin Immunoreactivity is Related to Up-regulated P/q-type Voltage Gated Ca\(^{2+}\) Channel Expression in the Rat Hippocampus Following Status Epilepticus

Ji-Eun Kim\(^1\), Sung-Eun Kwak\(^1\), Duk-Soo Kim\(^1\), Moo-Ho Won\(^1\), Hui-Chul Choi\(^1\), Hong-Ki Song\(^2\), Yeong-In Kim\(^3\), and Tae-Cheon Kang\(^1\)*

\(^1\)Department of Anatomy and Neurology, College of Medicine, Hallym University, Chuncheon, Korea
\(^2\)Department of Neurology, Kangnam St. Mary’s Hospital, The Catholic University of Korea, Seoul, Korea
\(^3\)Department of Neurology, Kangnam St. Mary’s Hospital, The Catholic University of Korea, Seoul, Korea

To identify the relationship between P/Q-type voltage gated Ca\(^{2+}\) channel (VGCC) and parvalbumin (PV) immunoreactivities are connected to impairs of inhibitory transmission during epileptogenesis, we performed temporal- and spatial-specific alterations in VGCC immunoreactivities within PV positive neurons in the rat hippocampus following status epilepticus (SE). PV immunoreactivities were markedly reduced in the CA1 region and in the dentate hilar region, as compared with the control. One week after SE, PV immunoreactive neurons in the hippocampus was rarely detected, since PV immunoreactivity in the cell bodies of interneurons was significantly reduced. Whereas, VGCC immunoreactivity was mainly detected in the pyramidal cells in CA1-3 regions and in the granule cells of the dentate gyrus in the control. Thirty min 7 days after SE, VGCC immunoreactivity was enhanced in the hippocampus. Our results showed that reduced PV immunoreactivity was accompanied by enhanced VGCC immunoreactivity. These findings indicated that enhanced VGCC expression maybe a cause of reduction in PV immunoreactivity following SE, and this may be involved in the impairs of inhibitory transmission during epileptogenesis.

Key words - Voltage-gated Ca\(^{2+}\) channel, Ca\(^{2+}\) binding protein, Parvalbumin, Epilepsy, Hippocampus

Anti-glutamatergic Effect of Riluzole; Comparison With Valproic Acid

Ji-Eun Kim, Duk-Soo Kim, Sung-Eun Kwak, Moo Ho Won, and Tae-Cheon Kang*

Department of Anatomy, College of Medicine, Hallym University, Chuncheon, Korea

Riluzole is viewed as a candidate supplementary medications for epilepsy, but its anti-epileptic effect has been evaluated mainly in normal animals. Therefore, we compared the effects of riluzole and valproate (VPA) in the pilocarpine-induced temporal lobe epilepsy model (a complex partial seizure model) and in a γ-hydroxybutyrate lactone (GBL)-induced absence seizure models. We applied immunohistochemical study for vesicular transporter 1 (VGLUT1) and extracellular recording in the rat dentate gyrus of both pilocarpine- and GBL-induced seizure modelsto measure effects of riluzole and VPA. Both VPA and riluzole treatments reduced VGLUT1 immunoreactivity. Riluzole treatment completely inhibited pre-ictal spikes and spike-wave discharges in the pilocarpine- and GBL-induced epilepsy models, whereas VPA partially inhibited these phenomena. In both seizure models, the anti-epileptic effects of VPA and riluzole are basically related to anti-glutamatergic (reducing field excitatory postsynaptic potential slope and excitability ratio), not GABAergic (paired-pulse inhibition) effect. Riluzole was more effective at reducing seizure activity in both epilepsy models than VPA. These results suggest that riluzole is a potential AED with activity against complex partial seizure and absence seizure.

Key words - Riluzole, Valproate, Pilocarpine, γ-hydroxybutyrate lactone, Epilepsy, Glutamate, VGLUT,
**[P-7]**

Mannose-binding Lectin Alters Lps-mediated Cytokine Secretion from Human Endothelial Cells

**Hee Jung Kang**¹, **Sun-Mi Lee**¹, **Hyeon-Hwa Lee**², **Ji Yeon Kim**¹, **Byung-Chul Lee**³, **Jung-Sun Yum**⁴

Hong Mo Moon⁴ and **Bok Luel Lee**²

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²National Research Lab., College of Pharmacy, Pusan National University, Busan, Korea
³Dept. of Neurology, Hallym University College of Medicine, Anyang, Korea
⁴Dobeel Corporation, Seongnam, Korea

Coupling between certain pathogen-associated molecular patterns and corresponding pattern recognition receptors of endothelial cells is important for the mediation of vascular inflammatory responses. Mannose-binding lectin (MBL) recognizes certain carbohydrate structures of microbes and subsequently activates the complement system as well as facilitates the phagocytosis of targets. We investigated if MBL can intervene in the interaction between bacterial lipopolysaccharide (LPS) and endothelial cells to modulate subsequent inflammatory responses. In response to LPS, human umbilical vein endothelial cells (HUVEC) produced various cytokines. Addition of the purified human MBL/MBL-associated serine proteases (MASP) complex or recombinant human MBL enhanced LPS-mediated cytokine secretion of HUVEC, including interleukin (IL)-8, IL-6 and monocyte chemotactic protein-1 in a dose dependent manner. This enhancing effect was ameliorated by addition of anti-MBL antibody or mannan. Among the cytokines we analyzed, IL-6 showed the greatest increase of secretion in the presence of native MBL/MASP complex or recombinant MBL. MBL, regardless of its association with MASP, alters LPS-mediated cytokine secretion of HUVEC. Besides the well-known functions of MBL, to activate the lectin-complement pathway and to facilitate clearance of targets, enhancement of IL-6 secretion may provide an additional role for MBL in modulating vascular inflammation.

**Key words** - MBL, endothelial cells, LPS, cytokine, interleukin-6

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**[P-8]**

The Cellular Prion Protein Prevent Autophagic Cell Death in Neuronal Cell-line

**Jae-Min Oh**¹ and **Yong-Sun Kim**¹²

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Recently, we reported the cellular prion protein (PrPᵣ), a glycosylphosphatidylinositol (GPI)-anchored membrane protein, is involved in anti-apoptotic roles against neuronal cell death induced by serum deprivation in prion protein gene (Prnp)-deficient neuronal cell line. However, the exact cellular mechanisms are still controversial. Autophagy is an intracellular bulk degradation system, which delivers cytoplasmic components to the lysosome/vacuole. To elucidate the mechanisms in which PrPᵣ is involved in autophagic cell death pathway, we compared expression patterns of microtubule-associated protein 1 light chain-3 (LC3), an autophagy marker, in Prnp-deficient (Prnp⁻⁻) neuronal cell line to those with wild-type (WT) neuronal cell line. The expression level of LC3-II was increased in the Prnp⁻⁻ neuronal cell line compare to controls under serum deprivation conditions, but not in normal growth medium cultured cells. Interestingly, co-localization of LC3 and lysosomal membrane glycoprotein-2 (LAMP-2), indicating the presence of autophagolysosomes, were present in Prnp⁻⁻ neuronal cell line under serum deprivation conditions but not in WT neuronal cell line. Our data suggest that PrPᵣ is implicated in autophagic cell death pathway in neuronal cell line.

**Key words** - prion, autophagy, microtubule-associated protein 1 light chain-3 (LC3)
Rap1 May be Essential For Temporally Tgf-β1-mediated Cell Migration And Regulation Of Mip-1α mRNA Level In Macrophage

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TGF-β1 is a multifunctional cytokine, both a stimulator and an inhibitor of cellular function, and it possesses the capacity to control the production of many genes. This potent cytokine is known to modulate cell differentiation, apoptosis, cell cycle progression, cellular migration and extracellular matrix production. In macrophage, TGF-β1 reveals timely dual function; short or long-term treatment of it stimulate or inhibit cell migration, respectively. Short treatment of TGF-β1 activates the level of GTP-RhoA, whereas long term treatment inhibits it. The cell migration of macrophage in response to TGF-β1 seems to depend on RhoA activity. RhoA inactivated by PKA inactivated by PKA and p190RhoGAP causes inhibitory cell migration. In addition, we found 8-(4-chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate (8CPT-2Me-cAMP), a specific stimulator of Epac (Guanine nucleotide exchange factor (GEF) for Rap1), inhibited the expression of macrophage inhibitory protein-1α (MIP-1α) and cell migration, suggesting that Rap1 may be involved in the regulation of MIP-1α expression in response to TGF-β1. Furthermore, we examined whether Rap1 inhibits RhoA in response to TGF-β1.

Key words - TGF-β1, MIP-1α, Rap1, cell migration

Transforming Growth Factor (Beta)-1 Regulates Nuclear Factor (Kappa) B Pathway Via Rhoa

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Transforming growth factor (TGF)-β1 is a pleiotropic cytokine that regulates numerous physiological process, including cell proliferation, differentiation, apoptosis, and extracellular matrix protein synthesis. We have previously reported that regulates cell migration and RhoA plays an important role the regulatory step. Activated RhoA induces MIP-1α, a chemokine, in response to TGF-β1. The transcriptional factor nuclear factor kappa B (NF-xB), may induce MIP-1α expression. The present study explored whether NF-xB is a target of TGF-β1 and which signaling pathways are involved in the activation of NF-xB are in response to TGF-β1. We demonstrated that TGF-β1 increased the transcriptional activity of NF-xB in macrophages, and TGF-β1 produced a concomitant increase in the phosphorylations IkBa with a subsequent degradation of IkBa. TGF-β1 mediated NF-xB activation was blocked by Tat-C3 exoenzyme and Y27632. Suggesting that inhibition of RhoA, RhoA-activated kinase significantly blocks the expression of NF-xB activity in TGF-β1 treated macrophage cell. Moreover, WT-RhoA and CA-RhoA increased and DN-RhoA reversed the effect of NF-xB activity induced by TGF-β1. Therefore we suggest that RhoA signaling plays a critical role in TGF-β1-induced NF-xB pathway.

Key words - TGF-β1, RhoA, NF-xB
[P-11] Involvements of Small Gtpases in Neurite Outgrowth of PC12 CELLS

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In PC12 rat pheochromocytoma cells, nerve growth factor (NGF), basic fibroblast growth factor (bFGF) or db cAMP induces neurite outgrowth. NGF-induced neuronal outgrowth required inactivation of RhoA. The action of NGF, bFGF or cAMP in PC12 cells seems to use similar signal transduction pathways for neurite outgrowth, although detail pathway remains to be clarified. We found that inhibition of RhoA by the C3 toxin (Clostridium botulinum toxin) promotes NGF-, bFGF-, or db cAMP-induced neurite outgrowth in PC12 cells, whereas lysophosphatidic acid (LPA), an activator of RhoA, inhibited it. This indicates that inactivation of RhoA is required for neurite outgrowth in response to NGF, bFGF, and db cAMP. Therefore, we tried to elucidate the mechanism of RhoA inactivation involved in neurite outgrowth of PC12 cells. Dominant negative p190RhoGAP was unable to attenuate the level of GTP-RhoA in neurite outgrowth in PC12 cells, suggesting that p190RhoGAP inactivates RhoA in response to stimuli. Remarkably, Rap1 have been implicated in neurite outgrowth of PC12 cells by NGF. Here we also showed that the functional relationship between Rap1 and RhoA in NGF, bFGF, or db cAMP-induced neurite outgrowth.

Key words: PC12 cells, Neurite outgrowth, RhoA, Rap1

[P-12] RAP1 GTPase Regulates of Superoxide and Phagocytosis in Raw 264.7 Macrophage Cells

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The NADPH oxidase is a complex enzyme consisting of at least 6 components: cytochrome b558 (a heterodimer comprised of gp91-phox and p22-phox), p47-phox, p67-phox, p40-phox, Rac 1 or Rac 2, and Rap1A. Furthermore, there are multiple signal transduction pathways leading to activation of the NADPH oxidase. Generation of reactive oxygen species (ROS) through NADPH oxidase is relevant to immune response including inflammation reaction or oxidative stress. Rap1 is a member of small molecule weight GTP binding protein, which is known to be implicated in ROS production by NADPH oxidase. Like other Ras-related GTPases, Rap1 is activated depending on GTP-binding state, whereas GDP-bound form is inactive state. When 8-CPT-2Me-cAMP, an activator of Epac (GEF for Rap1), was pretreated to macrophages and the cells were exposed to serum and IgG-opsonized zymosans, production of ROS was stimulated. Transfection of constitutively active Rap1 V12 into macrophages also stimulated ROS production. However, N17 Rap1 dominant-negative proteins abrogated ROS formation. This suggests that activation of Rap1 may be required for the activation of NADPH oxidase in macrophage. The mechanism of Rap1 engagement for the NADPH oxidase activation is being studied.

