慈 濟 大 學

99 年度校內研究成果

發表會手冊

教師暨博士生
研究成果發表期間：99 年 5 月
研究成果發表地點：慈濟大學第二、三教學研討室

研究生暨大學生
看板論文展覽日期：99 年 5 月 10 日~99 年 5 月 14 日
看板論文展覽地點：慈濟大學文化走廊

主辦單位：慈濟大學研發處

慈濟大學 99 年度學術研討會時程

【教師研究成果發表：整合型】

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/5 (三)	12:20~ 12:25	曾義雄 (總)	TCIRP 98003	鮑氏不動桿菌噬菌體之基礎與應用研究	第二教學研討室	微生物學科
	12:25~ 12:45	曾義雄	TCIRP 98003-01	Molecular biological study of *Acinetobacter baumannii phage* Abp53		微生物學科
	12:45~ 13:05	林念璁	TCIRP 98003-02	Molecular-level interactions between phages and their host *Acinetobacter baumannii*		微生物學科
	13:05~ 13:25	曾俊傑	TCIRP 98003-03	Application of *Acinetobacter baumannii* Specific Phages for Nosocomial Infection Control		公共衛生學系
5/7 (五)	12:20~ 12:25	曾國藩 (總)	TCIRP 98006	Mechanisms Underlying the Modulation of Dendritic Spines and Afferents in the Receiving and Output Neurons of the Cerebral Cortex Subjected to Compression	第二教學研討室	解剖學科
	12:25~ 12:45	曾國藩	TCIRP 98006-01	An Investigation of the Mechanisms Underlying the Compression-induced Dendritic Spine Retraction and Postsynaptic Receptor Remodeling of Cortical Pyramidal Neurons		解剖學科
	12:45~ 13:05	王曰然	TCIRP 98006-02	Modulation of Cortical Neuronal Dendritic Spines: Interactions between Compression, Cholinergic Innervations, Nerve Growth Factor and Estrogen		解剖學科
	13:05~ 13:25	劉培新	TCIRP 98006-03	Effects of Exogenous Nerve Growth Factor on Compression-induced Alterations of Thalamocortical Connections		解剖學科
5/10 (一)	12:20~ 12:25	陳俊堯 (總)	TCIRP 96003	Physiological Adaptation and Gene Regulation of *Vibrio* Species to Various Environmental Stress	第二教學研討室	生命科學系
	12:25~ 12:45	陳俊堯	TCIRP 96003-01	Physiological adaptation and gene regulation of *Vibrio* spp. to chemical and nutritional changes.		生命科學系
	12:45~ 13:05	林玲君	TCIRP 96003-02	Physiological adaptation and gene regulation of *Vibrio* spp. to oxidative stress and oxygen deprivation.		微生物學科
	13:05~ 13:25	余美萱	TCIRP 96003-03	Physiological adaptation and gene regulation of *Vibrio* spp. to temperature.		微生物學科
	13:25~ 13:45	林光慧	TCIRP 96003-04	Physiological adaptation and gene regulation of *Vibrio* spp. to pH variation.		微生物學科

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/11 (二)	12:20~ 12:25	羅時燕 (總)	TCIRP 96004	Structural Proteomics of Hepatitis C Virus.	第二教學研討室	醫學檢驗生物技術學系
	12:25~ 12:45	劉哲文	TCIRP 96004-01	Atomic force microscopy of hepatitis C virus proteins.		生化學科
	12:45~ 13:05	李惠春	TCIRP 96004-02	Spectroscopic Studies of Structural Proteins of HCV.		生化學科
	13:05~ 13:25	賴孟君	TCIRP 96004-04	A bioinformatic approach to study the viral entry and morphogenesis of HCV.		醫學檢驗生物技術學系
	13:25~ 13:45	羅時燕	TCIRP 96004-05	Study on the morphogenesis of hepatitis C virus.		醫學檢驗生物技術學系
5/12 (三)	12:20~ 12:25	徐雪瑩 (總)	TCIRP 96005	苦瓜對肝細胞病生理影響之研究	第二教學研討室	生命科學系
	12:25~ 12:45	徐雪瑩	TCIRP 96005-01	Investigation of molecular mechanism on anti-tumor effect of *Momordica charantia*.		生命科學系
	12:45~ 13:05	葉日弋	TCIRP 96005-04	A Study on the Antigluconeogenesis Activity of *Momordica charantia*.		家庭醫學科
	13:05~ 13:25	鄭靜明	TCIRP 96005-05	Isolation and characterization of terpenoid synthases and ribosome inactivating proteins from *Momordica charantia*.		生命科學系
5/13 (四)	12:20~ 12:25	彭致文 (總)	TCIRP 96006	Insight of the Molecular Model of EBV Latent Infection and Development of the Anti-EBV Strategies Using Potential Compounds Isolated from Green Tea and Other Natural Products.	第二教學研討室	生命科學系
	12:25~ 12:45	彭致文	TCIRP 96006-01	Investigation of the Transcription Machinery Mediated by EBV Nuclear Antigen 2 and Leader Protein (LP) and Development of High Throughput Assay Systems for Screening of Potential Anti-EBV Drugs Targeting to EBNA2 and EBNALP from Green Tea.		生命科學系
	12:45~ 13:05	林麗鳳	TCIRP 96006-02	Mechanistic insight into EBV nuclear antigen 1 mediated episomal maintenance and transcription activation and development of high throughput assay systems for screening of potential anti-EBV drugs targeting to EBNA1 from green tea.		生命科學系
	13:05~ 13:25	陳泓吉	TCIRP 96006-03	Mechanistic insight of cyclooxygenase-2 induction by latent membrane protein 1 in EBV associated cancers, and effects of green tea catechins on LMP1-associated signaling.		生命科學系

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/14 (五)	13:30~ 13:35	許木柱 (總)	TCIRP 96001	正向心理的發展與實踐：科際整合研究	第二教學研討室	人類發展學系
	13:35~ 13:55	何縕琪	TCIRP 96001-01	大學生品格長處、正向情緒與行為之關係與介入成效研究		教育研究所
	13:55~ 14:15	張景媛	TCIRP 96001-02	問題導向服務學習對師培生正向心理的影響		教育研究所
	14:15~ 14:35	陳畹蘭	TCIRP 96001-03	The role of positive emotions on cognition, cardiocascular reactivity of stress, and adjustment.		人類發展學系
	14:35~ 14:55	許木柱	TCIRP 96001-04	慈濟志工之正向心理研究		人類發展學系
5/14 (五)	15:00~ 15:05	張景媛 (總)	TCIRP 97002	兒童語文讀寫萌發之發展與教育應用	第二教學研討室	教育研究所
	15:05~ 15:25	張景媛	TCIRP97 002-01-P	大學教師專業學習社群之運作方式及其成效之研究		教育研究所
	15:25~ 15:45	何芮瑤	TCIRP97 002-02-P	幼兒讀寫萌發發展中的文字概念初探		兒童發展與家庭教育系
	15:45~ 16:05	施淑娟	TCIRP97 002-03-P	繪本教學對幼兒的語言發展之研究		兒童發展與家庭教育系
	16:05~ 16:25	鄭雅莉	TCIRP97 002-04-P	繪本閱讀方案對發展遲緩兒同儕溝通成效研究		兒童發展與家庭教育系
	16:25~ 16:45	李雪菱	TCIRP97 002-05-P	創意看圖作文對兒童寫作表現之影響		兒童發展與家庭教育系
	16:45~ 17:05	羅廷瑛	TCIRP97 002-06-P	原住民子女「傳說、科學、圖畫詩」親子創作之研究		兒童發展與家庭教育系
	17:05~ 17:25	胡美智	TCIRP97 002-07-P	新住民子女子親共讀下對讀寫萌發影響之研究		兒童發展與家庭教育系
5/17 (一)	12:20~ 12:25	詹銘煥 (總)	TCIRP 97001	The Basic Studies of Methamphetamine in addition, Toxicity and Treatment	第二教學研討室	藥理學科
	12:25~ 12:45	詹銘煥	TCIRP 97001-01	Therapeutic effects of GDNF inducing agents on methamphetamine-induced neuropsychological impairment		藥理學科
	12:45~ 13:05	郭昶志	TCIRP 97001-02	Effect of methamphetamine on the neuronal activities of the forebrain nuclei		生理學科
	13:05~ 13:25	袁宗凡	TCIRP 97001-03	The mechanism of stress-promoted response to methamphetamine		生理學科
	13:25~ 13:45	林恂恂	TCIRP 97001-04	The Central Mechanisms of Cardiovascular Toxicity Induced by Methamphetamine		生理學科

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/19 (三)	12:20~ 12:25	張新侯 (總)	TCIRP 98001	登革非結構性蛋白 NS1 及抗 NS1 蛋白所誘發之交叉反應性抗體的病理作用	第二教學研討室	分子生物暨人類遺傳學系
	12:25~ 12:45	張新侯	TCIRP 98001-01	Dengue virus non-structural protein 1 (NS1), anti-NS1 antibody and the immuocomplex-induced pathogenic response		分子生物暨人類遺傳學系
	12:45~ 13:05	蘇淑惠	TCIRP 98001-02	Protective Roles of Chinese Herbs in TNFα/DV-NS1 Antibodies-mediated Damages of Endothelial Cells		醫學研究所
	13:05~ 13:25	王士廉	TCIRP 98001-03	Mechanisms of Immune Suppression Post Immunization of Dengue Virus Non-structural Protein One		免疫學科
	13:25~ 13:45	吳文陞	TCIRP 98001-04	The signal mechnisms for pathological effect of platelet and endothelial cell triggered by NS1 Ab and NS1 protein		醫學檢驗生物技術學系
	13:45~ 14:05	孫德珊	TCIRP 98001-05	Role of dengue viral NS1 protein and anti-NS1 antibody on the differentiation and cellular function of the progenitor cells of platelet and monocyte		分子生物暨人類遺傳學系
5/20 (四)	12:20~ 12:25	楊昆達 (總)	TCIRP 98002	運動引發生理病理變化之機轉探討	第二教學研討室	生理學科
	12:25~ 12:45	楊昆達	TCIRP 98002-01	Exercise alters $Ca2^{+}$ and H^{+} handling in rat cardiomyocytes		生理學科
	12:45~ 13:05	孫宗伯	TCIRP 98002-02	The combined effects of exercise and hyperbaric oxygenation on cardiovascular neural regulation in diabetic individuals		外科
	13:05~ 13:25	賴靜蓉	TCIRP 98002-03	Effects of Exercise on Hypersensitivity of Pulmonary C Fibers in Rats		生理學科
	13:25~ 13:45	謝坤叡	TCIRP 98002-04	Effects of exercise on circadian-clock genes expression in digestive systems during time zone travel (jet lag)		生理學科
5/24 (一)	12:20~ 12:25	李茹萍 (總)	TCIRP 98004	由生理與心理議題探討影響酒精中毒之預防與治療趨勢：從生理表現到分子機轉	第二教學研討室	護理學系
	12:25~ 12:45	李茹萍	TCIRP 98004-01	以清醒鼠模式探討抗憂鬱劑合併酒精中毒的生理作用與炎症反應		護理學系
	12:45~ 13:05	徐邦治	TCIRP 98004-02	清醒鼠模式下探討酒精在不同模式急性腎衰竭的作用		內科
	13:05~ 13:25	怡懋・蘇米	TCIRP 98004-03	探討急慢性酒精中毒合併大失血情況下之合理輸液策略		護理學系
	13:25~ 13:45	楊福麟	TCIRP 98004-04	Dexmedetomidine 影響急性酒精中毒合併失血性休克的反應機制		外科