Key words - NADPH oxidase, Rap1, Superoxide, Phagocytosis
[P-13]
DNA Damage And Repair In Fibroblast Cells Of Mammals With Different Life Span

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Normal mammalian fibroblasts in culture have a limited life span and the maximum life span of species is well proportional to
the maximum population doubling of their fibroblasts. However, the mechanism underlying replicative senescence has not been
ecluciated clearly. We attempted to explore the mechanism of cellular senescence among mammalian fibroblasts through
investigation on responses of cells to DNA damaging agents: H₂O₂, cisplatin, camptothecin, and etoposide. The result showed that
cell viability after treatment of DNA damaging agents was decreasing in the order of human, dog, rat and mouse fibroblasts. Levels
and activities of molecular markers were well correlated with the cell viability results. We also found that base excision repair (BER),
cellular DNA repair capacity (DRC), double-strand break repair (DSBR) assay and NHEJ (Non-homologous end joining) assay were
higher in human embryonic fibroblasts than in other mammalian fibroblasts. These results suggest that the decrease in sensitivity
for DNA damage and the increase in repair activity are possibly related to the longevity of cellular level.

Key words: replicative senescence, DNA repair, fibroblasts, life span, aging

[P-14]
The Role of Foxo3a in Replicative Senescence of Human Fibroblasts

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Although FOXO and SIRT1 have been well characterized as longevity genes in yeasts and nematodes, the aging-related role of
FOXO or SIRT1 has not been elucidated in mammalian cells. To elucidate the role of FOXO3a in replicative senescence of human
diploid fibroblast, we tested first the expression levels of FOXO3a and its target proteins (p27, GADD45, and MnSOD) in aged cells.
When cells are aged, protein levels of p27, GADD45, and MnSOD were decreased. The transfection of fibroblasts with the luciferase-promoter reporters of p27, GADD45, and MnSOD showed that the transcription levels of these genes also decreased in aged cells
comparing to young cells. Western blot analysis using anti-phospho-FOXO3a and anti acetyl-Lys antibodies showed that
phosphorylation (Thr 32) and acetylation of FOXO3a were increased in aged human fibroblasts. Treatment of human diploid fibroblast
with LY294002 and Wortmannin, specific inhibitors of PI3 kinase Inhibitors decreased the level of phospho-FOXO3a (thr32) in both
young and aged cells, indicating that the phosphorylation of FOXO3a is mediated by Akt. Transfection of TM, a unphosphorylated
form, mediated nuclear localization of FOXO3a and increased transcription of MnSOD in both young and aged cell. K568R, a mimic
of deacetylated form, showed increased transcription of MnSOD. Taken togther, results suggested that decrease in FOXO3a activity
through phosphorylation by Akt and deacetylation by SIRT1 mediates inactivation of FOXO3a in aged cells.

Key words - replicative senescence, FOXO3a, MnSOD, GADD45, p27
Rap1 Signaling Regulates Microglia Adhesion To Amyloid-beta Peptide Via Integrin β2

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Microglia are the principle immune effector and phagocytic cells in the CNS. These cells are associated with β-amyloid fibrils (fAβ) containing plaques found in the brains of Alzheimer disease patients. Microglial interaction with extracellular is mediated through an ensemble of cell surface receptors including the B-class scavenger receptor CD36, the α6β1-integrin, and the integrin-associated protein/CD47. The binding of fAβ to this receptor complex has been shown to drive NF-κB signaling leading to production of tumor necrosis factor α (TNFα) and glutamate. As a result, TNFα-dependent expression of nitric-oxide synthase (iNOS) and neuronal apoptosis. Small G proteins serve as critical control point in signal transduction, integrating a wide range of stimuli to dictate cellular outcomes. Rap1 is a small G protein, belonging to Ras superfamily. Like all small G protein, Rap1 exists in a GDP-bound state and is activated when GDP is exchanged for GTP. Rap1 can regulate cell proliferation, differentiation, and cell adhesion thought distinct mechanisms. Rap1 has received recent attention for its role in exchanging integrin-dependent signals. Therefore we investigated Rap1 role for the altered adhesion associated with fAβ mediated intergian β2 in microglia. 8-CPT-2Me-CAMP, an activator of Epac (RaP GEF), reduced cell binding to fAβ. In addition antibody neutralization experiments showed that β2 integrin is a main receptor of BV2 cells to bind fAβ. Thus, we examined whether Rap1 regulates β2.

Key words - Alzheimer disease, Beta-amyloid, Microglia, integrin β2, Rap1

Identification of Novel Components Involved in Chk2 Signaling

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Multicellular organisms respond to DNA damaging agents by arresting the cell cycle or undergoing apoptosis. This response is mediated by a checkpoint kinase, Chk 2, also known as a tumor suppressor gene. In order to identify novel components involved in Chk2 signaling, we used transgenic flies that overexpress Chk2 in the eye. These flies had rough eye phenotype. From a screen of 2,300 EP, we isolated 30 EP lines that specifically suppressed rough eye phenotype of Chk2. Among these, 2 lines [EP(3)3587, EP(3)3574] showed partial defects in DNA damage induced cell cycle arrest. Ten lines also showed genetic interaction with another checkpoint kinase, Chk1. In conclusion, we isolated 30 Chk2 specific interactors by genetic screening based on Chk2 induced rough eye phenotype. Among these, 2 lines were shown to be directly involved in DNA damage induced cell cycle arrest.

Key words - Chk2, DNA damage checkpoint, tumor suppressor gene, Chk1

Hepatitis C Virus Nonstructural Protein 5 Activates Beta-catenin-dependent Transcription Activity

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HCV infection often leads to liver cirrhosis and hepatocellular carcinoma. The majority of HCV infection progress to chronic hepatitis. HCV nonstructural protein 5 (NS5) consists of 5A and 5B. The NS5A protein is a multifunctional protein and promotes
tumor growth and a number of cell cycle regulatory genes. The NS5B is an RNA-dependent RNA polymerase (RdRp) that catalyzes the replication of HCV. To elucidate the molecular mechanism of HCV-induced hepatocellular carcinoma, we examined the effects of HCV NS5 proteins on Wnt/beta-catenin signal transduction cascades. We show that beta-catenin-mediated transcription activity was elevated in replicon cells as determined by luciferase assay. We confirmed this result using a transient transfection experiment in hepatoma cell line. To further investigate the role of NS5A and NS5B in beta-catenin-mediated transcription activation, we examined the protein-protein interaction between NS5A/5B and beta-catenin. Both in vitro and in vivo binding data show that both NS5A and NS5B protein directly interact with beta-catenin in replicon and hepatoma cell lines. We also found that NS5A and NS5B proteins were colocalized with beta-catenin in the cytoplasm. These results indicate that both NS5A and NS5B proteins activate beta-catenin-dependent transcriptional activity through interaction with beta-catenin and NS5A/5B may be involved in Wnt/beta-catenin mediated neoplastic transformation.

Key words - Hepatitis C virus, Nonstructural 5A protein, Wnt/beta-catenin signaling

[Page 18]

Ginsenosides Attenuate Kainate-induced Mitochondrial Damage; Involvement of Adenosine A2A Receptor

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It has been reported that antioxidant properties of ginsenosides mediate its neuroprotective effects in various neurotoxic/excitotoxic models, such as glutamate- or ischemia-evoked neurotoxicity in vitro and in vivo. Because mitochondrial dysfunction is involved in kainate (KA)-induced excitotoxicity, we examined the effect of ginsenosides on the kainate-induced mitochondrial dysfunction and oxidative damage in this study. Ginsenosides (50 and 100 mg/kg, i.p.) was administered five times at 12-h intervals. One hour after the last administration of ginsenosides, KA (10 mg/kg) was injected intraperitoneally. Pretreatment with Ginsenosides significantly delayed seizure onset time and attenuated seizure score in a dose-dependent manner. KA-induced hippocampal intramitochondrial Ca²⁺ accumulation and decreases in mitochondrial superoxide dismutase (Mn-SOD) expression and mitochondrial transmembrane potential (MTP) were also significantly attenuated by pretreatment with ginsenosides. Consistently, lipid peroxidation, protein oxidation and reactive oxygen species (ROS) accumulation in hippocampus 4 hours after KA were also decreased by ginsenosides. Since our previous data demonstrated adenosine receptor modulation by ginsenosides, we applied selective antagonists against adenosine receptor subtypes to evaluate which adenosine receptor subtype mediate the protective effects of ginsenosides in response to KA. The adenosine A2A receptor antagonist, 1,3,7-trimethyl-8-(3-chlorostyryl) xanthine (1.0 mg/kg, i.p.), significantly reversed the effect of ginsenosides. However, adenosine A1 receptor antagonist, 8-cyclopentyl-1,3-dimethylxanthine (25 and 50 µg/kg, i.p.), and adenosine A2B receptor antagonist, alloxazine (1.5 and 3.0 mg/kg, i.p.), could not reverse the effect of ginsenosides. Combined our results suggest that pretreatment with ginsenosides protect KA-induced neurotoxicity by restoring mitochondrial function and adenosine A2A receptor modulation [Supported by a grant of the Korea Health 21 R & D Project (A020007) and by a grant (M103KV010013-06K22010310) from the Brain Research Center from 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Republic of Korea].

Key words - Ginsenosides. Kainate, Mitochondrial damage, Adenosine A2A receptor

[Page 19]

Repetetd Intracerebroventricular Infusion of Nicotine Prevents Kainate-induced Neurotoxicity by Activating The α7 Nicotinic Acetylcholine Receptor

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We examined whether infusion can affect kainic acid (KA)-induced neurotoxicity in rats. Although treatment with a single nicotine infusion (0.5 or 1.0 µg/side, i.c.v.) failed to attenuate KA-induced neurotoxicity, repeated nicotine infusions (1.0 µg/side/d for 10d) attenuated the seizures, the severe loss of cells in hippocampal regions CA1 and CA3, the increase in activator protein (AP)-1 DNA binding activity, and mortality after KA administration. Bungarotoxin and mecamylamine blocked the neuroprotective effects of nicotine. These results suggest that repeated nicotine treatment provides α7 nicotinic acetylcholine receptor-mediated neuroprotection against KA toxicity [Supported by a grant (M103KV010013-06K22010310) from the Brain Research Center from 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Republic of Korea, by a grant of the Korea Health 21 R & D Project (A020007), Ministry of Health & welfare, Republic of Korea, and by BK 21 Project, Korea Research Foundation. Equipment at the Institute of Pharmaceutical Science (Kangwon National University) was used for this study].

**Key words** - Nicotine, Neuroprotection, Kainic acid, AP-1 DNA binding activity, Hippocampus, α7 nicotinic acetylcholine receptor

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**[P-20]**

Interleukin-6 Prevents Trimethyltin-induced Learning Impairment by Activating Muscarinic M1 Receptor

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The trimethyltin (TMT)-induced neurotoxicity shows by a behavioural syndrome that includes tremor, convulsive/epileptic episodes, and hyperactivity combined learning deficiencies. Accumulating evidences raised a possibility that interleukin (IL)-6 plays a role in the neuronal damage mechanism. Our previous study demonstrated that recombinant IL-6 is a possible neuroprotectant in response to TMT insult in mice. In this study, we observed that TMT-induced learning impairment was potentiated by the muscarinic receptor antagonist in both IL-6 (+/+) and IL-6 (-/-) mice. Consistently, the decreases in gene expression of the muscarinic M1 receptor and choline acetyltransferase (ChAT), while the increases in acetylcholinesterase (AchE), were detected in both IL-6 (+/+) and IL-6 (-/-) mice after TMT treatment. Simultaneously, we found that pretreatment with monoclonal IL-6 receptor antibody (IL-6R) (1 mg/mouse, i.p and 50 µg/head, i.c.v) potentiated TMT-induced convulsive behaviours and memory impairments, correlate with the changes in muscarinic M1, ChAT and AchE expression in the hippocampus of the IL-6 (+/+) and IL-6 (-/-) mice. Our results reflect that IL-6 gene's cognitive enhancing effect, which may be via activating the muscarinic M1 receptor in response to TMT insult in mice. [Supported by a grant (M103KV010013-06K22010310) from the Brain research Center from 21st Century Frontier Research Program funded by the Ministry of Science and Technology, by a grant of the Korea Health 21 R and D Project (A020007), Ministry of Health and welfare, Republic of Korea, and by BK 21 Project, Korea Research Foundation].

Key words: Trimethyltin, learning and memory, and interleukin-6.