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/25 (二)	12:20~ 12:25	李哲夫 (總)	TCIRP 98005	脂肪酸與神經血管功能	第二教學研討室	生命科學系
	12:25~ 12:45	李哲夫	TCIRP 98005-01	Palmitic acid methyl ester (PAME) is the perivascular adipose tissue-derived relaxing factor (PVATRF)		生命科學系
	12:45~ 13:05	李原傑	TCIRP 98005-02	The mechanisms of action of methyl palmitate as a retina-derived relaxing factor		眼及視覺學科
	13:05~ 13:25	賴志嘉	TCIRP 98005-03	Role of Fatty Acids in Central Sympathetic Control of Cardiovascular Function		藥理學科
	13:25~ 13:45	林家禾	TCIRP 98005-04	Role of Fatty Acid Methyl Esters in the Regulation of the Hippocampus and Amygdala Functions		藥理學科
	13:45~ 14:05	賴滄海	TCIRP 98005-05	Investigation of the PAME Formation by Mass Spectrometry		醫學檢驗生物技術學系

【教師研究成果發表：個人型】

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/14 (五)	09:00~ 09:20	江錦玲	TCMRC-P-96005	Nursing Guide for Parkinson's Disease: Developing and Testing Multimedia VCD and Printed Booklet	第二教學研討室	護理學系
	09:20~ 09:40	劉嘉卿	TCMRC-P-97003	台灣歸化植物的風險評估		生命科學系
	09:40~ 10:00	邱鐵雄	TCMRC-P-97005	Mechanisms of bladder cancer inhibition by thalidomide and TrkB siRNA		藥理學科
	10:00~ 10:20	沈祖望	TCMRC-P-97006	The longitudinal studies of cardiovascular disease in end-stage renal disease （ESRD） undergoing long-term dialysis		醫學資訊學系
	14:00~ 14:20	胡馨丹	TCMRC-P-96001	中國現代小說的「寫實」與「擬寫實」問題研究—以 1918 至 1949 年間的小說為研究對象	第三教學研討室	東方語文學系
	14:20~ 14:40	張堯欽	TCMRC-P-96010	被壓抑的記憶—卡內提自傳中的猶太認同問題		英美語文學系
	14:40~ 15:00	許智香	TCMRC-P-96015	經典閱讀讀書會提升大學生閱讀理解及批判思考之研究		教育研究所
	15:00~ 15:20	潘靖瑛	TCMRC-P-96016	多元閱讀策略教學提升大學生英文閱讀理解成就及批判思考能力之研究		教育研究所
	15:20~ 15:40	廖心玫	TCMRC-P-97002	Connecting Principled Information with Worked Examples: Can'T Do or Don'T Do?		人類發展學系

【醫學研究所博士生研究成果發表】

日期：99年5月15日(星期六)

地點	時　間	姓　名	口　頭　發　表　題　目	論文指導老師
E704	08:30~ 09:00	耿念慈	Interaction of Ethanol with NMDA Receptor Antagonists on Spinal NMDA-induced Pressor Responses in Rats	賴志嘉
	09:00~ 09:30	邱鴻義	Cocaine- and Amphetamine-regulated Transcript (CART) Peptide Activates ERK Pathways via NMDA Receptors in Rat Spinal Cord Dorsal Horn in an Age-Dependent Manner	賴志嘉
	09:30~ 10:00	曾慧玲	Changes of Circadian-clock and Cytokine Genes Expression in the Liver and Spleen by Streptozotocin-induced Diabetic Rats	謝坤叡
	10:00~ 10:30	曾子玲	Post-treatment with an Active Component of Scutellariae Radix (Tchi-2) Reduces LPS-Induced Acute Lung Injuries and Fatality	李哲夫
	10:40~ 11:10	劉家瑞	Bubbles and Bowel Sounds	陳幸一
	11:10~ 11:40	陳穎信	The Molecular Mechanism of Amiodarone, an Anti-Arrhythmia Drug, to Cause the Defective Formation of Cardiac Valves During Zebrafish Embryogenesis	胡勝川
	11:40~ 12:10	廖家信	Pharmacologically Enhanced Imaging of ^{18}F-FDG PET for Evaluation of Parkinson's Disease in Rats.	郭重雄
E717	08:30~ 09:00	邱勝軍	The Induction of Endoplasmic Reticulum Stress Protein GADD153/CHOP Expression by *n*-butylidenephthalide as Pharmaceuticals on Prostate Cancer Therapy	馮清榮
	09:00~ 09:30	黃欣儀	Molecular Mechanisms Underlying Urocortin-Induced Restriction of Proliferation in Neural Stem Cells	郭重雄
	09:30~ 10:00	劉大瑋	FGF Signaling in Intestinal Cell Differentiation	王文柄
	10:00~ 10:30	陳沅孟	The Role of Parkin in Cell Progression	莊育裡
	10:40~ 11:10	林士淳	No Need, No Demand, No Cost? – Establishing Individual Accountability to Contain Medical Expenditures	曾英傑
	11:10~ 11:40	林碧莉	Factors Associated with the Avoidance Behavior of Household Environmental Tobacco Smoke among Pre-school Children'S Mothers	徐祥明
	11:40~ 12:10	江慧珠	Experiencing the Body of Hemodialysis Patients	許木柱

目　　錄

【教師成果發表論文摘要：整合型】

【教師成果發表論文摘要：個人型】

【博士生 口頭報告論文摘要】

【研究生　看板論文摘要－醫學院】

【研究生　看板論文摘要－生命科學院】

【大學生　看板論文摘要】

O01

(計畫名稱：鮑氏不動桿菌噬菌體之基礎與應用研究)

Bacteriophages of *Acinetobacter baumannii*-from Basic Research to Application

Yi-Hsiung Tseng[1*], Nien-Tsung Lin[1], Chun-Chieh Tseng[2]

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曾義雄[1*]、林念璁[1]、曾俊傑[2]

慈濟大學微免暨分子醫學研究所[1]

慈濟大學公共衛生學系暨研究所[2]

Acinetobacter baumannii is an important gram-negative opportunistic pathogen causing nosocomial infections. Pandrug resistant *A. baumannii* (PDRAB) isolates have been found to increase in Taiwan since its first report in 1998 on the island. Directed towards phage therapy using *A. baumannii* phages, this integrated research project consists of three sub-projects: 1) Isolation and characterization of the lytic phage Abp53 of *A.baumannii*, 2) Molecular-level interactions between phages and their host *Acinetobacter baumannii*, and 3) Application of *A. baumannii* specific phages for nosocomial infection control, which are carried out by Yi-Hsiung Tseng, Nien-Tsung Lin, and Chun-Chieh Tseng, respectively. The results obtained so far are as follows.

Subproject 1 has completed characterization of phage Abp53, including morphology, growth of the phage and sequencing of a phage fragment of about 10 kb. Bioinformatics analysis indicates that phage-homologous regions are present in the host genome. Occurrence of these Abp53-homologous sequences in the host suggests that horizontal gene transfer may have occurred between the phage and its host. However, presence of homologous DNA regions in host disfavors the use of Abp53 as the therapeutic agent in control of *A. baumannii* infections. The results will be presented in a poster at 110th General Meeting of American Society for Microbiology, May 23-27, 2010.

Subproject 2 isolated 10 phages from wastewater and designated as ϕAB1 to ϕAB9 and ϕAB11. Electron microscopy revealed that ϕAB1–7 and ϕAB9 possess an icosahedral head and a short tail that are typical of the family *Podoviridae*. Phages ϕAB8 and ϕAB11 have a contractile tail and an icosahedral head, a morphology characteristic of members of the family *Myoviridae*. They all have short life cycles and large burst sizes. In addition, these phages can collectively lyse 89% (113/127) of the isolates, among which 97.3% (110/113) are MDRAB. These properties suggest potential use of the phages in phage therapy. The results have been accepted for publication in the Research in Microbiology.

Subproject 3 has evaluated effects of chemical and physical factors on phage stability. The phage was found to 1) be stable at low temperatures (-20^{o}C, -80 ^{o}C and 4 ^{o}C) for 8 weeks, 2) lose infectivity at acidic pHs (pH 2 and pH 4) after an 11-week incubation, and 3) be stable in chloroform (0.5% to 2%) for 3 weeks. These results are useful for development of the agent for biocontrol of *A. baumannii*

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O02
(計畫名稱：Molecular biological study of Acinetobacter baumannii phage Abp53)

Isolation and Characterization of the Lytic Phage Abp53 of *Acinetobacter baumannii*

Chia-Ni Lee[1], Yung-Chieh Fu[1], Shu-Fen Weng[1] and Yi-Hsiung Tseng[2*]Institute of Molecular Biology, National Chung Hsing University, Taichung[1], and Institute of Microbiology Immunology and Molecular Medicine[2], Tzu-Chi University, Hualien, Taiwan
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A lytic phage specifically infecting *A. baumannii* was isolated from a sputum sample collected and designated as Abp53. This phage was able to infect 8 PDRAB strains, causing complete lysis, and 4 other PDRAB strains giving turbid clearing zones. This phage, possessed a double-stranded DNA genome of 95 kb, had an isometric head (60 nm in diameter) and a contractile tail (143-nm long, 14-nm wide) with tail fibers, resembling members of the *Myoviridae* A1 family. It exhibited a latent period of about 10 min and an average burst size of 100 PFU/cell. More than 20 protein bands were visualized upon separation of the virion proteins in gel, with the most abundant 47-kDa band predicted to be the major capsid protein. Sequence analysis of a 10-kb fragment from its genome revealed 8 genes, ORF251-ORF163-ORF313-ORF336-ORF1176-RF309-ORF132-ORF201, encoding maturation protease, protein of no similarity, phage related protein, 3 tail components, and 2 hypothetical proteins, respectively. The 3 tail proteins (ORF336, ORF1176 and ORF30) shared 26, 21 and 53% identity with 3 discontinuous regions within protein ACICU_02717 from *A. baumannii* strain ACICU, while hypothetical proteins ORF132 and ORF201 were similar to ACICU_02164 and ACICU_02163, respectively, which were 597 kb apart from ACICU_02717 on the bacterial chromosome. Occurrence of these Abp53-homologous sequences in the host suggests that horizontal gene transfer may have occurred between the phage and its host. However, presence of homologous DNA regions in host disfavors the use Abp53 as the therapeutic agent in control of *A. baumannii* infections. The largest gene *orf1176* encoded 1176 amino acid long product almost possesses 34% of the 10-kb DNA fragment. Interestingly, Domain duplication was found in ORF1176 (1176 aa) and its similar proteins encoded by *ACICU_02717* and *AB57_1286* from *A. baumannii* strains ACICU and AB0057 as well as *gp21* of *Klebsiella oxytoca* phage phiKO2. Compared with ORF1176 individually, the deduced proteins ACICU_02717 (3702 aa), AB57_1286 (3449 aa) and gp21 (3433 aa) revealed 5, 1 and 6 similar regions (about 300 aa) with ORF1176, respectively, with similarities from 36% to 43%. These similar regions almost arranged one by one in ACICU_02717 and gp21, hence, we assume that the increasing tail length may attribute to tail domain duplication. Furthermore, we also found that three similar regions of 72-amino acid with 43% identities exit inside ORF1176.