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**[P-21]**

Glutathione Peroxidase (Gpx)-1 Deficiency Potentiates Learning Impairment Induced by Methamphetamine

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It is well-known that peroxidative stress is involved in the learning impairment. Glutathione peroxidase (GPx)-1, a selenium dependent enzymatic antioxidant, plays a crucial role in scavenging peroxides in the brain. Recent evidence suggests that methamphetamine (MA) produces learning impairment. In this study, we examined whether GPx-1 gene affects on the MA-induced
learning impairments. To achieve better understanding on our study, we employed GPx-1(+/-) and GPx-1(-/-) mice in the presence of MA exposure. We examined Y maze test, novel object recognition test, water finding test, hidden platform test, passive avoidance test and conditioned fear learning test in the GPx-1(+/-) and GPx-1(-/-) mice suffering from MA dependence or MA neurotoxicity. Our result consistently indicate that GPx-1(-/-) mice are more susceptible to learning impairment induced by MA dependence or MA neurotoxicity as compared to those GPx-1 (+/+ ) mice. Our data suggest that GPx-1 gene is an essential factor in maintaining cognitive function in our experimental condition [Supported by a grant (M103KV010013-06K22010310) from the Brain research Center from 21st Century Frontier Research Program funded by the Ministry of Science and Technology, by a grant of the Korea Health 21 R and D Project (A020007), Ministry of Health and welfare, Republic of Korea, and by BK 21 Project, Korea Research Foundation].

Key words - Glutathione peroxidase, Methamphetamine, Neurotoxicity, Dependence

[P-22]

Characterization of Monoclonal Antibodies Specific for Nonstructural 5 (Ns5) Protein of HCV

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Hepatitis C virus (HCV) nonstructural 5 (NS5) consists of NS5A and NS5B proteins. Both NS5A and NS5B of HCV are the key proteins involved in HCV RNA replication. NS5A is a multifunctional phosphoprotein involved in both viral RNA replication and regulation of cellular signal transduction pathways. NS5B is an RNA-dependent RNA polymerase (RdRp) with a key role in HCV replication. To characterize the functional roles of both NS5A and NS5B in HCV replication, we generated monoclonal antibodies (MAbs) specific for either NS5A or NS5B protein from mice immunized with recombinant baculovirus-expressed NS5A and NS5B proteins. The epitope of one group of NS5B MAbs is localized in the middle region (aa 240-263) of NS5B protein. The epitope of the other MAbs is mapped to the aa 67-89 at the N-terminal region of NS5B protein. However, we were unable to generate MAb specific for the C-terminus of NS5B protein. To examine the effects of MAbs on HCV RNA replication, we performed in vitro RdRp assay using 3'UTR + X RNA of HCV as a template in the presence of each MAb. Most of the MAbs had no effect on RdRp activity. Preliminary data showed that MAb specific for the N-terminus of NS5B suppressed the RdRP activity in a dose-dependent manner, indicating that the epitope of this MAb may contain important biological activities for HCV replication. Characterization of NS5A-specific MAbs is in progress. Taken together, these MAbs will be useful to elucidate the mechanism of HCV replication and to further characterize the biochemical functions of NS5 proteins.

[P-23]

Effects of Selenoprotein W on Differentiation of C2c12 Cell Lines

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Selenoprotein W (SelW), a member of the selenoprotein family, protects cells from oxidative stress by catalyzing (GSH). SelW expression is the highest in skeletal muscle, however its specific functions in muscle are hardly characterized. To investigate the muscle specific function of SelW, we used C2C12 mouse skeletal muscle cell line that differentiate into multinucleated myotube. During C2C12 differentiation, endogenous SelW expression was increased until differentiation medium (DM) 1 day and then was gradually decreased. C2C12 cells overexpressing SelW showed the increased expression levels of MyoD, a key transcription factor of muscle differentiation and cdk inhibitor p21 than the control C2C12 cells. In addition, the expression levels of myosin heavy chain (MHC) that makes up myotubes also increased, enhancing skeletal muscle differentiation. Conversely abrogation of SelW reduced the levels of MyoD, p21 and MHC, inhibiting C2C12 muscle cell differentiation. MyoD and p21 participate in cell cycle arrest and this process is critical for early muscle differentiation. Taken together, these data first molecular evidence that Selenoprotein W can be involved in regulating the differentiation in skeletal muscle cells. [c00086]

Key words - Selenoprotein W and differentiation, C2C12 cell, MyoD
Anti-inflammatory Effect of PPAR Gamma on Human Dental Pulp Cells

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Dental pulp, which consists of cells, ground substance, and neural & vascular supplies, is a loose, mesenchymal tissue almost entirely enclosed within dentin. Damage to dental pulp by mechanical, chemical, thermal, and microbial irritants can provoke various types of inflammatory response, and these pulpal inflammation leads to tissue degradation, mediated in part by matrix metalloproteinases (MMPs), through accelerated extracellular matrix degradation. Peroxisome proliferator activated receptor gamma (PPARγ), as one of the nuclear hormone receptors superfamily, plays an critical role in the control of inflammatory responses. However, there are few evidences on the role of PPARγ in LPS-induced pulpal inflammation. Thus, we investigated the effect of PPAR on pulpal inflammation by LPS. Human dental pulp cells when exposed to various concentrations of LPS (1-10 µg/ml) showed the elevated levels of MMP-2 and MMP-9 on 24 hr culture medium. LPS also stimulated the production of ICAM-1 and VCAM-1. However, when we treated with rosiglitazone, a PPARγ agonist in the same culture system, production of adhesion molecules was markedly inhibited together with lowering the secretion of MMPs. PPARγ adenovirus showed more significant inhibitory effect on inflammation than rosiglitazone. These result may offer us a possible attempt of using PPARγ as one of anti-inflammatory modulators in human dental pulp cell.

Key Word - Inflammation, MMPs, Adhesion molecules, PPAR gamma.

Tis21 and Induced Cellular Senescence

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TIS21/BTG2/PC3 belongs to the antiproliferative (APRO) gene family and regulates G1-S and G2-M progressions of cell division cycle through the inhibition of G1 phase cyclin biosynthesis, and the delayed degradations of cyclin B1 and cyclin A, respectively. It has also been reported that TIS121 down regulates cyclin D1 and cyclin E-associated CDK activities depending on the presence and absence of pRB in the cells, respectively. Therefore, we have already suggested that TIS21 might play a role as a pan-cell cycle regulator and an important factor for cellular senescence. However, there are no experimental evidences of TIS21 as a regulator of cellular senescence. In this study, we observed that the changes of TIS21/BTG2/PC3 expression in the replicative and induced senescences of HDF cells; mRNA expression was high in the senescent cells with increased senescence associated-ß-galactosidase activity. Furthermore, when we treated Huh-7 hepatoma cells with H2O2, expression of BTG2/TIS21 mRNA was highly induced with the accompanying senescence phenotypes. To investigate a role of TIS21/BTG2/PC3 in the process of cellular senescence, Huh-7 and 293 cells were transfected with TIS21 cDNA construct and the followings are observed Growth of the Huh-7 cells and the 293 cells were significantly decreased, cell shapes were changed from the small and cylindrical young cell like to the large and flat cells, and percentages of the cells with senescence associated-ß-galactosidase activity were significantly increased. When HDF cells were treated with H2O2, induction of TIS21 expression was accompanied with loss of mitochondrial membrane potential differences, when evaluated by FACS with JC-1 staining. The above data implicate that TIS21 might sensitizes cells to mitochondrial damage in response to reactive oxygen species, the levels much higher in the old than the young cells, which might induces senescencephenotypes in response to ROS stimulation.[R01-2006-000-10311-0(2006)]

Key words - TIS21/BTG2/PC3, cellular senescence
Actin Accumulation in Nuclei And Expression of Exportin 6 in the Senescence of Human Diploid Fibroblast

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Actin is a highly conserved, ubiquitous cytoskeletal protein, which contains two leucine-rich nuclear export signal (NES) sequences in the middle of the molecule. It has been known that actin is exclusively kept in the cytoplasm by exportin 1-driven nuclear export. However, we recently reported that actin was accumulated into the nucleus during replicative senescence of human diploid fibroblast (HDF) cells. We are going to elucidate the possible involvement of exportin 6 (Exp6) in the regulation of nuclear actin accumulation using DNA damage-induced cellular senescence model. Doxorubicin, a well-known cellular senescence inducing reagent, induces actin accumulation in nuclei with a concomitant reduction of Exp6 expression. Treatment of hydrogen peroxide also showed the similar effect on the expression of Exp6 mRNA after Doxorubicin treatment. At the same time, increase of doubling time of HDF cells was accompanied by the reduction of Exp6 expression. These results may suggest that Exp6 is involved in the regulation of actin accumulation in nuclei during cellular senescence. Employing Exp6 shRNA construct in HDF old cells, we are going to prove whether the actin accumulation in nuclei is a cause or effect of cellular senescence event.

Key words - Exportin 6, Replicative senescence

Abrupt Inhibition of Tgase 2 by Antisense ssDNA and Interfering dsRNA Produced Apoptotic Nuclear Changes in Organ-cultured Salivary Gland of Mouse Embryo, Pha-activated Mouse Lympocytes and Snu-1cells

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Although the TGase 2 knockout mouse showed no critical abnormality in postnatal life, in the previous study we observed that the antisense inhibition of TGase 2 produced the aberrant growth of organ-cultured submandibular glands of mouse embryos. The present study is aimed to elucidate the essential roles of TGase 2 in the cellular changes of organ-cultured submandibular glands of mouse embryos, PHA-activated mouse lymphocytes and SNU-1 cell culture, when the TGase 2 was abruptly inhibited by antisense ssDNA and knockdown by dsRNA. Severe apoptotic change was found in ductal cells of mouse embryos salivary gland, PHA-activated mouse lymphocytes, and SNU-1 cells when treated with TGase 2 antibody, TGase 2 antisense oligonucleotide, and TGase 2 dsRNA through Western blot, immunohistochemistry and FACS analysis. And more, the apoptotic cells also showed dramatic nuclear explosion phenomenon with abnormal aggregation of nuclear chromosome in M stage. Therefore, we presumed that the decrease of TGase 2 expression may affect in the cross-linking of nucelosomes for nuclear organization and also in histone modification to package the chromosome.

Key words - TGase 2, Antisense inhibition, dsRNA interfering, Mouse embryo salivary gland, Apoptosis
Algin-cellulose Hybrid as a Bioinert Carrier Pack Material for Skin and Mucosa

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We developed a new pack material, algin-cellulose hybrid, to carry biological materials on skin and mucosa. Algin is a polymer composed of D-mannuronic acid and D-glucuronic acid, and cellulose is also a polymer of 1,4-glucuronic acid. Both of them are from sea weeds and squirts, respectively, which were utilized for the protection of their bodies, because they are greatly resistant to strong acid and alkali salts. However, many hydroxyl groups are reactive with different cationic salts to produce an aggregated precipitation, of which reaction was reversible by salt-chelation. When tricalcium phosphate (Ca₃(PO₄)₂) and calcium oxide were applied in the mixture of algin and cellulose, an algin-cellulose hybrid intermediated by three-dimensional structure of calcium phosphate apatite. This algin-phosphate hybrid connected by calcium phosphate apatite is biologically more inert, stable and resilient than other algin-cellulose aggregations produced by different salts. The algin-cellulose hybrid retains large volume of water together with different hydrophilic and hydrophobic molecules, so that it would be a good applicant carrier pack to apply directly on the skin and mucosa. Here, we tested the chemical and physical properties of algin-cellulose hybrid containing different phytochemicals for wound healing and antioxidants. The algin-cellulose hybrid showed high diffusion efficiency on semi-permeable cellulose membrane and can work up to 10 hours in a single use, so that it would be a good carrier pack materials for different usages on aging skin and mucosa.[R11-2002-097-07004-0]

Key words - Algin-cellulose hybrid, tricalcium phosphate, carrier pack, aging skin and mucosa

Effects of L-dopa on Dopamine Biosynthesis in Pc12 Cells

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3,4-Dihydroxyphenylalanine (L-DOPA) is the precursor of dopamine and is mostly used for Parkinson's disease. In this study, the mechanisms of L-DOPA-induced increase of dopamine biosynthesis in PC12 cells were investigated. L-DOPA treatment (20-200 µM) increased dopamine content by ca. 2.2-5.1 folds for 3-6 h. L-DOPA could increase intracellular L-DOPA content until 6 h. TH activity was decreased at 1 h, then increased at 3 h, and retained the activity until 24 h. While AADC was increased at 1-3 h, and then decreased rapidly from 6 h and recovered to control levels at 24 h. In these conditions, the intracellular cAMP levels increased by ca. 3.4-5.7 folds for 0.5-1 h. When pretreatment with SCH 23390, which was D1 receptor antagonist, cAMP levels and dopamine content were decreased. In addition, pretreatment with H89, which was PKA inhibitor, dopamine content was inhibited significantly as compared with L-DOPA alone, whereas GF 109203X, which was PKC inhibitor, had no significant effect. And then PKA, CREB phosphorylation and TH protein were measured. Taken together, L-DOPA increased dopamine biosynthesis in PC12 cells at least through two kinds of pathways; one way was that L-DOPA directly entered into cells to convert to dopamine biosynthesis by increasing AADC activity, in this step, TH activity was inhibited; the other way was that L-DOPA transmit the D1 receptor/cAMP/PKA and Ca²⁺/PKC pathways to control dopamine biosynthesis, and PKA pathway was more effective than PKC pathway.

Key words - L-DOPA, TH, AADC, cAMP, Dopamine biosynthesis, PC12 cells

Increase of Transglutaminase C Expression and Activity Confers Resistance to Apoptosis in Senescent Hdfs

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Alteration of apoptotic response against various stimuli is one of the most well-known aging cell phenotype. As a possible
mechanism for such irresponsiveness in senescent cells, we tried to suggest the meaning of over-expression of Transglutaminase C (TGC) enzyme during cellular senescence. There are a lot of evidences that TGC plays a regulatory role in the process of apoptosis. In this study, we tested the hypothesis that TGC over-expressed in senescent cells can attenuate apoptosis against various stimuli. Increase of TGC expression and activity during cellular senescence were reconfirmed in HDFs by Western blotting, IF staining, northern blotting, and in situ TGase activity assay. To investigate the role of TGC in ameliorating apoptotic response, we over express the TGC in young HDFs by RA (all trans retinoic acid) treatment or by using adenoviral system expressing human TGC. No matter what the methods, TGC overexpression in young fibroblasts protect cells from apoptotic stimuli, staurosporin and heat-shock. The cleaved caspase-3 and PARP are decreased in TGC over-expressed young cells as compared with mock infected young cells toward staurosporine treatment and heat induced apoptosis. At the PE conjugated annexin V staining analysis by flow cytometry showed also that TGC attenuates cellular apoptotic response towards staurosporin stimulation and heat-shock. With those results, we could suggest that TGC overexpressed in senescent fibroblasts might play an important role in altered apoptotic response in cellular senescence.

Key words - Cellular senescence, apoptosis, TGC

[P-31]
Estimating Epistasis with Mixed Model Method and Restricted Partition Method
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Epistatic effect greatly contributes the variation of quantitative traits. The objective of this study was to compare estimated epistatic effects by restricted partition method (RPM) and mixed model approach. RPM was recently suggested to efficiently estimate epistasis by partitioned multilocus genotypes that can explain a significant proportion of the observed trait variation. A mixed model approach was introduced to estimate the genetic parameters. Genotypic effects were estimated as random effect and environment effects were estimated as fixed effect. Data were simulated with designs of variance within genotype, sample size, balance of design, and existence of fixed effects. For comparison, we used predictional error (PE) as sum of squares between the estimated genotypic mean and the simulated genotypic mean. The PE obtained from mixed model were smaller (P < 0.05) than the corresponding PE from RPM regardless of variance within genotype, sample size, balance of design. The differences of PEs between the two methods were larger when the variance within genotype was larger and the sample size was smaller. The PE increased (P < 0.05) either with a large variance within genotype or with a small sample size for both methods. The unbalanced design simulated in this study also led to an increment (P<0.05) of PE in every possible combination from different variances within genotype and sample sizes. Although PEs obtained from mixed model were increased with additional fixed effects, their statistical significances were absent (P > 0.05). However, PEs obtained from RPM dramatically increased comparing to PEs from the data without fixed effects (P < 0.0001). As a result, the discrepancy between PEs from mixed model and RPM became considerably increased with the data simulated with fixed effects. The patterns of increases and decreases by variance within genotype, sample size, balance of design in the data with fixed effects were largely corresponding to those in the data without fixed effects. The number of merged groups resulted from RPM ranged from 2.10 to 2.90 using the data without fixed effects and from 1.78 to 1.90 using the data with fixed effects. The PE increased as this number of merged groups decreased throughout all simulations without fixed effects. The simulation study implied that mixed model approach was more plausible in estimation of epistatic effects than the RPM.

Key words - Complex trait, Epistasis, Restricted partition method, Mixed model

[P-32]
Transcriptional Regulations by Light Promoter SNPs are Associated with Ischemic Stroke
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LIGHT, the tumor necrosis factor superfamily 14 (TNFSF14), modulates T cell immune responses by signaling through herpes virus entry mediator (HVEM) and lymphotixin b receptor (LTrβ). Also, it has been implicated in T cell development and homeostasis, dendritic cell maturation and atherogenesis. We identified that four SNPs (-770C>T, -607G>T, -543G>A and -399A>G)
in hLIGHT promoter regions might alter transcription factor binding site from TRANSFAC analysis. Their rare allele frequencies in 120 unrelated Koreans were 29.0%, 48.0%, 38.0%, 5.0%, respectively. Six naturally occurring haplotypes (CGGA, TTAA, CTGA, CTAA, CGGG and TTGA) in the population were cloned into a luciferase expression plasmid, transfected into HEK293 (human embryonic kidney 293) cells, and reporter activity measured at 24 and 48 h. Three haplotypes (CGGA, TTAA and CTGA, respectively) showed average expression that was 2.44-fold (p < .0001), 1.12-fold (p < .0001) and 1.96-fold (p < .0001) higher than the lowest expressed haplotype TTGA at 24h. We also measured transcriptional activity between TGGA and CGGA in order to investigate the alteration of gene expression by the first SNP, showed that TGGA decreased by 53% compared with CGGA (p < .0001). We further investigated a gender-related association of the haplotypes as well as SNPs with ischemic stroke and vascular dementia. The current study suggested that transcriptional regulation of the hLIGHT gene might be associated with LAA and CE as well as vascular dementia in female.

**Key words** - SNP, haplotype, LIGHT, vascular dementia, ischemic stroke

[**P-33**]

**Impact of a Common Variant in Transforming Growth Factor-β1 Gene On Ischemic Stroke And Vascular Dementia**

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Transforming growth factor-β1 (TGF-β1) is an anti-inflammatory cytokine that plays an important role in cerebrovascular pathophysiology with protective activity against ischemia-induced neuronal death. We investigated the association of the polymorphism in TGFβ1 with ischemic stroke and vascular dementia. Three sequence variants and around promoter and exons of TGFβ1 gene were identified in 30 Koreans. Pro10Leu out of them was selected for association study and screened in patients with ischemic stroke (n = 271) and vascular dementia (n = 207) and in control subjects (n = 207). Subjects carrying Leu/Leu were susceptible to both ischemic stroke (OR = 1.63; P < 0.05) and vascular dementia (OR = 1.88; P < 0.01). Analyses with stroke subtypes showed a strong association with small vessel occlusion (SVO, n = 110; OR = 2.07; P < 0.01). Further analysis of SVO data partitioned by gender revealed the female-specific association with Pro10Leu (OR = 2.70; P < 0.05). The Pro10Leu of TGFβ1 might be a risk factor of ischemic stroke and vascular dementia, especially for SVO in females. [A020007]

**Key words** - Genetics, Ischemic stroke, Vascular dementia

[**P-34**]

**Analysis of Genes Causing Parkinson's Disease in a Korean Cohort**

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Mutations of several genes inherited in Mendelian fashion have been identified to cause Parkinson's disease (PD), which include SNCA, PARKIN, UCHL1, PINK1, DJ-1 and LRRK2. Besides PARK genes, mutations of some genes implicated in spinocerebellar

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ataxia such as ATXN2, ATXN3, SCA8 or TBP were also reported as a cause of familial PD especially in Asia. To investigate frequencies of mutations of the above genes in Korean PD population, we have been analyzing mutations of SNCA, PARKIN, PINK1, DJ-1, LRRK2, ATXN2 in EOPD or familial PD. Genomic DNA from peripheral lymphocytes in sixty-eight PD (fifty-six EOPD and twelve familial PD) were extracted. Homozygous deletion of exon 4 in PARKIN was found in one patient. In the analysis of PINK1, we found three heterozygous mutations (G32R, R279H, and F385L) in three patients, and a compound heterozygous mutation in one patient (N367S and K520RfsX522). A heterozygous mutation of SNCA (A53T) was found in one familial PD. However, no mutation in DJ-1 and exon 41 of LRRK2 was detected. There was no abnormal expansion of trinucleotide repeats in ATXN2 in our series. In conclusion, mutations of PINK1 in EOPD seems to be more common than those of other genes in Korea, although future studies in a larger cohort including gene dose analysis are needed. [KRF-2005-042-E00123]

Key words - Parkinson’s Disease, mutations, Mendelian

[Page 35]
Genotyping of the Jc Virus in Urine Samples of Healthy Korean Individuals and Herpes Zoster Patients

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Herpes zoster is a neurocutaneous disease caused by the varicella zoster virus (VZV) as a consequence of declining cell-mediated immunity or by immune suppression and conditions with immunodeficiency. Reactivation of JC virus (JCV) may be linked with immunodeficiency or immunosuppressive therapy. To evaluate the relationship between the herpes zoster and JCV reactivation, the prevalence of the JCV was investigated in 102 Korean herpes zoster patients and 100 healthy Korean individuals. There are no significant differences in the incidence of JCV between the Korean herpes zoster patients and healthy controls (P = 0.5391). In order to investigate genotypes of JCV, we analyzed 45 JCV isolates amplified from Koreans by DNA sequencing and nucleotide sequence analysis. Six distinctive JCV strains were identified in the VT-intergenic region. Based on phylogenetic analysis, JCV types 1 (11%), 2A (22%), and 7B (67%) were found in these Korean patients. Interestingly, JCV type 1 found in Koreans was sub-classified into Type 1C subtype, which is different from type 1A and B substrains prevalent in European. These results indicate that herpes zoster may not play an important role in JCV reactivation and are not associated with JCV types.

Key words - JC virus, Herpes zoster, Genotype

[Page 36]
Altered Expression of Type 1 Inositol 1,4,5 Triphosphate Receptor in the Ngsk Prnp Nulll Mice

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Some lines of PrP knockout mice showed ataxia and Purkinje cell degeneration in the cerebellum caused by the ectopic expression of the prion-like doppel protein (Dpl), which prevented by expression of the cellular prion protein (PrPC). But physiological functions of Dpl and PrPC and underlying mechanisms are unknown. In this study, to clarify the possible implication of both proteins on the intracellular calcium homeostasis, we have investigated the expression levels of sarco/endoplasmic reticulum Ca2+ ATPase type 2b (SERCA 2b) and IP3 receptor type 1 (IP, R1), the major calcium-release channel in the cerebellum, in cerebella Ngsk mice and wild type mice. Both mRNA and protein compared with wild mice, but that of SERCA 2b gene was not changed. Interestingly the level of IP, R1 mRNA expression was down regulated at early time in parallel with protein levels. The results of this study suggest that Dpl may effect on the expression of IP, R1 in Purkinje cell degeneration, and as a result, the onset of ataxia in Ngsk mice.

Key words - Prion protein, Doppel, Type 1 inositol 1,4,5 triphosphate receptor, Ataxia, Purkinje cells
The Detection of Human Endogenous Retroviruses (HerVs) in the Cerebrospinal Fluids of Individuals with Sporadic Creutzfeldt-Jakob Disease (CJD)

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Human endogenous retroviruses (HERVs) constitute approximately 8% of the human genome. The possible involvement of retroviruses in prion disease has been suggested by many studies. In this study, we investigated the expression of 10 HERV families from the cell-free cerebrospinal fluids (CSFs) of individuals with 87 sporadic Creutzfeldt-Jakob disease (CJD) by RT-PCR analysis. The retroviral RNAs of HERV-W, HERV-K, HERV-FRD, ERV-9, HERV-T, HERV-L, HERV-H, HERV-F, HERV-I, and HERV-E were found in 86 (98%), 76 (87.4%), 62 (71.3%), 53 (60.9%), 33 (37%), 21 (24.1%), 12 (13.8%), 11 (12.6%), 7 (8.0%), and 2 (2.3%) CSF samples of individuals with sporadic CJD, respectively. Interestingly, the detection rate of HERV-T and HERV-L in CSFs of sporadic CJD patients was significantly increased than in those of 14-3-3 negative samples. Our results suggest that the expression of HERVs may be associated with the development of prion diseases, and it might lead to improve methods for the diagnosis of sporadic CJD.