O03

(計畫名稱：Molecular-level interactions between phages and their host Acinetobacter baumannii)

Isolation and Characterization of Bacteriophages of *Acinetobacter baumannii*

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Multidrug-resistant strains of *Acinetobacter baumannii* (MDRAB) are increasingly being reported worldwide. Bacteriophage therapy is a potential alternative treatment for MDR bacterial infections. Although *A. baumannii* infection has been experimentally treated with phages, no MDRAB-specific phage has been characterized. In first-year study, we used 125 clinical isolates of *A. baumannii* as indicator hosts for the isolation of lytic phages. Altogether, 10 phages were isolated from wastewater and designated as ϕAB1 to ϕAB9 and ϕAB11. To classify the phages into morphotype-specific groups, phage particles were examined by transmission electron microscopy. Phages ϕAB1–7 and ϕAB9 possessed an icosahedral head and a short tail that are typical of the family *Podoviridae*. Phages ϕAB8 and ϕAB11 showed a contractile tail and an icosahedral head, a morphology characteristic of members of the family *Myoviridae*. To analyze the host range, the phage lysates were spotted separately onto the lawns formed by *A. baumannii* clinical isolates (125 from our collection plus 2 type strains, ATCC17978 and ATCC19606), 1 *A. calcoaceticus* (ATCC33305), 10 *E. coli*, 6 *K. pneumoniae*, and 3 *P. aeruginosa* strains. The results indicated that phage ϕAB1, ϕAB2, ϕAB3, ϕAB4, ϕAB5, ϕAB6, ϕAB7, ϕAB8, ϕAB9, and ϕAB11 specifically infected 25, 25, 4, 20, 28, 4, 29, 9, 21, and 39 isolates of *A. baumannii*, respectively; collectively lysed 89% (113/127) of the isolates, but not any of the other bacteria tested. It is worth mentioning that 97.3% (110/113) of the susceptible isolates were MDRAB. To visualize the virion proteins, purified phage particles were subjected to SDS-PAGE separation, respectively. At least 7 distinct protein bands, with molecular masses ranging from 20 to 110 kDa, were visualized after the gels were stained with Coomassie brilliant blue. The most abundant one in the gel was a 35 kDa protein, most likely the major coat protein of ϕAB1–7 and ϕAB9. In addition, ϕAB1–7 and ϕAB9 possessed the similar properties such as rapid adsorption (>99% adsorbed in 8 min), a short latent period (<10 min), and a large burst size (ca. 200 PFU per infected cell), indicating that these phages may be good candidates as a therapeutic/disinfectant agent to control nosocomial infections caused by MDRAB.

O04

Application of *Acinetobacter baumannii* Specific Phages for Nosocomial Infection Control

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The rise of antibiotic-resistant agents was one of the most significant public health problems from 20th century. In Taiwan, multidrug-resistant bacteria can cause serious disease and has been an important public health problem now. Therefore, interest is growing in controlling the spread of these antibiotic-resistant bacteria through environmental disinfection methods. Up to now, bacteriophages have been demonstrated to be capable to inactivate multidrug-resistant bacteria on inanimate objects/surfaces. Therefore, application of multi-drug resistant bacteriophages for nosocomial control is a potential method. The purpose of this study is to investigate the stability of the phage which against to *Acinetobacter baumannii* in the prepared solution and to evaluate the biocontrol effectiveness of bacteriophages for nosocomial control.

In the first year, the chemical and physical factors that influencing the phage survive in detergent-disinfectants were evaluated in a batch-based study. The evaluated chemical factors involved in this study included phage stability at various pHs and chemicals. In addition, the stability of phage at different levels of storage temperature was also investigated. Our results demonstrated the phage was stable at low temperature (-20 °C, -80 °C and 4 °C) for 8 weeks, but the phage stability at acid pHs (pH 2 and pH 4) would be lost after 11 weeks cultivation. Moreover, the phage was observed to be stable in the concentration of 0.5% and 2% chloroform suspension for 3 weeks. In our study, the importance of temperature and pHs to the stability of the phage might be determined, certain of the stability experiments are still under investigation.

O05

Mechanisms Underlying the Modulation of Dendritic Spines and Afferents in the Receiving and Output Neurons of the Cerebral Cortex Subjected to Compression

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Head trauma and brain tumor can affect cerebral cortex via mechanical as well as chemical factors. In practice, compression is often left untreated for some duration since asymptomatic meningioma is often observed for months before definite treatment. Today, although we have better understanding of the pathology of meingiomas, the effects of compression on neurons of the underlying cortex remain largely unexplored.

Using a rat model of focal cortical compression of epidural bead implantation we found mechanical compression causes the underlying cortical pyramidal neurons, the major output neurons of the cortex, to quickly remodel their dendritic arbors and retrieve dendritic spines. However, how does it affect the receiving neurons of the underlying cortex and the associated thalamocortical connection and the mechanisms of dendritic spine retraction and its interaction with other spine-regulating factors such as gonadal hormone and cholinergic innervations remains unknown? With this in mind, in the first year of this integrated project we explored (1) the molecular mechanisms underlying dendritic spine retraction by first developing the methodology to isolate synapse-associated postsynaptic fraction and subsequent studies of the changes of the postsynaptic glutamate receptor subunit expressions and it regulatory mechanisms; (2) effects of cholinergic innervations, gonadal hormone, aging and compression on the regulation of cortical neuronal dendritic spine densities; (3) regulation of the expressions of excitatory, AMPA and NMDA glutamate subunits, and inhibitory, $GABA_A$ and $GABA_B$ receptor subunits in layer IV stellate cells, the receiving neurons of the cortex. Details of the findings and their significance are presented in each report. It's obvious that the effect of compression on output and receiving neurons of cerebral cortex that we revealed will better our understandings on the pathophysiology of cortex affected by an expanding mass such as tumor and providing hints to clinical decision-making regarding patient treatments.

O06

An Investigation of the Mechanisms Underlying the Compression-induced Dendritic Spine Retraction and Postsynaptic Receptor Remodeling of Cortical Pyramidal Neurons

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Cerebral cortex is often compressed in trauma or diseases, however how does compression affect underlying cortical neurons remains largely unexplored. Using an epidural bead implantation model in rats we found epidural compression distorted the dendrites of underlying neurons instantly and active dendritic modeling was accomplished in 3 days so that dendrites were effectively shortened. Dendritic spines on these neurons were reduced within 1 day of compression and failed to recover following decompression suggesting long-term functional influences via downregulation of excitatory connection. We subsequently explored the molecular changes associated with the reduction of dendritic spines.

First we explored the isolation of the postsynaptic components associated with excitatory synapses. We have worked out a protocol to prepare synaptoneurosomes from the studied cortex and had verified the purity of this fraction with both electron microscopy and immunoblotting of the expression of proteins specific to the postsynaptic structures. We are now exploring the molecular changes underlying the retraction of the dendritic spines. Preliminary data show that compression changed the level of postsynaptic glutamate receptor subunits and their activation appeared to underlie the retrieval of dendritic spines. We are now in the process of finding out whether compression alters the phosphorylation machinery associated with the postsynaptic structures and the polymerization of actin. These findings are expected to shed light on the molecular mechanisms responsible for the quick modulation of cortical neuronal dendritic spines and in addition offer thoughts on how this may be prevented in clinical cases of compression. The fast onset of these pathophysiological molecular changes demonstrated are expected to prompt clinicians to reassess whether to postpone treatment until syndromes occur following compression.

O07

Modulation of Cortical Neuronal Dendritic Spines: Interactions between Compression, Cholinergic Innervations, Nerve Growth Factor and Estrogen

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Using a rat epidural bead implantation model, we found lately that compression reduced the dendritic spines on cortical pyramidal neurons in days and decompression failed to restore this. Since estrogen has been implicated in the regulation of dendritic spines on hippocampal neurons, we studied whether dendritic spines on cortical pyramidal neurons changed during estrous cycle and found that their densities were cyclically regulated during different phases of the estrous cycle, with higher density corresponding to phase of high serum level of estrogen. Ovariectomy reduced the dendritic spines on these neurons and exogenous estrogen restored their densities. These demonstrate that estrogen supports cortical neuronal dendritic spines. Since decreased level of estrogen is a hallmark of aging and the latter is often accompanied by dementia and linked to the loss of cholinergic neurons in the basal forebrain that project to the cerebral cortex. We subsequently investigate the role of basal forebrain cholinergic neurons on the maintenance of cortical dendritic spines. Knowing that these cholinergic neurons express NGF receptors, we first tested the effect of NGF and found it increased the dendritic spines on cortical pyramidal neurons following decompression and this appears to mediate through basal forebrain cholinergic neurons as lesion of the latter knocked down the effect of NGF. We are now exploring whether epidural compression further reduces the dendritic spines as well as dendritic arbors of cortical pyramidal neurons of ovariectomized animals to find out how estrogen and the physical force of compression interact with each other on the regulation of cortical neurons. Results obtained are expected to shed light on how and whether dendritic spines on cortical neurons are regulated independently by blood level of estrogen and the force of mechanical compression.

O08

Effects of Exogenous Nerve Growth Factor on Compression-induced Alterations of Thalamocortical Connections

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Compression of cerebral cortex is usually caused by brain tumor, epidural or subdural hematoma, intracranial hemorrhage, or head trauma. This can cause diverse clinical manifestations such as headache, nausea, vomiting, seizure, focal neurological symptoms and even mortality. We recently reported that physical compression of primary somatosensory cortex (S1) leads to short-term sensory deficits of the affected rats, evidenced by behavioral and electrophysiological tests (Lin et al., in press). These findings prompted us to investigate whether S1 compression elicits sensory deficit through disturbing the thalamocortical connections. After S1 compression, male SD rats were grouped and allowed to survive for 1 day, 3 days, 1 week, 2 weeks, and 3 months. Using immunohistochemical labeling, we found that the expressions of glutamate receptor subunits glutamate receptor 1 (GluR1) and *N*-methyl-D-aspartate receptor 1 (NMDAR1) were slightly decreased in layer IV stellate cells, the main thalamocortical receiving neurons, at 1 day and 3 days following cortical compression. However, the expressions of GluR2 and GluR4 were unaltered at any given time point. In contrast, the expressions of γ-aminobutyric acid A ($GABA_A$) and $GABA_B$ receptor subunits were increased at 1 day and 3 days following S1 compression. These findings suggest that the mechanism underlying S1 compression-induced sensory impairment may involve the regulation of the thalamocortical connections. In addition, we used the Golgi stain technique to illustrate the dendritic arbor of layer IV stellate neurons, and carried out the immunohistochemistry and the anterograde tracing to reveal the thalamocortical fibers in layer IV of S1. Analyses of these results are in progress.

O09

Physiological Adaptation and Gene Regulation of *Vibrio* Species to Various Environmental Stress

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Vibrios adapt themselves to changing environments with various life styles, being free-living, parasitic or symbiotic to multicellular hosts. The aim of this project is to identify the mechanisms vibrios use to cope with various environmental stress.

In subproject 1, we demonstrated vibrios had more mutations when incubated under various chronic stress using *Vibrio vulnificus* model. Cell density and duration of stress are two important factors to influence phenotypic variation in starvation. Many starvation survivors have *rpoS* mutations, being either nonsense or deletion mutant. Although acute stress cross protection experiment did not show overlap in physiological response to different environmental stress, we showed that cells evolved under one stress had increased resistance to other stress, suggesting chronic stress can facilitate the development of cells resistant to multiple stress.