Key words - Prion disease, Creutzfeldt-Jakob disease (CJD), Human endogenous retrovirus (HERV), Cerebrospinal fluid (CSF)

The Effect of Simian Virus 40 (SV40) Large T Antigen in Glioblastoma Multiforme

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During the initial manufacture of the salk polio vaccine from 1954 to 1960, the vaccine was grown on rhesus monkey kidney cells, which were then treated with formalin to inactivate virus infectivity while preserving antigenicity. In 1960, there was fairly rapid recognition of SV40 contamination of these lots because SV40 is found frequently in rhesus monkey but is more resistant to formalin inactivation than polio. We gained the brain tumor tissues from surgery which were diagnosed as glioblastoma multiforme (GM). Tissues were cultured as primary condition with culture media, consequently obtained three naturally immortalized cell clones from one patient and analyzed the cells using molecular biological methods. Immortalized cells were characterized as oligodendroglia cells, where viruses usually anchor and proliferate. Immunofluorescence results clarified the location of SV40 large T antigen as nuclear. SV40 large T antigen was detected in brain tissue, blood sample, cultured cells, and cell cultured media by PCR method, however we failed to find virus particles by electronic microscope (EM). Instead, using cytogenic research we found the tumor cell’s chromosomes were enormously changed when compared with normal cells. SV40 large T antigen is postulated to be effect on chromosomal changes in oligodendrogial cells of brain tissue. Since the patient was 60-years-old, thus we suspect that this GM patient was vaccinated with SV40 contaminated polio vaccine and induced the tumorigenesis.
[P-39]

**Generation of Prion Protein (Prp) Transgenic Drosophila**

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Although many prion protein (PrP) transgenic or knockout animal models have been generated for studying the biology and etiology of prion diseases, the neurodegenerative mechanisms of these diseases and the function(s) of prion protein remain unclear. It is suggested that a possible role of PrP_C may be masked by functional redundancy of other proteins. Since Drosophila has several advantages for investigating in the etiology and pathogenesis of many neurological disorders, we evaluated transgenic (Tg) Drosophila expressing wild or mutant type of human (Hu-) or mouse (Mo-) prion protein. When various forms of PrP were expressed in Drosophila neurons, the Mo-PrP was localized in pre-synaptic terminals as well as axon tracts, and the expression of mutant Mo-PrP (P101L) was found to be highly localized to presynaptic terminals compared to that of normal PrP. It is of interest that only mutant type flies exhibit bang sensitivity in early stages. These results suggest that PrP Tg Drosophila models may be a good model for investigation of predisposing defects in early stages as well as neurodegenerative mechanisms of prion diseases.

**Key words** - Prion protein, Neurodegenerative mechanisms, Synapse, Transgenic Drosophila

[ P-40 ]

**Change and Translocation of Hsp60 in the Gerbil Hippocampal Ca1 Region Induced by Transient Ischemia and Its Protective Effect Against Ischemic Damage**

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In the present study, we observed changes in heat shock protein 60 (HSP60) immunoreactivity(IR) and level in the gerbil hippocampal CA1 region after transient forebrain ischemia and its protective effect against ischemic damage. HSP60-IR was increased at 3 h after ischemia and peaked 6 h after ischemia. At these time points, HSP60-IR was detected in the mitochondria. At 24 h after ischemia, HSP60-IR was detectable in the cytoplasm of hippocampal CA1 region. Thereafter, HSP60-IR was nearly disappeared in the hippocampal CA1 region. At 4-10 days after ischemia, its immunoreactivity was detected in the glia. In Western blot study, HSP60 protein level of mitochondrial fraction was increased 3-6 h after ischemia, in contrast HSP60 protein level of cytosolic fraction was significantly increased at 12-24 h after ischemia/reperfusion. The HSP60 transfection via the adenovirus significantly increased the neuronal survival after transient forebrain ischemia. This result suggests that the translocation and decrease of HSP60 may be associated with neuronal cell death and the induction of HSP60 protects neurons from the ischemic damage.

**Key words** - Hippocampal CA1 region, Transient ischemia, Delayed neuronal death, HSP60
[P-41] Transient Ischemia-induced Changes Of Interleukin-2 And Its Receptor β Immunoreactivity And Levels In The Gerbil Hippocampal CA1 Region

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Interleukin-2 (IL-2) is an important cytokine in the brain: IL-2 and its receptors are involved with inflammatory processes. Chronological changes in IL-2 level in serum, and IL-2 and its receptor (IL-2 receptor β, IL-2Rβ) immunoreactivities and levels were examined in the hippocampal CA1 region after transient forebrain ischemia in gerbils. IL-2 level in serum significantly decreased 12 h after ischemia/reperfusion. IL-2 immunoreactivity was detected in the somata of pyramidal cells in sham-operated group. At 15 min after ischemia, IL-2 immunoreactivity was shown in non-pyramidal cells as well as pyramidal cells. One day after ischemia, IL-2 immunoreactivity was lowest, and IL-2 immunoreactivity is shown in non-pyramidal cells from 2 days after ischemia. Four days after ischemia, IL-2 immunoreactivity was shown in dying pyramidal cells. IL-2Rβ immunoreactivity in the sham-operated and 15 min-3 min post-ischemic groups is detected in the cell membrane of pyramidal cells. From 3 h after ischemia, IL-2Rβ immunoreactivity is found in cytoplasm and nuclei, but not in cell membrane. IL-2Rβ immunoreactivity decreases from 6 h after ischemia, and is shown mainly in non-pyramidal cells from 3 days after ischemia. The data of Western blot analyses for IL-2 and IL-2Rβ was similar to the immunohistochemical data. IL-2 infusion into cerebrospinal fluid did not protect hippocampal neurons from ischemic damage. These results suggest that IL-2 and IL-2Rβ show malfunction from 3 h after ischemia, and exogenous IL-2 does not protect ischemic neuronal damage.

Key words - Interleukin-2, Interleukin-2 receptor β, Transient ischemia, Hippocampal CA1 region, Pyramidal cells

[P-42] Ribosomal Protein S3 is Related to Delayed Neuronal Damage and Has Neuroprotective Effect in the Gerbil Hippocampal CA1 Region Induced by Transient Ischemia

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Some ribosomal proteins play an important role in the regulation of development and DNA repair. In the present study, we investigated ischemia-induced changes in rpS3 mRNA and protein levels in the hippocampal CA1 region of the gerbil after ischemia. RpS3 immunoreactivity and its protein level in the CA1 region significantly increased at 15 min post-ischemia, and then decreased by 3 h post-ischemia. They significantly increased again at 6 h post-ischemia, and then continuously decreased with time. RT-PCR analysis also showed that mRNA also significantly increased in the ischemic hippocampus at 6 h after transient ischemia. To elucidate the role of rpS3 on ischemic damage, we developed a delivery vector (Pep-1) and its rpS3 fusion protein (Pep-1-rpS3). Pep-1-rpS3 treatment in ischemic brains significantly increased the neuronal survival in the stratum pyramidale of hippocampal CA1 region dose-dependently. In addition, the treatment reduced the number of TUNEL positive CA1 pyramidal cells in the stratum pyramidale dose-dependently. These results indicate that rpS3 show a potential neuroprotective effect against ischemic damage.

Key words - Transient forebrain ischemia, CA1 pyramidal cells, Ribosomal protein S3 Pep-1 vector, Neuroprotection
[P-43]  
The Significant Increase of Thiol-proteins in the Hippocampal Ca1 Region After Transient Forebrain Ischemia  

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In the present study, we investigated chronological changes of hyperoxidized peroxiredoxins (Prx-SO3) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH-SO3), thiol-containing antioxidants easily oxidized in cells treated with ROS after transient forebrain ischemia to identify the functional changes of Prx and GAPDH induced by ischemic damage. At 6-12 h after ischemia/reperfusion, GAPDH-SO3 and Prx-SO3 was significantly increased in the hippocampal CA1 region and decreased 24 h after ischemia/reperfusion. At 2-3 days after ischemia/reperfusion, GAPDH-SO3 and Prx-SO3 immunoreactivity nearly disappeared in the hippocampal CA1 region. At 4 days after ischemia/reperfusion, GAPDH-SO3 immunoreactivity was predominantly detected in the non-pyramidal cells of hippocampal CA1 region, while Prx-SO3 immunoreactivity was detected in the glial components. At 5-7 days after ischemia/reperfusion, GAPDH-SO3 and Prx-SO3 immunoreactivity was detected in the glial component of hippocampal CA1 region. These results suggest that the thiol-rich antioxidants experienced the function modification after transient forebrain ischemia and this modification may be associated with neuronal death induced by ischemic damage.  
Key words - Peroxiredoxins, Glyceraldehyde-3-phosphate dehydrogenase, Hyeroxidation, Reactive oxygen species, Ischemia, Gerbil  

[P-44]  
The Differential Paired-pulse Responses between the Ca1 and the Dentate Gyrus are Related to Altered Clc-2 Immunoreactivity in the Pilocarpine-induced Rat Epilepsy Model  

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The epileptic hippocampus shows differential paired-pulse responses between the dentate gyrus and the CA1 region. However, little data are available to explain this phenomenon. In the present study, we identified the relationship between regional differences of paired-pulse response and voltage gated Cl(-) channel 2 (CLC-2)/vesicular GABA transport (VGAT) expression in a pilocarpine-induced rat model. During epileptogenic periods, paired-pulse inhibitions in the dentate gyrus and the CA1 region were markedly reduced. After recurrent seizure onset, paired-pulse inhibition in the dentate gyrus was markedly enhanced, while that in the CA1 region more reduced. Unlike VGAT, CLC-2 immunoreactivity was markedly reduced in the hippocampus during epileptogenic periods and was re-enhanced only in the dentate gyrus after recurrent seizure onset. Linear regression analysis showed an inverse proportional relationship between alterations in CLC-2 immunoreactivity and changes in normalized population spike amplitude ratio within the CA1 region and the dentate gyrus. Therefore, our findings suggest that the regionally specific alterations in CLC-2 immunoreactivity after SE may determine the properties of paired-pulse responses in the hippocampus of the pilocarpine-induced rat epilepsy model.  
Key words - CLC-2, Dentate gyrus, Hippocampus, Epilepsy, Paired-pulse inhibition
The Epileptogenic Roles of Astroglial Death and Regeneration in the Dentate Gyrus of Experimental Temporal Lobe Epilepsy

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Since altered morphological organization or functionality of astrocytes induced by status epilepticus (SE) were closely related to the abnormal neurotransmission via altered vesicular glutamate, GABA transporter expressions, and mossy fiber sprouting of the dentate gyrus, we investigated whether these alterations is responsible for epileptogenesis. The glial responses (reactive microgliosis followed by astroglial death) in the dentate gyrus to pilocarpine-induced SE preceded the neuronal damage. These alterations were closely related to the abnormal neurotransmission via altered vesicular glutamate and GABA transporter expressions, and mossy fiber sprouting of the dentate gyrus. In addition, newly generated astrocytes showed down-regulated glutamine synthase, glutamate dehydrogenase and glial GABA transporter expressions. These findings suggest that glial responses after SE may contribute to epileptogenesis and the acquisition of the properties of the epileptic hippocampus. Similar to inhibition of neuronal deaths, thus, it is worth considering that targeting inactivation of microglia or protection against astroglial loss may be involved in new therapeutic approaches of epileptogenesis.

\textbf{Key words} - Epilepsy, Astrocyte, Microglia, GFAP, vimentin, VGLUT, VGAT, GS, GDH, GAT-3, P2X, receptor

Increased Task-1, Not Task-2, Immunoreactivity in the Astroglia is Related to Seizure Activity in the Gerbil

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TASK-1 is a highly modulated pH-sensitive 'leak' K$^+ \text{ channel expressed in the brain. In the present study, we investigated the distributional patterns of TASK immunoreactivities and effects of seizure activity and various antiepileptic drugs (AEDs) on them in the hippocampus of gerbil, a genetic epilepsy model. Following various AED treatments, we investigated alteration in TASK-1 and TASK-2 immunoreactivity in the hippocampus by immunohistochemical and double immunofluorescence methods. In the hippocampus, TASK-1 immunoreactivity was mainly observed in astroglia, whilst TASK-2 immunoreactivity was detected both in neurons and in astroglia. In seizure sensitive (SS) gerbils, TASK-1 immunoreactivity in the hippocampus was higher than that in seizure resistant (SR) gerbils. However, there was no difference of TASK-2 immunoreactivity in the hippocampus between SR and SS gerbils. Following spontaneous seizure on-set, elevated TASK-1 immunoreactivity was markedly reduced. In addition, TASK-1 immunoreactivity was decreased by some anti-epileptic drugs. TASK-2 immunoreactivity was unaffected by spontaneous seizure or AEDs. These findings suggest that the elevated TASK-1 immunoreactivity may be involved in generating or spreading seizure activity, and TASK-1 may be one of potential target molecules for AEDs.

\textbf{Key words} - TASK-1, Astroglia, Hippocampus, Epilepsy, Gerbil
**Epithelial to Mesenchymal Transition of Mesothelial Cells in the Tuberculosis Pleurisy**

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Tuberculous pleurisy is the most frequent extra pulmonary manifestation of tuberculosis. Residual pleural thickening of 2 to 10 mm has been reported in 20% to 50% of cases in spite of adequate treatment. Pleural resident fibroblasts have historically been considered to be the primary cells involved in the development of pleural fibrosis. Recently, several studies of kidney diseases demonstrated that fibroblast are derived from renal tubular epithelium via epithelial to mesenchymal transition (EMT). However, a possible direct involvement of pleural mesothelial cells in tuberculous pleurisy has not been examined. We studied 1) whether cytokines such as TGF-β1 or IL-1β elevated in tuberculous pleurisy induce mesothelial cells EMT in vitro, 2) whether pleural mesothelial cells are involved in the fibrosis of tuberculous pleurisy, 3) whether transcription factor, snail expression, is associated with E-cadherin loss. we demonstrated below results.

1. Mesothelial cell transdifferention in vitro was induced by TGF-β1 and TGF-β1+ IL-1β
2. Both stimuli of TGF-β1 and IL-1β show synergistic effect on morphologic changes and snail expression
3. Snail expression show close relationships with E-cadherin repression
4. Cell cultures from effluents from tuberculous Pleurisy show mainly fibroblast-like cells features, but two patients show both features of mesothelial and fibroblast cells
5. Some elongated fibroblast-like cells positive for cytokeratin and vimentin were founded embedded in the fibrotic tissue (11 cases positive among 22 cases).

In conclusion, we demonstrated that mesothelial cells undergo an epithelial-to-mesenchymal transition in the fibrosis of tuberculous pleurisy This work was supported by clinical research fund of Hallym university medical center.

**Key Words** - EMT, TGF-β1, Tuberculous Pleurisy

**Characterization of Sod1 Mutant And Hsp27 in Familial Amyotrophic Lateral Sclerosis**

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the selective death of motor neurons. Approximately 10% of ALS cases are familial (FALS) and about 25% of FALS patients inherit autosomal dominant mutations in the gene encoding Cu,Zn-superoxide dismutase (SOD). In spite of many studies in which FALS-related SOD mutations provoke a new toxic gain of functions, the specific mechanism of this remains unclear. To investigate the roles of FALS-related SOD mutants, the human wild type (WT), mutant type SOD and HSP27 genes were fused with PEP-1 peptide in a bacterial expression vector to produce a genetic in-frame PEP-1-SOD (WT and mutants) fusion protein, respectively. The expressed and purified PEP-1-SODfusion proteins can transduced efficiently into neuronal cell in a time- and dose-dependent manner when added exogenously in culture media. The mutant PEP-1-SOD showed more vulnerability to oxidative stress induced by paraquat than that of WT proteins. However, the transduced HSP27 into neuronal cells protects against cell death by mutant SOD. These results suggest that the protective effect of transduced HSP27 fusion protein against the FALS-associated SOD disease mutant may be of potential therapeutic important.

**Key words** - SOD1 mutant, HSP27, FALS, Protein transduction
Lithium Aluminum Hydride Cause Reductive Cleavage of Oxidized Molecules Leading to A Reducton of Prion Infectivity And Levels of Prp$^{sc}$

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Previous evidence suggested that RNA molecules can function as cellular co-factors for PrP$^{sc}$ formation. An approach to this issue would employ chemicals that can cleave phosphodiester bonds of RNA and then assess the effects on the infectious agent. Lithium aluminum hydride (LiAlH$_4$) is a reducing agent that can induce reductive cleavage of oxidized molecules such as carbonyls, carboxyl acids, esters, and phosphodiester bonds. We found that LiAlH$_4$ destroyed PrP$^{sc}$, extended the scrapie incubation period and markedly reduced total RNA concentration. These findings prompted us to investigate whether RNA molecules are co-factors for PrP$^{sc}$ propagation. RNase A treatment of partially purified-PrP$^{sc}$ and of 263K brain homogenates was sufficient to increase sensitivity of PrP$^{sc}$ to PK, providing the first evidence that RNA molecules are a component of PrP$^{sc}$. However, RNase A alone and PrP$^{sc}$ degradation by RNase A plus PK in vitro, did not show profound loss of scrapie infectivity compared with LiAlH$_4$, suggesting oxidized molecules can be important in the scrapie agent replication process. Our data suggest that RNA molecules can be important in maintaining the structure of PrP$^{sc}$. Chemicals that cleave highly oxidized molecules, such as LiAlH$_4$, have a profound effect on infectivity. [A020007]

Key words - PrP$^{sc}$, RNA, LiAlH$_4$, Oxidized molecules

Jak-stat Signaling Pathway Mediates Astrogliosis in Brains of Scrapie-infected Mice

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Scrapie is characterized histologically, in part, by astrogliosis in brain and spinal cord. However, the mechanisms of astrogliosis in brain injury occurring during prion infection are not well understood. Here, we investigated the expression levels and cellular localization of Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signaling molecules and growth factors such as leukemia inhibitory factor (LIF) and ciliary neurotropic factor (CNTF) by Western blot analysis and immunohistochemistry. We found that expression levels of LIF and CNTF were increased in scrapie-infected brains, and phosphorylated (p)-JAK2, p-STAT1 (Ser727, Tyr701), p-STAT3 (Tyr705) and glial fibrillary acidic protein (GFAP) were expressed strongly in scrapie infected brains. Moreover, we found that p-STAT1 and p-STAT3 were found in the nucleus in scrapie-infected brains. Immunohistochemically, p-STAT1 was colocalized with LIF, CNTF and p-JAK2 in many reactive astrocytes in scrapie-infected brains. Immunostaining for p-STAT3 in the hippocampus of scrapie-infected brains was also found in astrocytes, but p-STAT3 was colocalized with the embryonic intermediate filament protein, nestin. Taken together, our results suggest that activation of JAK2-STAT1 signaling pathway occurred in reactive astrocytes, and activation of STAT3 may be related to expression of nestin in astrocytes in hippocampus of scrapie-infected brains.

Key words - Scrapie, Astrogliosis, JAK-STAT signaling pathway
Increased Expression of VEGF in the Brains of Scrapie Infected Mice

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Prion diseases are one of fatal neurodegenerative diseases in human and animals, which are characterized by neuronal cell death, astrogliosis, spongiosis and accumulation PrPSc in the brains. In addition to its major roles in angiogenesis, vascular endothelial growth factor (VEGF) acts as a pro-inflammatory cytokines. Increased expression of VEGF has been described in other neurodegenerative disease including Alzheimers disease. In order to understand the possible roles of the VEGF in prion disease, we investigated the expression levels of VEGF and VEGF-R2 in ME7-infected mice by using Reverse transcription (RT)-PCR, Western blot analysis, immunofluorescence analysis and immunohistochemistry analysis. The expression levels of VEGF and VEGF-R2 were increased in brains of ME7-infected mice compared with control group at the terminal stage. In addition, the immunoreactivity of VEGF was colocalized within glial fibrillary acidic protein (GFAP) positive reactive astrocytes of hippocampus in scrapie-infected mice. In conclusion, we found that VEGF was upregulated and secreted in reactive astrocyte in hippocampus of ME7 infected-mice. Also, we found that VEGF-R2 was upregulated in hippocampus of ME7-infected mice compared with control group at the terminal stage.

Key words - Prion diseases, VEGF, Reactive astrocytes, Scrapie

Comparison of Appearance between Origin and Korean Outbreak on Experimentally Transmit Chronic Wasting Disease Agent Into ElkPrp Tg Mice

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Chronic Wasting Disease (CWD), a transmissible neurodegenerative disease caused by unusual pathogens called prions, has been raged in North America's cervidae population recently. Also, in Korea, it has been reported that outbreak from imported Canadian elk and deer. The aim of this study was to document which entity of CWD agent have entered Korean and to study distinction of CWD agent in elkPrp tg mice by lesion profiling. The homozygous elkPrp tg mice were infected with three CWD agents (originated from one Korean and two North American cevidae) and carried out an experiment on incubation time, neuropathological lesion profile, pattern of PrPsc deposition and western blot profile. Interestingly, we found that the elkPrp tg mice infected with Korea CWD case's brain homogenate showed more short incubation period and severe vacuolar degeneration than control, as well, amount of PrPsc deposit as a hallmark in prion diseases. These results suggested that homozygous elkPrp tg mice efficiently transmit in short incubation periods are valuable not only as research tool of cervid prions but also as reliable diagnostic tools.

Key words - CWD, Cervidized mice, Incubation time

Galectin-3 Expression Correlates with Abnormal Prion Protein Accumulation in Murine scrapie

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To investigate the involvemment of galectin-3, a novel substrate for matrix metalloproteinases (MMP)-2 and -9, in the process of...
neurodegeneration in prion diseases, the expression and cellular localization of galectin-3 were studied in the brains of a mouse model of prion disease, scrapie. Reverse transcriptase polymerase chain reaction (RT–PCR) and Western blot analysis showed that the expression of galectin-3 protein and mRNA was induced in scrapie-affected brains, particularly at the time when the abnormal prion protein, PrP\textsuperscript{Sc}, began to accumulate in the brains. Galectin-3 was found to be co-immunoprecipitated with MMP-2, but not with MMP-9. Immunohistochemically, immunostaining for galectin-3 was found mainly in B4 isolectin-positive cells (presumably activated microglia/macrophage), but not in astrocytes. Galectin-3 immunoreactivity was mainly localized in areas of PrP\textsuperscript{Sc} accumulation and neuronal death in scrapie infected brains. These findings suggest that induction of galectin-3 is associated with the chemotaxis and phagocytosis of apoptotic cells and other particles by activated microglia/macrophage in prion disease.

**Key words** - Prion disease, Galectin-3, Matrix metalloproteinase-2, Microglia, Macrophage

**[P-54]**

**Atm Mediateds Nuclear Actin Accumulation and Leads to Cellular Senescence with \( G_2 \) Arrest of Normal and Cancer Cells**

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Signal pathway and mechanism of actin accumulation in nuclei, a marker of cellular senescence, were investigated. When human diploid fibroblasts were treated either with H\(_2\)O\(_2\), X-ray irradiation, or anthracyclins, but not with Ara-C, senescence phenotypes appeared along with cellular enlargement, slow growth, G2 phase arrest, foci formations of \(\gamma\)-H2AX, p53\(^{Ser15}\) and Brca-1, and actin accumulation via ATM-Chk2-p53-p21\(^{WA F1}\). Caffeine pretreatment significantly reversed all of the phenotypes. Employing mononuclear cells of normal and leukemia patients, actin accumulation was found to be more sensitive to anthracyclins in the partially committed progenitor cells than the committed and the early stem cells, resulting in cell cycle arrest or death. When investigated the mechanism of actin accumulation, H\(_2\)O\(_2\) and anthracyclins significantly reduced G-actin polymerization, whereas Ara-C triggered dynamic steady state, indicating that nuclear actin accumulation might be due to loss of actin dynamics. In summary, DNA damage inhibits G-actin polymerization, which induces nuclear actin accumulation via ATM activation, resulting in cellular senescence with G2/M phase arrest.[R11-2002-097-05002-0(2006)]

**Key words** - Actin dynamics, reactive oxygen species, anthracyclins (doxorubicin, daunorubicin), Ara-C, Acute Myeloid Leukemia, Huh7 cells

**[P-55]**

**Immuno-membrlot, As A Diagnostic Tool For Human Saliva**

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Immuno-Membrlot is a technique for detecting, analyzing, and identifying proteins, similar to the Western blot technique but differing in that the protein samples are not separated electrophoretically but are spotted through circular or slot templates directly onto the membrane. Recently we developed a new Immuno-Membrlot (IMB) method, applying immunoreactions and coloring procedures directly in the wells of MemBlot apparatus with canal drainage system. This study is aimed to evaluate the analytical accuracy of IMB using different biological assay, and applied in the saliva obtained from different subjects. In the sensitivity test of IMB the monoclonal antibody can clearly detect the 30 ng (about 12 pM) of Mucocidin peptide (35 mer), and is also available to detect at least 10 ng (about 4 pM) of Mucocidin peptide (35 mer). This IMB is useful to screen large number of specific samples with ease and accuracy in a short time. In the screenings for the presence of Mucocidin in saliva the quantitative comparison is
conspicuous among 200 persons depend on the different conditions of gender, drinking and smoking habits, and oral diseases. Therefore, it is presumed that, even though the target proteins were partly degraded, a specific epitope can be detected if a monoclonal antibody was still reactive. Conclusively, these data suggest that the IMB can be useful in the primary qualitative and quantitative analysis of proteins in various fluids, i.e., blood, saliva, tear, urine, etc., especially is available to the specimens obtained from debilitating patients and aging people. [R11-2002-097-07004-0]

**Key words** - Immuno blot, Immuno-MemBlot, Primary screening, saliva, Aging people

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### P-56

**Inhibitory Effects of Tat-sod on Uvb-induced Expression of Mmp-9 in Keratinocytes**

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Keratinocyte is one of major cell types involved in inflammatory skin diseases, such as atopic dermatitis. keratinocyte can be induced by UVB to express MMP-9, which can contribute to inflammatory events within the skin. Matrix metalloproteinases (MMPs) are known as important enzymes involved in tissue metabolism. Among MMP-9 is termed gelatinases, but their specific roles in vivo are still unknown, including their expression patterns following ultraviolet (UV) irradiation. In this study, we examined the effect of a cell-permeable superoxide dismutase. Tat-SOD on UVB-induced MMP-9 expression in an human keratinocyte cell line HaCaT. We observed that (1) Tat-SOD was efficiently transduced into human keratinocyte cell line HaCaT in dose- and time-dependent manners; (2) Transduced Tat-SOD abolished generation of ROS in HaCaT cells exposed to UVB; (3) Tat-SOD inhibited translocation of NF-kappaB and up-regulation of MMP-9 expression in HaCaT exposed to UVB. Induction of MMP-9 secretion is related to the inflammation including inflammation of keratinocytes resulting from UVB irradiation. These results suggest that Tat-SOD might be a useful tool in therapy of skin inflammation induced by UVB.