In subproject 2, we examined further the role of VPA0768, which is one of the two katG homologs found in *V. parahaemolyticus* genome. Using zymogram we demonstrated VPA0768 is a bifunctional catalase. Its expression was increased in starved cells, which correlated to increased survival when starved cell received H_2O_2 treatment. Growth inhibition in hyper- and hypo-osmotic stress was observed in VPA0768 mutant. These results indicated that VPA0768 can protect *V. parahaemolyticus* from various environmental stress.

In subproject 3, the roles of two sigma factors, *rpoS* and *rpoE*, were studied. *RpoS* mutant, *rpoE* mutant and double mutant of *V. parahaemolyticus* were created. The *rpoE* and double mutants, but not *rpoS* mutant, have reduced growth rates. When tested using stationary phase cells, *rpoS* mutant is more susceptible to starvation, oxidative and acid stress, but *rpoE* mutant has only increased susceptibility to heat stress. When tested using exponential phase cells, *rpoS* and *rpoE* mutants were more susceptible to acid and heat stress, respectively.

In subproject 4, we applied proteomic and RT-PCR approaches to identify the genes needed to combat acid stress in *V. parahaemolyticus*. Two genes, *csdA* and *rafH*, were found to have increased expression, but expression of *rpoS*, the sigma factor considered major regulator of stress, remained unchanged. In a screen of Tn*10*-derived *V. parahaemolyticus* mutant library, 6 mutants showed reduced acid resistance and failed to survive at pH 5. However these genes have not been reported to be involved in acid resistance and may represent novel mechanism for acid stress survival.

We have shown that vibrios have some unique features in responses to various compared to the *Escherichia coli* model. Since *Vibrio* is considered a marine environment-adapted heterotrophic genus, the meaning and evolution of stress-related physiological response should be considered in appropriate natural environments. We expect to pursuit that in future studies.

O10

(計畫名稱：Physiological adaptation and gene regulation of *Vibrio* spp. to chemical and nutritional changes)

Evolution and Phenotypic Variation of *Vibrio vulnificus* after Various Longterm Chronic Stress Conditions

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Vibrio vulnificus is an important human and fish pathogen. Studying their response to stress may help us understand how this pathogen persists in the environment. In this study we examined the response of *V. vulnificus* to starvation.

High proportion of cells survived for long-term starvation demonstrated altered hemolytic, proteolytic activities, and motility. RAPD analysis of 13-months survivors indicates that the survivors are genetically heterogeneous, even if they are phenotypically similar. Surviving populations from duplicate experiments receiving identical inoculum were different in genetic composition, indicating that survivors evolved independently in each experiment, not persisting members of the inoculum population. Bacteria starved for 2 months under low cell density remained high viability and wild type phenotype, therefore high cell density is required for mutation and survival during prolonged starvation. Re-examine the phenotypic variation in cells starved under various densities showed that variation did increase with cell density and duration of starvation. We found that many starvation survivors have *rpoS* mutation, being either has nonsense mutation or deletion. This may lead to the observed increased competitiveness when being co-cultured with wild type strain.

We have examined the acute stress effect with cross-protection experiments and found that stress combination generally caused higher mortality compared to single stress. We then cultured the cells under stress condition for prolonged period of time, allow them to evolve and fix the genetic change, before test them for other stress condition. Similar to what was found in prolonged starvation, phenotypic variation was significant in other stress treatments. We also found increased resistance to other stress in the evolved population. This result suggests that chronic but persistent stress may facilitate mutation. Although the acute stress result suggests that generally no cross protection between two types of stress, our chronic stress result indicates that some genetic modification could provide benefit to different stress.

O11

(計畫名稱：Physiological adaptation and gene regulation of *Vibrio* spp. to oxidative Stress and oxygen deprivation)

Characterization of a Bifunctional Catalase in *Vibrio parahaemyolitcus* and Its Role in Response to Acid, Starvation, Osmotic and Oxidative Stress

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Vibrio parahaemyolitcus is an important foodborne pathogen. The genomic study revealed there are two copies of bifunctional catalases (VPA0768 and VPA0453) exist in *V. parahemolyticus* genome. In this study, we constructed the VPA0768 mutant and assessed the influences that might arise by this genetic defect. Zymogram assay showed one of three detectable catalase activities was significantly impaired, which was designated as Group C, by comparing with electromobility pattern of wild type. In addition, the group C catalase was also confirmed as a bifunctional catalase (KatG homolog) by double staining. Under acid stress, Group C catalase can be induced promptly and confer a protection role. To test the survival of exponential and stationary phase cells of wild type and VPA0768 mutant in starvation condition, cells were treated with artificial sea water for 24 hr. The protein extracts of starved cells showed the other pattern exhibiting high level of catalase activity in zymogram gel, suggesting it might be involved in responding to starvation stress. Besides, the location of electromobility of this pattern was closer to that of the Group C catalase. The starved cells were also subjected to hydrogen peroxide and confirmed a role in protecting cells from second stress. Moreover, the growth inhibition of VPA0768 mutant was observed when cells were cultured in hyperosmotic or hypoosmotic conditions. Taken together, we suggest VPA0768 has a potential role in protecting cells from several environmental stresses.

O12

(計畫名稱：Physiological adaptation and gene regulation of *Vibrio* spp to temperature)

Response of *Vibrio parahaemolyticus* to Environmental Stresses: Role of RpoS and RpoE in Stress Survival

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Vibrio parahaemolyticus is a halophilic gram-negative bacterium that usually inhabits in coastal and estuarine waters. In Taiwan and Japan, it is the important seafood-borne pathogen. Patients usually caused acute gastroenteritis by consumption of seafood contaminated by *V. parahaemolyticus*. Since they are widely distributed in marine environment and exhibit several life types, such as free-living organisms, symbioists of marine animals, and pathogens of human and marine animals. In response to changes in environmental conditions, they need to continually modulate gene expression by alternative sigma factors. In this study, we aim to clarify the role of two alternative sigma factors (RpoS and RpoE) in environmental fitness of *V. parahaemolyticus*. It has been known that RpoS is responsible for stationary-phase and stress response gene expression and RpoE, an Extracytoplasmic function (ECF) sigma factor, is involved in bacterial adaptation of envelop stress. Compared to the growth curve of wild type, the *rpoS* mutant showed similar growth pattern, and both of the *rpoE* mutant and the *rpoS rpoE* double mutant showed the reduced growth rate, but they reached similar optical density with wild type in late exponential and stationary phases. In the outer membrane protein profile of *rpoE* mutant, two distinct bands were abolished compared to that of wild type. In stationary-phase cells, *rpoS* mutants were more sensitive to oxidative, starvation, and acid stress conditions, but *rpoE* mutants were only sensitive to heat stress. In contrast, *rpoS* and *rpoE* is not required for exponential-phase cells to survive under conditions of cold, oxidative stress and starvation, but they are important for acid stress and heat stress, respectively. Besides, a novel gene (VP2094) encoding a molybdenum cofactor biosynthesis protein that are related to cold resistance was identified by the approach of mutant library screening.

O13

(計畫名稱：Physiological adaptation and gene regulation of *Vibrio* spp to pH variation)

Physiological Adaptation and Gene Regulation of *Vibrio* spp. to pH Fluctuation

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Most *Vibrio* species are natural inhabitants of estuaries and sea water, and can cause food borne disease and wound infection. In response to variety of environmental conditions, *Vibrio* spp. should equip with the capacity to sense, and adapt to environmental fluctuations. Low-salinity challenge, oxidative stress, temperature changes are the most popular topics to be addressed in *Vibrio* species. In this study, we focus on the effect of pH fluctuation on *Vibrio* species. First of all, proteomic approach was applied to study the protein expression profiles in bacterial cells cultured under different pH. Beside the proteomics approach, we have examined the RNA transcription profiles for *oxyR, ompR* and *rpoS* after acid treatment, to identify the possible regulatory genes of *Vibrio* species under pH fluctuation. Results demonstrated that *csdA* and *rafH* showed differential transcripts while in counter acid treatment under different temperature. However a globe regulator, *rpoS*, revealed no significant difference after acid stress. In combination of proteomics and RNA expression patterns, it will be very helpful for us to understand gene regulatory mechanism of *Vibrio* spp. while encounter stress environment. Mutant library of *Vibrio* species were constructed by using a Tn*10*-dervied transposon. After screening of at least 3,000 transposon insertion mutants of *V. parahaemolyticus*, we had isolated 6 mutants lost the ability to survive in the mild acid condition (pH5). These mutants showed different survival ability encounter different stress condition and demonstrated different biofilm forming ability. We will focus on several novel genes which were identified from mutant library to understand regulatory pathway of stress response of *V. parahaemolyticus*. Results of this study might provide further insight into the interaction of *Vibrio* species to environmental fluctuation.

O14

Structural Proteomics of Hepatitis C Virus

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Infection with hepatitis C virus (HCV) can cause severe liver diseases. The HCV carrier population in the world is about 2%. Neither an effective treatment for chronic HCV infection nor a vaccine to prevent HCV infection is available right now. At present, the treatment of HCV-infected patients is using alpha-interferon. Only about 50% of HCV-infected patients are responsive to the treatment of alpha-interferon (plus ribavirin). Therefore, the developments of vaccines and new anti-HCV drugs are urgent. Structural information of HCV proteins will help vaccine development and search for anti-viral agents.

It takes a multi-discipline collaboration to study the structural proteomics of HCV. During the past two and half years, we discussed research information and shared the research materials: Projects 1 and 3 are conducting the study on the HCV core protein structure using E.M. and AFM; Projects 1, 2, 3 and 5 are conducting the study on the lipid raft structure for HCV replication; Projects 2 and 4 are conducting the study on the fusion between HCV envelope proteins and cell membrane; Projects 4 and 5 are conducting the study on HCV NS3 and RdRp. (Note: we did not continue project 3 in the third year, 20090801-20100731). Through this collaboration, we will understand more regarding structural information of HCV proteins.

O15

(計畫名稱：Atomic force microscopy of hepatitis C virus proteins)

Structural Analysis on Hepatitis C Virus Core Assembly and Core Protein-lipid Interactions.

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Hepatitis C virus (HCV) has become one of the major concerns in public health. Despite the seriousness of the problems caused by this virus, the HCV is among the least understood viruses to date. In order to have a better understanding to this virus, it is crucial to have the structural and dynamic information of the virus and the virus proteins. Because of the small size of the virus, it is traditionally very difficult to image the virus and obtain direct information of the virus proteins on virus surface and within. A naval approach for this purpose is therefore required.

Atomic Force Microscope (AFM), a research tool in nanotechnology, has become increasing important in biological and biomedical research. The sample preparations for the AFM imaging are relatively simple, no harsh physical or chemical treatments are required. Thus, the disruption of the samples during the preparations is minimised. Among all, the most important feature of the AFM is the fact that it permits the observation of samples in buffer solutions, so that biological samples can be studied at nanometre scales in their native and functional states under their physiological conditions, allowing not only their structure, but also their dynamics to be analysed. This study applied the AFM to study the HCV core assembly and the importance of the hydrophobic C terminal of the core protein in the assembly process. As the virus core in mature virus is covered with a layer of phospholipids obtained from the host cells, the correct structural conformation of the core protein might also affected by the interactions between the core protein and lipids. This study also investigated the structural changes of the core protein upon interaction with the lipid DMPC by means of biophysical techniques such as fluorescence wavelength shifts and circular dichroism (CD). According to the AFM data, the virus cores assembled by full length core protein 1-191 had a diametre of approximately 38 nm which is very close to the size of *Flaviviridae* viruses and is significantly larger than the cores assembled by core protein 1-116 with hydrophobic tail truncated (approximately 22 nm in diametre) indicating that the hydrophobic tail of the protein did contribute to the assembly of the virus cores. CD data revealed a 2.4 fold increase in α-helix and 25% decrease in β-sheet structures in the core protein upon interacting with the DMPC meaning that the protein-lipid interactions also played an important role in the core protein structuring in virus particles.