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### P-57

**Regulation of Synaptic Architectures and Synaptic Vesicle Pools by Nervous Wreck at Type I Glutamtergic Drosophila Larval Nmjs**

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Nervous Wreck (NWK), a SH3 domain and FCH motive protein present at Type 1 glutamtatergic boutons as reticular structures is a *Drosophila* homolog of human sGAP3 protein, linked to X-linked mental retardation. Circles in NWK reticulum enclosed synaptic vesicle markers or exo/endocytosis components at confocal immuno-fluorescence microscopy. At EM levels, NWK proteins localized at the edge of synapses and synaptic vesicle pools. Synergistic morphological phenotypes observed from eag<sup>1</sup>;nwk<sup>2</sup> NMJs suggested that NWK regulates synaptic plasticity in a different manner from the classical activity-dependent Hebbian forms of plasticity. Even though mean synaptic areas in eag<sup>1</sup>;nwk<sup>2</sup> boutons were not significantly different those of nwk<sup>2</sup>, the numbers of readily releasable synaptic vesicle pools (RRPs) were significantly increased up to those of CS. In addition, 3 dimensional High Voltage EM tomography analysis proved that synaptic vesicle density and sizes at T-shaped active zones were altered and membrane ruffles at the edges of synapses were observed from nwk mutant synapses. Taken together, NWK reticulum is important for maintaining synaptic architectures and SV sizes and pools at T-shaped active zones. [KRF2R05-0101-012-S0001]

**Key words** - Synapse, Active zones, Endocytosis, Exocytosis, Plasticity
DFY Mutation in Htora Induces Abnormal Behavior and Altered Synaptic Architectures

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Although two autosomal in-frame deletion mutations in human torsinA (HtorA) are associated with early-onset torsion dystonia (EOTD), most studies have focused on investigating how a glutamic acid deletion (DE302/303; DE) in HtorA affects EOTD pathogenesis. We examined the molecular and cellular etiologies that underlie the deletion of the six amino acid residues (DF323Y328; DFY) in HtorA, as well as the unknown roles of mutant HtorA in muscles with respect to synaptic plasticity. Even though DFY HtorA forms no protein clusters, flies expressing DFY HtorA in muscles manifested a similar but delayed onset of adult locomotor disability and had fewer aberrant ultrastructures at synapses, such as free-electron dense bodies, compared to flies expressing DE HtorA in muscles. In addition, the area of the functional synapses and the Drosophila p-21 activated serine/threonine kinase immunofluorescence signals were significantly enlarged in the DFY and the DE HtorA flies. The behavioral and anatomical aberrations observed in the DFY and the DE HtorA flies suggest that the DFY mutation in HtorA may be responsible for dominant gain-of-function phenotypes, and the presence of mutant HtorA in muscles may also underlie EOTD in Drosophila.

Key words - DYT1, Neurological Disorder, Drosophila, Synapse, Plasticity

Regulation of Schlafen-2 Gene Expression in Response to Toll-like Receptor-mediated Stimulations

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Recent studies have gained attention to obtain information how host gene expression is altered during infections. To analyze genes that are specifically expressed at LPS-inducible inflammation, we examined LPS-induced differentially expressed genes (DEGs) in RAW 264.7 cells by using a differential display PCR method that is based on annealing control primers (ACPs). We selected several clones from LPS-induced DEGs encoding numerous inflammatory genes. We focused on the one clone, which was identified as Schlafen-2 (slfn-2) known as the member of Slfn family, the regulator of T cell development. We have examined expression and regulation of slfn-2 in CpG-DNA-treated and LPS-treated macrophages. Furthermore, we define a transcriptional start site in the slfn-2 gene. We focused on the first time a ~1.8 kb region upstream of the transcription start site for the gene. Sequence analysis indicates consensus sites for AP-1 and NF-κB in this region. Comprehensive mutant analyses, ELISA-based transcription factor activation assay, ChIP assays reveal that functional interaction of AP-1 and NF-κB with the promoter element is necessary for the TLR-mediated slfn-2 gene expression by CpG-DNA and LPS treatment in macrophages. In summary, we identified for the first time a slfn-2 promoter and demonstrated that CpG-DNA and LPS triggers slfn-2 gene expression by activating NF-κB and AP-1 pathways in macrophages.

Key words - Schlafen-2, NF-κB, AP-1, macrophages

Fate of Microglial Cells in Transient Cerebral Ischemia

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In the present study, we investigated the morphological change and viability of parenchymal microglial cells after transient ischemia and reperfusion. Focal cerebral ischemia was produced by intraluminal occlusion of the right middle cerebral artery...
(MCAO) for 2 h. Increased OX42-immunoreactivities were observed in ipsilateral side after 3-h reperfusion following 2-h MCAO. OX42-positive cells in infarct core region seemed to be mainly hyper-ramified microglia. Twelve hours after ischemia, the OX42-positive cells were fragmented to death in the ischemic core region and highly hyper-ramified cells were found in an adjacent area containing morphological intact neurons. Twenty four hours after reperfusion, oval or ameboid OX42-positive cells were found in the ischemic core lesion, but ramified or hyper-ramified OX42-positive cells not observed in the infarct zone. Immunofluorescent studies showed that oxidized hydroethidine- and 8-OHdG-positive cells were co-localized with OX42-immunoreactive cells in the ischemic core region 6 h after reperfusion. Interleukin-1 receptor antagonist (5 µg/5 µl, i.c.v.) and caspase-1 inhibitor (Ac-YVAD-cmk, 1 µg/1 µl/hr, i.v.) reduced the ischemic infarction 24 h after reperfusion. Furthermore, hyper-ramified cells were found in the ischemic core lesion in brains injected with IL-1 receptor antagonist or caspase-1 inhibitor. γ-irradiation of rat body, not head, 5 days prior to MCAO significantly reduced both IL-1β expression and cerebral ischemic injury. The results suggest that the death of hyper-ramified OX42-positive microglial cells early (e.g. 6 h) after ischemia may be synergically caused by IL-1β secreted from peripheral cells and over-production of reactive oxygen species produced during cerebral ischemia.

Key words - Transient focal ischemia, microglia, IL-1β, ROS

The Activation of NF-κB Through Akt-induced Foxo1 Phosphorylation During Aging and Its Modulation by Calorie Restriction

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To explore the interaction of insulin-associated Akt, FOXO1 and NF-κB during aging, experimentations were carried out to determine the relationship between FOXO1 and NF-κB protein activation using HEK293T cells and aged kidney isolated from ad libitum fed (AL) and 40% calorie restriction (CR) rats. Results showed that phosphorylation of FOXO1 and NF-κB activation were significantly increased in old rats compared to those of young rats. Moreover, FOXO1 phosphorylation and NF-κB activation were shown to be significantly lower in the CR rats compared to 24-month-old CR rats. On the other hand, FOXO-dependent catalase decreased during aging. Our findings further documented that FOXO1- and NF-κB-binding activities are associated with age-related increases in serum insulin levels. To further explore the molecular link between FOXO and NF-κB, we performed transfection experiments with FOXO-mutant plasmid in cultured HEK293T cells. Treatment of the cell with insulin led to NF-κB activation through the phosphorylation of FOXO via the PI3K/Akt pathway. We conclude that the phosphorylation of FOXO1 regulates NF-κB translocation by activating PI3K/Akt during aging, which was suppressed by the hypoinsulinemic action of CR.

Key words - Aging FOXO1, insulin, NF-κB, PI3K/Akt pathway

Betaine Modulates Vascular Infalmmation by the Suppression of Lysophosphatidylcholine During Aging

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Lysophosphatidylcholine (LPC) is known to induce expression of proinflammatory molecules such as adhesion molecules (AMs) and cytokines. In the present study, we examined the anti-inflammatory effect of betaine on LPC in vascular aging in vivo and in vitro. Sprague-Dawley rats aged 7 or 21 months were used in this study and older rats were fed with betaine. The serum and aorta of each group were examined. The older rats showed increased levels of LPC and intracellular reactive species (RS), but betaine reduced both levels. The activation of 5-lipoxygenase (5-LO) which is the source of increased RS by LPC was modulated by betaine.
LPC induced AMs and activated NF-κB through IKK and MAPKs, which were suppressed by betaine. We conclude that betaine suppresses LPC-induced NF-κB signaling via IKK and MAPKs, and NF-κB related mechanisms. Betaine might be useful as a preventive agent against LPC induced inflammation during vascular aging.

**Key words** - Betaine, lysophosphatidylcholine, vascular aging, IKK and MAPKs, 5-lipoxygenase

**[P-63]**

**Significance of Ptk/ptp Shift in the Inflammatory Process:**

**Regulating NF-κB Signaling During Aging**

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The proposed molecular inflammation hypothesis of aging highlights the importance of the effects of reactive species (RS) on proinflammatory NF-κB modulation and its role in redox-dependent signaling regulation during aging. The present study delved into molecular questions concerning protein kinase/phosphatase balance, more specifically, how the protein tyrosine kinase (PTK)/protein tyrosine phosphatase (PTP) balance is affected by proinflammatory RS during aging. In present study, we assessed the effects of proinflammatory RS on the PTK/PTP balance in aged rats, utilizing (a) calorie restriction, (b) proinflammatory LPS injected rat, and in addition, cultured HEK293T cells. During aging RS increased PTK activation, but inactivated PTP, leading to a shift in the PTK/PTP balance. In experiments with HEK293T cells, we are able to show that a PTK/PTP imbalance by treatment of LPS and AAPH led to NF-κB activation. Results indicated that the PTK/PTP shift toward PTK was closely associated with NF-κB activation via phosphorylated NIK/IKK and MAPKs. These findings strongly suggested that age-related molecular inflammation elicited by increased activation of NF-κB via the NIK/IKK and MAPKs is due to age-related changes in the PTK/PTP balance. The significance of the current study is that we were able to document for the first time that the activation mechanism of NF-κB is influenced by altered PTK/PTP balance during aging.

**Key words** - PTK/PTP imbalance, NF-κB, Molecular inflammation, Aging, Reactive species

**[P-64]**

**Kaempferol Modulates Age-related NF-κB Activation**

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Phytoestrogens are a family of plant-derived compounds with weak estrogenic or anti-estrogenic dual properties. The antioxidant capacity of phytoestrogens has been proposed as one of the important mechanisms on the prevention of various estrogen-related diseases. Kaempferol is better known as a dietary antioxidant among phytoestrogens. In the current study, we examined the effect of kaempferol on the cytoprotective ability against ONOO or reactive species (RS) and activation of NF-κB cascade on aged rats fed kaempferol (2, 4, 9 mg/kg/day for 10 days). Kaempferol showed RS and ONOO scavenging abilities and suppression of translocation of NF-κB to nucleus and NF-κB-dependent proinflammatory mediators. Kaempferol inhibited NF-κB pathway through modulation of NIK/IKK and MAP kinases. From this study, we think that kaempferol can be a strong candidate not only anti-oxidative agent but also as an anti-inflammatory agent on aging.

**Key words** - Kaempferol, phytoestrogen, anti-oxidative, anti-inflammatory, aging, NF-kappa B
Modulation of Age-related NF-κB Signaling Via Ppar Activation by 3-methyl-1,2-cyclopentanedione

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Activation of the nuclear factor-kappa B (NF-κB) transcription plays a central role in inflammation and aging process through inducing pro-inflammatory genes. This pathway is very sensitive to signals related to oxidative stress. Peroxisome proliferator activated receptors (PPARs) belong to a subfamily of nuclear transcription factors and they have anti-inflammatory functions. 3-Methyl-1,2-cyclopentanedione (3-MCP) extracted from coffee has cyclopentane ring structure. In this study 3-MCP was investigated on the possibility as a selective regulator of inflammation via modulating PPARs using in vivo model. Young and old rats fed 3-MCP (4 and 8 mg/kg/day for 10 days) were examined on reactive oxygen species (ROS) generation, NF-κB signaling, as well as PPARs activation. Results indicated that the 3-MCP suppressed ROS level, NF-κB signaling and NF-κB related genes on aged rats. Also, 3-MCP increased PPARs on aged rats and PPAR activity in YPEN cell. The results strongly suggest that 3-MCP modulates age-related NF-κB signaling cascade via PPARs activation. Therefore, 3-MCP is proposed to be an effective anti-inflammatory agent which can be used in the therapeutic treatment of inflammatory diseases during aging.

Key words - 3-methyl-1,2-cyclopentanedione ROS, PPAR, NF-κB, Aging

Wen-pi-tang-hab-wu-ling-san Extract Inhibits the Release of Inflammatory Mediators from Lps-stimulated Mouse Macrophage Cells

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Wen-Pi-Tang is a Chinese herbal medication that has been used traditionally to treatment of variety if inflammatory diseases such as chronic renal failure, renal injury, renal tubular cell damage and diabetic nephropathy. In this study, we investigated the anti-inflammatory effects of modified Wen-Pi-Tang (Wen-Pi-Tang-Hab-Wu-Ling-San; WHW) extract in LPS-stimulated mouse macrophage cells, in addition, we sought to elucidate its pharmacological activity. We found that WHW extract had potent anti-inflammatory effects on the LPS-stimulated RAW264.7 cells and primary peritoneal macrophages. WHW extract greatly inhibited the LPS-induced nitric oxide production and protein expression of inducible nitric oxide synthase by blocking its gene transcription in RAW264.7 cells and peritoneal macrophages. WHW extract also caused a dose-dependent inhibition of the release of proinflammatory cytokines including TNF-α, IL-1β and IL-6 from both cell types. WHW extract abolished LPS-induced phosphorylation of p42/44 MAPK and JNK, and nuclear translocation of p65 NF-κB protein in both cell types. These data suggest that WHW extract attenuates inflammatory mediator synthesis of activated macrophages through an NF-κB-dependent pathway. Taken together, WHW extract may provide a potential biological reagent for treatment of inflammatory diseases including neurodegenerative disorders. [B050024]

Key words - Antiinflammation, interleukin-1β interleukin-6, iNOS, MAP Kinase, Nuclear factor-xB, nitric oxide, RAW264.7 cells, tumor necrosis factor-α, Wen-Pi-Tang-Hab-Wu-Ling-San extract
Effects of Growth Hormone Replacement Therapy on Quality of Life in Patients With Somatopause

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Objective: There is increasing interest in growth hormone (GH) replacement therapy to improve quality of life (QoL) of elderly with age-related decline in growth hormone level (somatopause). The aim of the study was to evaluate the effects of GH replacement on the QoL in patients with somatopause. Design: Case-controlled study Setting: Anti-aging clinic in Busan, South Korea Participants: 100 patients with the symptoms of growth hormone deficiency (GHD) were divided into GHD group (n = 56) and non-GHD group (n = 44) and compared with 41 normal population whose without symptoms of GHD. Interventions: The diagnosis of GHD was based on a peak GH response < 3 ng/mL during an insulin tolerance test (ITT). GH was replaced in GHD group over 6-month period. Main Outcome Measures: QoL was assessed by using three self-rating questionnaires: the Assessment of Growth Hormone Deficiency in Adults (AGHDA), the Nottingham Health Profile (NHP), and the Psychological General Well-Being Index (PGWBI).

Results: Significant impairment in QoL as measured by AGHDA, NHP, and PGWBI were noted in patients with somatopause compared with age and sex matched controls (p < 0.05). There was significant improvement in QoL after 6-month GH replacement (p < 0.05). Conclusions: Six months GH replacement induced an improvement in the QoL of patients with somatopause.

Key Words - Somatopause, Quality of life, Growth hormone replacement

The Effects of Human Adipose Tissue Derived Mesenchymal Stem Cells on Degenerative Change of Disc in Rabbit Model

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The aim of this study was to determine whether transplanted human adipose tissue derived stem cells (hATSCs) can survive and increase the amount of proteoglycans of degenerated intervertebral disc in rabbits. Lumbar disc degeneration was induced in thirty New Zealand white rabbits by injection of chondroitinase ABC. After 2 weeks, hATSCs were transplanted in degenerated disc in hATSCs group. Control group received phosphate buffered saline. The histologic grading and height of disc were measured at 2, 4, and 8 weeks after transplantation. The viability of donor cells was identified by using β-Actin gene polymerase chain reaction (PCR). At 4 and 8 weeks after hATSCs transplantation, the histologic grading showed significantly high score in hATSCs group (p < 0.05), but the amount of proteoglycans was not significantly different between the two groups. The change of disc height was not significantly increased in hATSCs group. In the β-Actin gene PCR analysis, positive signal in the hATSCs group was observed. In conclusion, hATSCs transplantation may be useful in decelerating disc degeneration in experimental rabbit models and provide new hopes for treatment of degenerative disc disease in humans.

Key words - Transplanted human adipose tissue derived stem cells, Disc degeneration, Rabbit

Failure of Stress-induced Down-regulation of Bcl-2 Contributes to Apoptosis Resistance in Senescent Human Diploid Fibroblasts

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We previously reported that senescent human diploid fibroblasts (HDFs) are resistant to apoptosis induced by H2O2, and staurosporine. We report here that senescent HDFs are resistant to thapsigargin-induced apoptosis as well. These agonists caused
the reductions in mitochondrial membrane potential (MMP) and in the apoptosis inhibitory protein, (Bcl-2) only in young HDFs but not in senescent HDFs. In addition, down-regulation of Bcl-2 increased the sensitivity of senescent HDFs to apoptosis induction, suggesting the significant role of Bcl-2 in apoptosis resistance of the senescent HDFs. We further found that P-CREB, a positive regulator of Bcl-2, decreased in stress-induced apoptosis of young HDFs but not in senescent HDFs and that Bcl-2 was markedly reduced in CREB siRNA transfected senescent HDFs. In addition, activity of protein phosphatase 2A (PP2A), which dephosphorylates p-CREB, significantly increased in young HDFs but not in senescent HDFs treated with H2O2, staurosporine or thapsigargin. Taken together, these results suggest that failure of stress-induced down-regulation of Bcl-2 underlies resistance of senescent HDFs to apoptosis. [R11-2002-097-05-001-0]

Key words - apoptosis, senescence, fibroblast, Bcl-2, p-CREB, PP2A

[P-70]
Effect of Brain Function-related Calcium-binding Protein on Protein Citrullination by Peptidylarginine DEIMINASE 2

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Alteration of intracellular Ca2+ distribution and Ca2+-related proteins has a potent role of synaptic dysfunction and neuronal cell death in neurodegenerative diseases. Protein citrullination, a post-translational modification catalyzed by Ca2+-dependent peptidylarginine deiminases (PADs) appears as a potential biomarker for degenerative diseases and its involvement in neurodegenerative diseases has been reported. To investigate the expression and activity of PAD2 in neurodegenerative conditions, we used ME7-scrapie infected mice as a mouse model for prion disease. Our preliminary data showed that PAD2 immunoreactivity and its expression levels of mRNA and protein were significantly increased in scrapie-infected brains and PAD2 was mainly distributed in reactive astrocytes. Since PAD activity is regulated by intracellular Ca2+, we were interested in the effect of brain function-related calcium-binding proteins such as calsenilin and S100B on protein citrullination. To investigate whether these Ca2+-binding proteins can modulate the protein citrullination in prion disease, we examined the citrullinated proteins by PAD2 in the brains of calsenilin or S100B wild and knock-out mice after ME7 scrapie infection. In consistant with our previous data, the expression and the activity of PAD2 were significantly increased in the brains of scrapie-infected mice. Current studies are focused on the involvement of brain function-related calcium-binding proteins in protein citrullination during neurodegeneration and the identification and characterization of new substrate for PAD2. [This work was supported by the Ministry of Health and Welfare (A020007) and the Korea Research Foundation grants funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-003-200287)]

Key words - Prion, PAD2, Citrullination, Calsenilin, S100B

[P-71]
Impact Of Elder Abuse On State Anxiety

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The purpose of this study is to determine elder abuse and assess its impact on anxiety. A total of 345 Korean elderly males, aged 65 and above, participated in the study. Elder abuse(Seo, 2000) was used to assess the 10-item subscale for emotional abuse, 7-item subscale for financial abuse, and the 8-item subscale for neglect. State Anxiety(Spielberger, 1970) was used to assess the degree of State Anxiety. Data were analyzed using SPSS for Windows V 11.0. The relationship between elder abuse and State Anxiety was analyzed based on Pearson's correlation coefficient. Pearson's correlation results showed that State Anxiety significantly correlated with the participant's emotional abuse(r = 0.571, p < 0.01), financial abuse(r = 0.304, p < 0.01), neglect(r = 0.468, p < 0.01). Reports on emotional abuse, financial abuse, neglect and State Anxiety correlated positively with one another. Multiple regression analysis was performed to determine the variables predicting State Anxiety. The dependent variables was State Anxiety, while the independent variables were participant's emotional abuse, financial abuse, and neglect. The R² value for the State Anxiety...
was .417. Multiple regression analyses showed emotional abuse ($\beta = .498$, $p = 0.000$), financial abuse ($\beta = 0.310$, $p = 0.000$), and neglect ($\beta = -0.193$, $p = 0.001$) were significant predictors for State Anxiety.

**Key words** - Elder abuse, State Anxiety

**[P-72]**

**Relationship between Elder's Dependency and Self-esteem**

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This study seeks to identify the relationship between elder's dependency and self-esteem. The sample consisted of 200 Korean elderly (140 females and 60 males) aged 65 to 87 (mean age 68.7 ± 0.69). Data were collected according to demographic characteristics. Elder's Dependency (Ahn, 1999) of the 8-items subscale for physical dependency, 5-items subscales for psychological dependency and the 4-items subscale for social dependency were used. Self-esteem was measured using the Self-esteem scale (Rosenberg, 1965). Descriptive statistics were used for analyzing demographic data; t-test, ANOVA were used for comparing means of variables according to demographic data. The relationship between dependency and self-esteem was analyzed based on Pearson's correlation coefficient. The results are as follows: physical dependency was significantly different according to gender ($t = -5.22$, $p = 0.000$), age ($F = 9.59$, $p = 0.000$), education ($F = 5.34$, $p = 0.000$), marital status ($F = 6.86$, $p = 0.000$), health status ($F = 15.18$, $p = 0.000$). Social dependency was significantly different according to gender ($t = -4.92$, $p = 0.000$), age ($F = 23.61$, $p = 0.000$), education ($F = 5.72$, $p = 0.000$), marital status ($F = 7.29$, $p = 0.000$), and health status ($F = 16.03$, $p = 0.000$). Psychological dependency was significantly different according to gender ($t = -4.421$, $p = 0.000$), age ($F = 13.87$, $p = 0.000$), education ($F = 3.95$, $p = 0.000$), and health status ($F = 13.71$, $p = 0.000$). Self-esteem was significantly different according to age ($F = 3.91$, $p = 0.022$). Self-esteem significantly correlated with physical dependency ($r = -0.156$, $p = 0.000$), social dependency ($r = -0.149$, $p = 0.000$), and psychological dependency ($r = -0.259$, $p = 0.000$).

**Key words** - Dependency, Self-esteem

**[P-73]**

**Non-smoking Groups Consume Diets Higher in Brown Rice, Vitamin B2, Kimchi, and Vegetables, but No Differences were Shown in Plasma Vitamin C, a and Homocysteine in Seoul and Its Vicinity**

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As one of the leading developing countries, Korea was reported as having the highest number of heavy smokers in the world. Now, lung cancer is one of the leading causes of death in Korea due to heavy smoking and pollution. Although the smoking problem has taken on serious proportions, especially amongst young people, a few studies have ever been made of the differences in the nutritional patterns between smokers and non-smokers in Korea. The purpose of this study is to compare the dietary habits and nutritional patterns of smokers with non-smokers in terms of their intake of nutrients, plasma levels of antioxidant nutrients, cancer preventive factors, cardio-protective nutrients as well as dietary components contributing to life style and health. The data was collected from selected groups in Seoul and its vicinity. The subjects, aged 24 to 26 years old (n = 755) participated through answering questionnaires. The results showed that non-smokers had a higher intake of brown rice, grains, fruits, vegetables, and kimchi, but no differences were shown in plasma levels of vitamin C, A, and homocysteine, an independent risk factor for cardiovascular disease. In conclusion, there were significant differences in dietary habits and nutrient intake between the smoking and non-smoking groups. As a result it is to be recommended that educational programs be developed for smokers, in other to guide them into adopting better dietary habits, which is aimed at improving and maintaining their health. Also, to study the relationship between smoking status and the plasma levels of preventive factors for cardiovascular disease and cancer will be a fruitful area for additional study.

**Key words** - Smoking, brown Rice, Plasma Vit C, Vit A, homocysteine