O16

Spectroscopic Studies of Structural Proteins of HCV

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Hepatitis C virus (HCV) is an enveloped, positive-stranded RNA virus classified in the Hepacivirus genus of the Flaviviridae family. The HCV genome encodes structural and nonstructural proteins. The nonstructural proteins and HCV RNA assemble in the endoplasmic reticulum membrane to form complex structure, termed HCV replicons. HCV replicons reside in detergent-insoluble subcellular domains or lipid raft. However, the morphology of HCV replicons and how their structures are affected by the replicon-related genes are not clear. In this project, atomic force microscopy (AFM) is used to characterize the HCV sub-genomic replicon structures. The membrane flotation assay was used to separate membrane fractions and western blotting was used to identify fractions containing the HCV sub-genomic replicons. Disk-like structures were observed by AFM from fraction 2 samples derived from HCVsub-genomic replicons but not those from parental Huh-7 cells. Circle-like structures were seen with TEM from the same fraction. Antibodies with gold-particles were used to confirm that these disk-like structures were HCV sub-genomic replicons. F2 samples from cells treated with interferon-α to reduce the expression of HCV sub-genomic replicons showed no disk-like structures by AFM. Finally, knock-down caveolin-2, a lipid raft-related gene, showed a different disk-morphology, indicating that the disk-like structures occurred in HCV sub-genomic replicons depended on caveolin-2.

O17

(計畫名稱：A bioinformatic approach to study the viral entry and morphogenesis of HCV)

Enrich the Detection of Sequence Similarities of HCV Proteins by Using Structural-based Substitution Matrix

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Sequence similarity search against existing databases has been a widely used approach to derive hypothesis concerning relatives and functions of a sequence. The performance of a search algorithm partly depends on the employed substitution matrix which scores the alignment of one residue against another. Various substitution matrices for protein similarity search, such as PAM and BLOSUM were 20×20 symmetric matrices derived from sequence alignments of related proteins. However, in sequence comparison, using a common matrix for all proteins regardless if they have known protein structures excludes the benefit might gain from the structural information.

Here, a combination of the sequence search tool, BLAST and the position-based structural substitution matrix is proposed. The environment of a residue in a protein structure is described by its secondary structure, solvent accessibility, and hydrogen bond forming pattern. Using the approach is hoped to detect the similar sequences in the twilight zone with sequence identity lower than 25%. The application on HCV proteins with solved structures will be presented.

O18

(計畫名稱：Study on the morphogenesis of hepatitis C virus)

Interactions between Hepatitis C Virus NS3 and Two Cellular Proteins

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Hepatitis C virus (HCV) is etiologically involved in liver cirrhosis, hepatic cancer, and B-cell lymphomas. Molecular mechanisms regarding HCV pathogenesis are not well understood. HCV NS3 protease domain but not helicase domain was found to interact with cytosolic 5'(3')-deoxyribonucleotidase (dNT-1) in yeast two-hybrid screening. Interaction between HCV NS3 and dNT-1 proteins was further demonstrated by IP-WB and confocal analysis in the cultured cells. Binding domains of these two domains were also determined using yeast two-hybrid system. Cellular dNT-1 activity was repressed by HCV NS3 protein in the transiently-transfected system. Furthermore, HCV would repress the dNT-1 activity but not down-regulate its expression while dNT-1 has no effect on the HCV replication and protein processing. Thus, our results suggest that HCV reduces the dNT-1 activity through NS3 and in turn causes diseases.

Genes differentially expressed (over-expressed or down-regulated) in HuH7 cells with or without HCV sub-genomic replicon were identified by dd-RT-PCR. Genes over-expressed in HCV replicon cells could be the factors facilitating HCV replication while down-regulated genes could be the factors repressing the HCV replication. Heparin cofactor II (HCII) identified by ddRT-PCR was down-regulated in HCV replicon cells. To determine whether HCII represses HCV replication in the HCV replicon systems, both loss-of-function (knockdown of HC II) and gain-of-function (over-expression of HC II) approaches were used. Our results showed HC II could indeed inhibit HCV replication. Furthermore, heparin could reverse the suppressive effect of HC II on HCV replication. HCII represses the HCV replication possibly through interacting with HCV NS3 protein. The binding domains of HCII and HCV NS3 proteins were also determined by yeast two-hybrid system. In conclusion, our studies suggested HC II could interact with HCV NS3 protein and in turn represses HCV replication. Further studies to characterize the mechanisms how HCV down-regulates the expression of HC II are needed.

O19

(計畫名稱：苦瓜對肝細胞病生理影響之研究)

The Pathophysiological Effects on Liver Cells by *Momordica charantia.*

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Liver diseases are considered to be an important health problem that result in serious health problems and exhaust lots of medical resources. It is becoming increasingly evident that chronic viral or metabolic liver diseases are at risk for the development of hepatocellular carcinoma. Obesity, a rapidly growing health issue, is a risk factor for cardiovascular, metabolic, neoplastic and sleep-disorder complications. Numerous efforts have been directed at the development of effective liver-specific therapeutic strategies by natural products. Diets rich in bioactive phytochemicals are used for both prevention and treatment of liver diseases recently. *Momordica charantia* was reported to have some biomedical activities such as anti-inflammation, anti-virus, anti-tumor and anti-diabetes. Our recent results showed that extracts of *Momordica charantia* inhibit the growth of Hep G2 and the viral containing Hep G2.2.15 cells. It also showed the anti-hepatitis B viral effects and anti-diabetic effects on 3T3/L1 adipocytes. Results of this study indicated that the effects of *Momordica charantia* are fluctant, the components extracted from the stem and leaves are more bioactive than others. There are several purified compounds isolated from what species of *Momordica charantia* showed bioactivities in previous investigations. Among all these compounds, MC-1, MC-9 and MC-10 are newly identified ones, MF-5, MS-1 and MS-2, didn't show inhibitory effects on anti-tumor effects. Both MC-1 and MF-D1 showed significant anti-tumor activities on Hep G2 and MDA231 tumor cells, respectively. MC-9 and MC-10 can protect normal liver FL83B cells from Cu^{2+}-induced cell damages. For the delayed purification of *Momordica charantia*, the amount of these compounds are not enough to undertake the anti-diabetic study on animals. In the third year, we have also established a possible system for exploring the molecular mechanism of NIH/3T3 adipogenesis and improved the method to induce obesity and insulin resistance in mice using a liquid diet during the second year. In addition, a yeast-constructed screen system was also establishing for the synthesis of terpenoid functional genes that can be used to analyze the second metabolites of *Momordica charantia.*

O20

(計畫名稱：Investigation of molecular mechanism on anti-tumor effect of Momordica charantia)

Investigation of Anti-tumor Mechanism on Hepatoma by *Momordica charantia*

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Momordica charantia, commonly called bitter gourd, was recently reported to have some biomedical activities such as anti-inflammation, anti-diabetes, anti-tummor and anti-virus effects. Our previous investigations revealed that ethanol crude extracts of *Momordica charantia* showed significantly effects on inhibiting the growth of some tumor cells. Hepatocellular carcinoma cell line is observed to be one of the tumor cell lines sensitive to the extracts of *Momordica charantia*.. To clarify the anti-tumor potential of *Momordica charantia* on hepatocellular carcinoma, we used Hep G2, Hep 3B, MSG2 and HepG2.2.15 cells to comparatively evaluate the cell growth inhibition of *Momordica charantia* at doses from 0.1 to 2mg/ml. Among all the crude extracts, fruits of *Momordica charantia* extracted by ethanol was found to be more effective on inhibiting the growth of hepatocellular carcinoma cells. According to different species, the effective doses used for inhibiting the cell growth are different. It showed from the results that the *Momordica charantia*-induced apoptosis in hepatocellular carcinoma were p53 and caspases-independent. To realize the anti-tumor mechanism of *Momordica charantia* on hepatocellular carcinoma, we further used five compounds purified from the stems and seeds, MC-1, MC-9, MC-10, MF-5 and MS-2, to investigate the possible anti-tumor components on Hep G2 cells. Among these five components, both MC-1 and MS-2, especially for MC-1 showed an inhibited cell growth on Hep G2 cells and a contrary effect was found by the other compounds. The expression of proteins indicated MC-1 might be one of the possible components in *Momordica charantia* resulted in cell apoptosis. To further investigate the possible biological roles of other components purified from *Momordica charantia*, MC-9 and MC-10 were used to evaluate their anti-oxidative activities by FL83B cells in this study. The expression of JNK, p38 MAPK, Erk and Akt indicated that MC-9 and MC-10 may play a modulated role in regulating copper-induced oxidative stress in hepatocytes.

O21

A Study on the Antigluconeogenesis Activity of *Momordica charantia*

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We found two crude extracts that could promote adipogenesis and enhance insulin stimulation and two other extracts that could inhibit adipogenesis during the first year of the work. The plan to purify the active principle in thes crude extracts was stalled because our collaborator could not produce enough extracts for animal study. We explored the molecular mechanism of NIH/3T3 adipogenesis and improved the method to induce obesity and insulin resistance in mice using a liquid diet during the second year. We continued the work in the third year.

The mice were fed with high sucrose liquid diet and regular chow ad libitum. The calorie intake was about 50% higher in mice fed with this diet. The average body weight was about 30% higher than the control group after 8-12 weeks. Fasting blood glucose and oral glucose tolerance test showed these animals were diabetic. The abnormality disappeared completely one month after they were fed with regular chow only. This animal model of obesity and insulin resistance is easy to induce using a non-invasive and non-toxic treatment and at a low cost. It serves as a platform to screen for in vivo hypoglycemic activity. In addition, this model provides an opportunity to understand when impaired glucose tolerance induced by overeating is irreversible and search for biochemical markers predicting the irreversibility of impaired glucose disposing ability.

O22

(計畫名稱：Isolation and characterization of terpenoid synthases and ribosome inactivating proteins from Momordica charantia)

Genetic Engineering of Plant Terpenoid Biosynthesis in *Saccharomyces cerevisiae*

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Genetic engineering of secondary metabolites in *Saccharomyces cerevisiae* could be used to study novel enzymes for several purposes: it provides insights into the evolution of these specific biosynthesis pathways and into the evolutionary relationships between fungi and plants; it provides attractive taxonomic markers for distinguishing between closely related plant species and between different batches of herbal preparations; and it may also provide bioactive compounds of medicinal importance.

Twelve and fourteen lines of *Saccharomyces cerevisiae* haploid mutants expressing the nourseothricin (Nat) or hygromycin (Hyg) resistance, respectively were constructed from sporulation of diploid yeasts carrying plasmid pRS416-erg7.

To directly select the terpenoid cyclase from plant cDNA libraries, a gateway plasmid pYES-52-Leu was constructed. Growing the haploid yeasts on leucine minus media containing 5-fluoroorotic acid (5-FOA), the yeast cell will excise plasmid pRS416-erg7 and maintain plasmid pYES-52-Leu with a proper terpenoid cyclase. The URA3 gene of pRS416-erg7 will convert 5-FOA into 5-fluorouracil that toxically arrests the yeast growth, and the survived plant terpenoid cyclase of pYES-52-Leu will complement the defect mechanism of yeast 2, 3-oxidosqualene cyclization.

This system could be generally applied as a direct and rapid screening tool for positive selection of terpenid cyclase from plant cDNA libraries.

O23

Insight of the Molecular Model of EBV Latent Infection and Development of the Anti-EBV Strategies Using Potential Compounds Isolated from Green Tea and Other Natural Products

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Epstein-Barr Virus (EBV) immortalizes human B lymphocyte to prevent cell death thus establishes the permanent infection of EBV in hosts. EBNA2 is essential for such an immortalization process and was further implicated in activation of EBV promoters and transcription of some cellular genes. EBNALP plays a major role in co-activation with EBNA2 and is also critical for EBV transformation. EBNA1 and one region of the viral genome, the latent origin of plasmid replication (oriP), were known necessary and sufficient for replication of the viral plasmid. When oriP is supplied with EBNA1 *in trans* will provide efficient duplication, partitioning and maintenance of plasmids bearing it. Latent membrane protein 1 (LMP1) appears to be the major transforming protein of EBV among all EBV latent proteins expressed during latency. LMP1 acts as a constitutively active receptor-like molecule that does not need a ligand and can induce a variety of cellular genes that enhance cell survival as well as adhesive, invasive, and angiogenic potential.

To have a better understanding of the molecular model of EBV latent infection and develop strategies for screening potential anti-EBV drugs, we organize a research team to perform this three-year research project. The three joined faculties are from department of life science and housed in the same floor. All of the three principle investigators have strong backgrounds in molecular biology and biochemistry to meet the requirements for pursuing this integrated research project.

O24

Investigation of the Transcription Machinery Mediated by EBV Nuclear Antigen 2 and Leader Protein (LP) and Development of High Throughput Assay Systems for Screening of Potential Anti-EBV Drugs Targeting to EBNA2 and EBNALP from Green Tea.

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Transcription of specific EBV promoters and cellular genes driven by Epstein-Barr Virus (EBV) nuclear antigen 2 (EBNA2) and leader protein (LP) is essential for immortalization of B lymphocytes by EBV infection. The interaction between EBNALP and p53 was documented previously and the interactions of p53 and EBNALP, p53 and EBNA2 were further emphasized in our study, suggesting p53 may contribute in cell defense to EBV infection. Among all three types of EBV latency infected cells, the expression levels of p53 were extremely low or barely detectable in the EBV infected cells in comparison with the phenotypes of EBV negative cells. Our results revealed that ectopically expressed p53 strongly down-regulates LMP1 promoter activity activated by EBNA2 and co-activated by EBNALP. Our current data suggested that p53 can recruit corepressor sin3A to repress EBNA2 mediated transcription which was implicated in cell protection from EBV infection.

The co-chaperone BAG family proteins, BAG3, is up-regulated in EBV latency infected cells at both mRNA and protein levels. In particular, our results demonstrated that EBNA3A can activate BAG3 promoter reporters but neither do other EBV latent proteins. Interestingly, we found over-expression of BAG3 are able to down-regulate EBNALP co-activation with EBNA2 while over-expression of BAG3 alone possess very limited up-regulating effects on EBNA2 response to the LMP1 promoter reporter. Our results revealed that the conserved BAG and PXXP domains of BAG3 are essential for maintaining of the repressing activity to EBNALP, whereas WW domain and serine-rich are dispensable. The interaction between EBNALP and BAG3 is implicated in down-regulation of EBNALP co-activation. In addition, a cell based LMP1 promoter reporter assay system is generated for screening cellular factors targeting to EBNA2.

O25

(計畫名稱：Mechanistic insight into EBV nuclear antigen 1 mediated episomal maintenance and transcription activation and development of high throughput assay systems for screening of potential anti-EBV drugs targeting to EBNA1 from green tea)

Mechanistic Insight into EBV Nuclear Antigen 1 mediated Transcription Activation and Development of High Throughput Assay Systems for Screening Potential Anti-EBV Drugs Targeting to EBNA1

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The Epstein-Barr Virus (EBV) nuclear antigen 1 (EBNA1) is the most prevalent EBNAs and can be detected in all EBV associated diseases. EBNA1 enables the persistence of the episomal viral genome, which is required for the initiation of DNA replication from the EBV latent origin (oriP) and the stable segregation of the viral genomes during cell division. In our preliminary results obtained from proteomic analyses, both nucleolin and ribosomal protein L4 (RL4) were identified as EBNA1 associated cellular proteins, and with the successful gain-of-function analyses we demonstrated that over-expression of either RL4 or nucleolin was sufficient to potentiate ENNA1 mediated transcription. The significant functional domains contributed to the interaction of each protein with EBNA1 were also addressed. Based on the functional features of EBNA1 in EBV infection cycle, we have generated two modes of cell based high throughput assay systems for screening of the potential anti-EBV drugs in the context of B lymphocytes. Our results demonstrated that two EBNA1/oriP based reporter cell lines can produce consistent and reliable measurements. Our preliminary results demonstrated that EGCG has a strong negative affect on EBNA1 activation of oriP based episomal reporter, suggesting EGCG possesses a good anti-EBV activity.

Taken together, we aim to uncover the molecular mechanism of EBNA1 mediated transcription from episome and DNA synthesis in EBV infection cycle and these results will lead us to find new potential drugs for treating EBV associated diseases. Furthermore, the cell based high throughput screening systems will appear as powerful tools to screen the potential anti-EBV drugs or natural compounds in the near future.

O26

(計畫名稱：Mechanistic insight of cyclooxygenase-2 induction by latent membrane protein 1 in EBV associated cancers, and effects of green tea catechins on LMP1-associated signaling)

Mechanistic Insight of Cyclooxygenase-2 induced by Latent Membrane 1 in EBV Associated Cancers and Development of High Throughput Assay Systems for Screening of Potential Anti-EBV Drugs from Compounds Isolated from Green Tea

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Among all 9 EBV latent proteins expressed during latency latent membrane protein 1 (LMP1) appeared to be the major transforming protein of EBV, thus far, LMP1 appeared as a potential drug target for EBV-associated malignancy. The C-terminal of LMP1 has been shown to be responsible for transducing LMP1 signals to activate NF-κB which is implicated in transformation outgrowth of B lymphoblasts upon EBV infection. In addition, NF-κB has been shown to play a critical role in regulation of Cox-2 expression and EGCG, the major catechin isolated from green tea, has been shown to inhibit Cox-2 activity through blocking NF-κB activation. During transient transfection, the expression of full length LMP1 in HEK293T cells induced the activation of NF-κB-luciferase reporter (3XκBL). Strikingly, our results demonstrated EGCG potentially causes a strong reduction of NF-κB activation mediated by LMP1 through increasing of LMP1 protein degradation. In addition, we found LMP1 can also induce formation of autophage. To facilitate the study of the mechanism by which LMP1 induced formation of autophage, a GFP-LC3 stably expressed cell line was generated and specific localizing pattern of GFP-LC3 can be visualized upon LMP1 expression is induced. In addition, a specific GFP shift was recorded using flow cytometry once autophage formation was induced with serum strvation. Further more, a cell based LMP1 induced NFκB reporter system was successfully generated. This high throughput system is further utilized for screening potential anti-EBV drugs targeting to LMP1/ NFκB signaling pathway. Our current data suggested that EGCG can target to LMP1 and appears as an anti-EBV drug to treat EBV associated malignancies.

O27

正向心理的發展與實踐：科際整合研究

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本研究從發展面、學習面、復原力及感恩心四個向度，探討正向心理特質建構的歷程與影響，研究方法包含量化研究與質性研究，研究對象包括大學生及成人。在大學生方面，主要是瞭解課程學習、服務學習及有關的訓練方案，對大學生及師培生的正向心理特質之影響。最後，則以壓力復原力的基礎研究，以及慈濟人投入環保志工的過程與主觀經驗，瞭解正向心理的轉化歷程與影響。

子計畫一：

研究目的：以大學生為對象，透過課程設計，了解學生接受教學後的正向情緒與正向行為表現。

研究方法：課程方案、量表施測、質性資料檢測。

研究發現：修課同學在樂觀、感恩、希望感、幸福感、快樂等正向情緒顯著優於對照組，且憂鬱感受顯著低於對照組，顯現接受教學後，實驗組比對照組更為正向，且能減低負向情緒。

子計畫二：

研究目的：探討「問題導向服務學習方案」對師資生的感恩心與利他行為的影響。

研究方法：約 20 名慈濟大學師資培育的大學生，使用感恩心量表及訪問資料。

研究發現：「問題導向服務學習方案」中，師資生紀錄在集訓時的省思、在觀摩教學上的省思以及最難忘與感恩的事。服務學習方案施行後，師資生在後續進行服務時，很快地就能規劃出自然科學體驗營的服務學習活動，也規劃出學習策略的服務學習活動。

子計畫三：

研究目的:探討壓力事件與情緒之關聯,哪些心理特質在面臨壓力時可產生正面反應，而最初的正向心理水平是否可預測未來的正向情緒與心理安適感。

研究方法：預定 120 位東部地區大學生志願參加，使用半結構問卷、量表施測。

研究發現：預定 6 月間可獲得初步分析結果。

子計畫四：

研究目的：探討為數眾多的慈濟環保志工，在何種情境下加入環保志工行列，如何發展出正向的情緒與認知（如包容與感恩），從而衍生出正向行為（如助人行為與利他精神）。

研究方法：以台灣北中南東四區的慈濟志工為對象，透過深度訪談與參與觀察，在各區總計訪問 80 位環保志工。

研究發現：根據訪問資料之初步分析，發現：(1)擔任環保志工有數種不同的因緣，包括工作環境的啟發、因鄰里關係的接觸、接觸慈濟月刊或靜思語等，但最終都因為受到證嚴法師「用鼓掌的雙手救地球」的感召；(2)在接觸過程中，多數志工認為比較會產生感恩、包容等正向的心理特質；(3)主要的影響在於對環境的觀念、人或生命的價值、人際互動關係，以及比較願意付出（助人）等。這些發現大致符合本計畫提出的概念架構。

O28

大學生品格長處、正向情緒與行為之關係與介入成效研究

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正向心理學以研究個人長處、建立正面情緒和美德為重點，旨在幫助個人找到內在的心理能量，隨時作為對抗挫折的緩衝與掌控逆境，使得個體在遇到困難時不會輕易落入憂鬱的狀態中，並藉由發揮長處，重新思考可以達成的目標，同時實踐關懷行動，開展生命正向的經驗。本研究以大學生為對象，除編製正向心理量表，並在大學開設課程，了解學生接受教學後的正向情緒與正向行為表現，第三年為正式課程的介入研究。

研究者以第二年探索性課程的實施為基礎，進行課程設計的修正：一、在理論概念上，除延續「樂觀」、「長處美德」、「希望感」、與「感恩」外，增加「幸福感」與「挫折容忍力」的學習。二、為強化大學生的實踐力和學習效能感，除延續「感恩」與「利他服務」行動外，將個人長處實踐改為「自我管理學習」方案設計與實踐。三、在感恩實踐上，指定以母親節或畢業學長姐為對象，設計創意感恩行動。四、設計學習單，引導學生在實踐行動上能更系統化的規畫與記錄。目前課程正持續進行中，學生的學習表現以探索性教學加以說明。

在探索性教學中，研究者以「大學生生活態度量表」為工具，並選擇對照組進行前後測差異比較，結果發現修課同學在樂觀、感恩、希望感、幸福感、快樂等正向情緒顯著優於對照組，且憂鬱感受顯著低於對照組，顯現接受教學後，實驗組比對照組更為正向，且能減低負向情緒。從質性的文件資料中發現，藉由正向人物的報告，學生學習到反省自身盲點、積極面對當下、勇敢迎向挑戰、涵養利他精神、以及培養實踐行動等面向。修課學生中，僅有 2 位修讀教育學程和 2 位人醫社的同學經常性的參與服務學習，因而本課程鼓勵進行 4 到 6 小時的服務，許多同學除於服務後感到快樂外，也對服務對象（一般國小、國中資源班、啟智班、偏遠學校學生）有更深一層的認識。在個人長處實踐上，學生依據 VIA 量表的分析結果，自選長處進行兩週的實踐，由於是規定作業，學生都表示完成了許多「不可能的任務」，更深刻體會「設定目標」能引導行動。不過，學生也懷疑自己未來是否仍有毅力持續堅持。本研究發現正向心理學的介入教學方案能有效引導大學生正向情緒的發展，但在正向行為表現上，建議未來可設計自我管理學習建構大學生以目標為導向的學習策略，並從實踐中強化自我效能感。

關鍵字：大學生、正向心理學、正向情緒、長處美德

O29

問題導向服務學習對師培生正向心理(感恩心)的影響~以慈濟大學師培生進行偏遠地區原住民學生課業輔導為例

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本子計畫主要的目的有四項：一是瞭解偏遠地區原住民學生課業學習困難的因素及大學師資生進行課業輔導服務學習的動機；二是探究「問題導向服務學習方案」的實施方法，師資生遇到的各項問題，以及解決問題的方法；三是分析「問題導向服務學習方案」對偏遠地區原住民學生課業學習上產生的影響與成效；四是省思「問題導向服務學習方案」中，師資生在感恩心上所產生的轉變及後續進行服務的情形。本研究對象為慈濟大學師資培育的大學生，共約 20 名。使用的工具包括：感恩心量表、問題導向服務學習方案、新生入學測驗、段考測驗卷、省思札記、原住民課業輔導教學方案、原住民學生學習表現、課業輔導滿意度調查表及訪談大綱等。資料分析包括質性的方析與量化資料的處理。第一年研究結果包括：1、部分偏遠地區原住民學生到學校的心態不佳，造成學習動機低落； 2、偏遠地區原住民學生家庭問題造成學生心態不平衡，需要有專任輔導老師進行長期的心理諮商與輔導；3、偏遠地區教師教學品質不一，用心教學的教師沒有成就感，長期下來教學熱誠逐漸消退；4、引進外界相關資源協助教師改善教學方法，宜運用對話式形成性評量改善學生學習表現，並促進教師專業成長。第二年研究結果包括：1、「問題導向服務學習」方案的設計原則包括：瞭解原住民學生的特性、加強師培生試教的訓練、師培生自主性的進行課程設計、與督導共同討論教材的適切性、實施課業輔導並依教學觀摩的結果予以修改、師培生紀錄每次感恩的事情並與大家分享：2、師培生省思個人在服務中成長的情形：看到原住民學生課業進步時感受到服務的意義與價值、在課程設計方面較能考量原住民學生的特性加以設計、在教學方法方面會運用多元的策略進行活動、在行政方面瞭解服務中的人際溝通與行政處理也是很重要的事、從服務中感受到要做好一件事是需要許多人的幫助。第三年由於經驗的累積，大部分均由有經驗的師資生帶領團隊進行「問題導向服務學習」，研究結果包括：1、問題導向服務學習分為六個步驟：分組、呈現問題與連結問題、建立學習結構發展學習議題、訪查問題、成果或表現及結論與省思，過程三和四是一個不斷循環的歷程。2、「問題導向服務學習方案」對偏遠地區原住民學生英語學習表現和數學學習表現的成果良好。學生接受「創意英語教學方案」，故可了解學生在接受教學後其後測得分高於前測得分，並存在顯著差異。「數學成就測驗」前測總分的影響排除後，共變數分析的結果為：，表示經過實驗處理後，兩組學生在「數學成就測驗」的後測總分存在顯著差異。3、「問題導向服務學習方案」中，師資生紀錄在集訓時的省思、在觀摩教學上的省思以及最難忘與感恩的事。4、「問題導向服務學習方案」後，師資生在後續進行服務時，很快地就能規劃出自然科學體驗營的服務學習活動，也規劃出學習策略的服務學習活動。

關鍵詞：問題導向服務學習、正向心理學、感恩心

O30

(計畫名稱：The role of positive emotions on cognition, cardiovascular reactivity of stress, and adjustment.)

A Prospective Study of Positive Emotions, Resilience, and Stress.

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Objective: Results of empirical studies on positive emotion concluded that positive emotions facilitate coping with adversity, undo the negative effects of stress, and promote psychological well-being. However, several questions remain to be further discussed. One major question is that most studies employed cross-sectional instead of diary approach to measure emotion and stress; therefore, the precise link between stressful events and emotions could not be investigated. Secondly, what psychological traits influence individual's capacity to maintain positive emotion when facing stressful event. Finally, whether initial levels of positive emotions predict future positive emotions and psychological well-being. The current study adopts a longitudinal design to address these questions.

Methods：Approximately 120 participants will be recruited from two universities in eastern county of Taiwan from March to mid-June in 2010. They are recruited in a variety of ways, including announcement in 2 Introduction to Psychology classes, and fliers posted at dorms and PTT Bulletin Board System. For inclusion in the study, participant has to meet the following criteria (a) age between 18 and 25 years, (b) college student, and (c) be able to fill out daily Web questionnaires for 14 days. An individual session is scheduled on campus for each participant. The main feature of the study include a brief semi-structured interview in which background information including age, living situation, and history of health will be obtained for the purposes of describing the characteristics of the research sample. In addition, the following measures will be obtained: State-Trait Anxiety Inventory; Beck Depression Inventory-II (BDI-II); Ego Resilience-scale; Positive and Negative Affect Schedule; and Perceived Stress Scale. Participants will be given website address containing emotion and stress questionnaires to be completed and instructed to begin filling them out in the evening on the day of meeting, and continue for another 13 consecutive days. Ten weeks following completing of the prospective daily emotion and stress reports, participants will be scheduled an individual appointment and asked to fill out BDI-II and health related questionnaire.

Results and Discussion: Theoretical and clinical implications of this study will be in-depth discussed after completing the data collection by the end of June.

O31

慈濟志工之正向心理研究

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本研究運用人類學的深度訪談法，探討為數眾多的慈濟環保志工，在何種情境下加入環保志工行列，如何發展出正向的情緒與認知（如包容與感恩），從而衍生出正向行為（如助人行為與利他精神）。本計畫以台灣北中南東四區的慈濟志工為對象，透過深度訪談與參與觀察，在各區總計訪問 80 位環保志工，最終目的在透過台灣本土資料，驗證並建構正向心理學的理論，以彰顯慈濟志工行為的學術意義。根據訪問資料之初步分析，發現：(1)擔任環保志工有數種不同的因緣，包括工作環境的啟發、因鄰里關係的接觸、接觸慈濟月刊或靜思語等，但最終都因為受到證嚴法師「用鼓掌的雙手救地球」的感召；(2)在接觸過程中，多數志工認為比較會產生感恩、包容等正向的心理特質；(3)主要的影響在於對環境的觀念、人或生命的價值、人際互動關係，以及比較願意付出（助人）等。這些發現大致符合本計畫提出的概念架構。

O32

兒童語文讀寫萌發之發展與教育應用
(子計畫一：大學教師專業學習社群之運作方式及其成效之研究)

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本研究目的為：一、瞭解「大學教師專業學習社群」對教師進行兒童讀寫萌發相關研究的影響；二、分析台灣幼兒家庭語文文化及幼兒正式書寫前的文字概念；三、研究幼兒繪本教學對其語言讀寫發展之影響；四、探討繪本閱讀方案對發展遲緩兒同儕溝通之影響；五、分析創意看圖作文對兒童寫作表現之影響；六、研究新住民子女在子親共讀下，其讀寫能力改變的情形；七、探究原住民親子共學下，學童在語文創作表現的情形。本研究主要採用行動研究的方法，針對上述問題進行初步的瞭解。使用的研究工具包括：幼兒畢保德圖畫詞彙測驗、幼兒文字覺察測驗、幼兒教師訪談、觀察紀錄表與教師省思札記等。研究結果發現：大學教師專業學習社群對研究的幫助，主要是提供研究設計上的建議，釐清各子計畫的研究目的與研究方法；台灣幼兒的書寫發展過程明顯的和西方拼音文字社會的幼兒不同；繪本教學能引起發展遲緩幼兒團體課程的注意力，也提昇一般幼兒和發展遲緩幼兒的專注力；創意看圖作文教學中的多元表達與多元評估策略,提升兒童語文能力;親子共學科學課程對原住民學童科學讀寫的仿作能力有所影響；新住民子女子親共讀對啟發幼兒聽、說、讀、寫能力有所幫助。

關鍵詞：語文萌發

O33

幼兒讀寫萌發發展中的文字概念初探

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近二十年來，幼兒的讀寫發展研究有驚人的發現。在 1930 年代，成熟論的學者們認為兒童在六歲前無法學會讀和寫; 接著,1960 年代的讀寫準備(reading readiness)取向，認為幼兒可以藉著階段性的準備課程，一步步的學會讀和寫。但是，1970 年代後，愈來愈多的幼兒發展研究顯示:幼兒的讀寫學習從出生就已經開始了。1986 年 Teale & Sulzby(1986)統合了相關的研究發現後，提出了讀寫萌發理論(emergent literacy)，強調幼兒的聽、說、讀、寫能力從出生就開始發展且是同時發展，而不是順序發展。這理論在歐美的拼音文字系統得到支持，但卻尚未在以圖形文字為主的中國文字被驗證。以台灣目前的幼兒讀寫學習現況來看，幼兒在學前階段雖不正式學習讀寫，但卻被鼓勵認字並學習寫自己的名字以及筆劃較少的字。本研究的主要目的便在觀察台灣幼兒在正式學習寫字之前的文字概念。

本研究以花蓮地區 67 位二至六歲的幼兒為主。研究工具包括: 幼兒名字書寫及辨認、幼兒畢保德圖畫詞彙測驗、幼兒文字覺察測驗、幼兒教師訪談、以及幼兒園教室語文教學觀察。研究資料經過質、量並行分析後發現:

1. 台灣幼兒隨著年紀的增長，對文字的形態概念愈加僵化。對三歲幼兒而言，サン(日文)、שָׁמַשׁ(希伯來文)、สปส (印度文)、□□ □□ 都可以是文字。但對五歲的幼童而言，若不是看起來像中文、英文或韓文，就不是文字，例如: الشمس حرارة (阿拉伯文) 被認為太過凌亂所以不是文字，而 **ดวง อาทิตย์** (泰文)則因為看起來像英文，所以是文字。文字概念會隨著對週遭印刷的接觸而定型。
2. 台灣幼兒園極力教導幼兒認字希望幼兒能盡快學會自己閱讀，但本研究顯示，台灣幼兒對於教育部所編的最常見的 20 個中文字的認識非常貧乏。由此可以大膽推論，台灣幼兒的閱讀仍須靠師長父母的多方協助。
3. 台灣有七成的四歲幼兒可以從班級名冊中指出自己的名字，但是將幼兒的名字拆開分散在教育部所編的最常見的 20 個中文字中，僅有三分之一能分別指認出自己的名字。由此可見，四歲幼兒是靠著字型來學習認字的。
4. 台灣幼兒能清楚的指認出自己的名字大約是在五歲之後。三個名字中，姓是幼兒最容易辨識，也是最早會寫的。
5. 台灣幼兒要等到六歲以後才能清楚的寫出自己的姓，和名字。
6. 在認識或書寫自己的名字之前，台灣幼兒大多可以辨識並書寫筆畫簡單的國字，例如: 一、大、人、小、日等等。
7. 台灣幼兒的書寫發展過程明顯的和西方拼音文字社會的幼兒不同。在拼音文化中長大的幼兒，他們的書寫發展過程包括了無方向塗鴉、線形塗鴉、字單位的出現、拼音錯誤等等，但台灣幼兒的書寫發展卻僅有線條交錯塗鴉、類中文字型、以及成熟的中文字型。中文字和拼音文字間的書寫發展差異，也暗示了中文字是必須刻意學習的，與幼兒的發音敏感度無關。

本研究提供了幼兒中文學習的重要觀察。讀寫是開啟幼兒心智的重要鑰匙，透過了解幼兒如何發展出文字概念，學前教育可以更有效的教導幼兒學會讀寫。

本研究因為收案的不足，以及受測對象地處東部而會影響到結論。後續的研究應該將幼兒的家庭語文教育納入調查，幼兒本身的精細動作發展也應考慮在內，畢竟，寫字和幼兒的精細動作能力有密切的相關。

關鍵字: 幼兒語文萌發、幼兒書寫發展、幼兒語文教育、幼兒家庭語文環境

O34

(計畫名稱：繪本教學對幼兒的語言發展之研究)

對話式閱讀對幼兒聽覺詞彙與口語表達能力之研究

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本研究對象為新住民之 3-6 歲幼兒，運用對話式閱讀進行 12 次的說故事活動，平均每人參與 9 次說故事活動，研究結果顯示參與對話式閱讀之幼兒可以增進其聽覺詞彙能力；再來，要提升幼兒的口語表達能力，可以運用幼兒的舊經驗及圖畫情境連結、五 W 的策略、回憶與完成等方式來進行說故事活動；最後，幼兒口語表達內容，幼兒是擁有解決問題的能力、並有認知與因果關係之概念。

（一）幼兒聽覺詞彙能力之差異性

未接受說故事活動計畫之前幼兒原始分數的結果，進行十二次說故事活動之後，明顯看出十位幼兒其原始分數增高之情況，顯示在進行對話式閱讀之後，幼兒聽覺詞彙能力增加，惟有兩位幼兒原始分數降低及一位幼兒呈現持平之情形，由此判斷進行對話式閱讀可增進幼兒之聽覺詞彙之能力。

在畢保德圖畫詞彙測驗中，針對問題中的名詞、動詞及形容詞之錯誤題數來分析，其結果為十位不同之幼兒在名詞、動詞及形容詞的認識有明顯的進步，但有三位不同之幼兒在名詞、動詞及形容詞的認識無明顯之進步，由此判斷進行對話式閱讀可增進幼兒之聽覺詞彙之能力。

（二）提昇口語表達能力之方法與策略

研究結果顯示運用幼兒的舊經驗及圖畫情境連結、五 W 的策略、回憶與完成的策略方式，可以提升幼兒口語表達能力。

（三）口語表達之內容

研究結果顯示，幼兒口語表達之內容具有解決問題的能力、認知概念與了解因果關係。

1.解決問題的能力

在說故事團體進行中，透過對話式的閱讀，幼兒清楚的表達解決迷路的方法；對於處理生氣的方法，都能以正向的方式來解決；鱷魚想放假無法載青蛙、兔子、鴨子去上學，幼兒都能為青蛙、兔子、鴨子提出一些具體解決的方法。2.認知概念

幼兒們提出他們的對男生與女生的看法，並在自我性別概念大部分是正確的；對於排行的序列關係是清楚的；並可以類推說出自己與他人的姓氏；也能理解影子的概念；也知道看電影是否需要買票的問題。

3.因果關係

當故事中討論到為何貓想要當獅子的原因，大部分的幼兒都可以直接聯想到獅子的特性；媽媽因為爸爸與小孩都沒有協助做家事，結果離家出走，但是最後媽媽還是回來了，討論媽媽回來的原因，幼兒都能以同理心來思考媽媽的想法。

關鍵字：對話式閱讀、口語表達能力、聽覺詞彙能力

O35

(計畫名稱：繪本閱讀方案對發展遲緩兒同儕溝通成效研究)

以繪本介入發展遲緩幼兒同儕互動成效之研究

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許多文獻指出特殊幼兒與一般同儕互動情況趨於正向(傅秀媚,2002;楊智雯,2000；鄒啟蓉，2004；鄭雅莉，2004)，國內也有許多關於特殊幼兒在融合情境中的社會行為與同儕的互動表現（王天苗，2002；許碧勳，2001；鄒啟蓉，2000；蘇雪玉，1996)，但是，對於幼稚園及托兒所教師在面對發展遲緩幼兒時的教學策略上未有長期的質性研究，是故，本研究藉由繪本介入，來探討發展遲緩幼兒及一般幼兒同儕互動的成效。本研究目的在於瞭解發展遲緩幼兒的人際互動，透過繪本閱讀方案的進行，檢視該方案對發展遲緩幼兒同儕互動之影響。研究對象是幼稚園或托兒所班級中安置有發展遲緩的班級,研究者分別選定兩所公立幼稚園中的大班,該兩班分別安置有發展遲緩幼兒各一名，使用研究者自編之「發展遲緩幼兒同儕互動評量表」來評量發展遲緩與一般同儕的互動情形，此量表將會在繪本閱讀方案介入前後進行測試，藉此瞭解繪本閱讀對發展遲緩幼兒同儕互動的影響。此外，研究者在繪本閱讀方案介入前後進行觀察，藉此瞭解觀察發展遲緩幼兒與同儕互動的頻率與情形，並且，在繪本閱讀方案介入後訪談班級教師來瞭解幼兒同儕互動的改變歷程。

研究發現繪本教學能引起發展遲緩幼兒團體課程的注意力，並且進一步提昇一般和發展遲緩幼兒的專注力，此外，透過繪本閱讀能使教師進一步瞭解一般幼兒與發展遲緩幼兒互動的品質。

關鍵字：發展遲緩幼兒、繪本教學、同儕互動

O36

(計畫名稱：創意看圖作文對兒童寫作表現之影響)

創意看圖作文教學在原住民小學的教學實踐

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本研究旨在探究原住民學童的認知與語言表達情形，並企圖從文化回應教學的理念出發，發展貼近原住民學童生活與文化世界的「看圖作文」教學方案。研究初期，研究團隊發現原住民學童擁有豐富的多元家庭生命經驗，卻在閱讀與寫作課程表現出不情願。發現問題後，我們決定將看圖作文教材融入多元家庭議題與社區文化圖片，將搭建學習鷹架視為教學者的重要任務。研究發現，響應學生文化的看圖作文教學設計，有助於激勵學生更熱中於學習；探究原住民學童的學習風格，有助於促進教學的良性循環；而凸顯看圖作文三階段，可幫助教師發展因應差異的閱讀寫作教學。最後，本研究所使用的多元表達與多元評估策略，有助於提升師生的語文教學成就。

關鍵字：看圖作文、文化回應教學、行動研究

O37

(計畫名稱：原住民子女「傳說、科學、圖畫詩」親子創作之研究)

親子共學科學課程對原住民學童科學讀寫之研究

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原住民文化原本就擁有許多數理科學的智慧，然而現今學校的課程、教材及教師轉化科學概念用語與其所擁有的族群文化有所差異，因而造成指導學童科學讀寫時，家長處於弱勢地位。因此本研究擬設計以自然科學低成就的原住民親子為對象，設計學習環模式的親子共學科學課程，以探究原住民學童參予「親子共學科學課程」其科學讀寫歷程、創意寫作表現及對課程的滿意度。

研究發現如下：

1.在科學讀寫歷程方面，學童從被動、無自信的狀況轉變為有讀寫動機，能完成科學的讀寫活動。

2.在科學創意寫作方面，原住民學童仿作能力強、呈現豐富的動物知識。

3.在親子課程滿意度方面，原住民親子對課程之滿意度頗佳。

最後依據結論提出建議，提供多元文化教育、原住民研究及國小教師相關親子教學及課程設計之參考。

關鍵字：原住民親子、親子共學科學課程、科學創意寫作、科學讀寫

O38

新住民子女子親共讀下對讀寫萌發影響之研究

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本研究採質性的行動研究，探討促進三位學前階段的新住民家庭與其子女，經由子親共讀對讀寫萌發歷程之發展與可能面臨的困難。本研究首先藉由訪談二位新住民幼兒的幼稚園教師及一位義工家長，探討幼兒園語文教學對於三位幼兒讀寫活動的影響。再以啟蒙計畫的概念所設計的到宅說故事活動及延伸活動，以為期八個月 15 次到宅閱讀活動，探究三位花蓮縣吉安鄉新住民家庭幼兒暨其母親讀寫萌發時的問題與困難。此行動研究的歷程中，整個行動研究研究團隊，也不斷對於自己所進行的活動，進行省思，並計畫下一階段的行動策略。研究進行的過程中，運用文件搜集、觀察、輔以錄影或錄音、訪談等方式進行行動研究資料的蒐集，並進行質性研究的分析。最後發現

（一）促進新住民幼兒讀寫萌發的因素，從過去家庭轉為由學前機構擔任起此項重要任務。透過教室內的高品質語文活動，將啟發幼兒聽、說、讀、寫的能力。並透過子親共讀的策略，幼兒園教師可逐漸邀請家長與幼兒共同學習閱讀及相關語文活動。

（二）採用美國啟蒙計畫（Head Start Program）概念下所設計的到宅閱讀活動，透過一對一的方式與新住民家庭幼兒與外籍母親閱讀導引，逐漸引發新住民家庭幼兒與外籍母親聽、說、讀等三部份的興趣與能力。

（三）行動研究團隊成員，應接受幼兒多元文化教育的薰陶，能在閱讀活動進行時，更肯定新住民母語存在的必要性，並適時提供新住民家庭適當的家庭社會資源的訊息。依上述的研究結論，本研究分別對於新住民家庭幼兒學前教育的教師、未來研究、政府及學校等三方面提出建議，期許大家透過各方的努力，以啟蒙計畫的概念，與幼兒園合作，能幫助新住民弱勢幼兒。學前階段幼兒園藉由提供幼兒正常化的教學與課程，發展幼兒讀寫萌發的技巧與能力。政府可鼓勵大專院校、非營利組織，加入啟蒙計畫，幫助新住民弱勢家庭。透過「子親共讀」的活動，幫助新住民家庭更融入幼兒園語文相關活動。進而，邀請新住民家庭成為幼兒園多元文化的推手，將新住民的本國文化及語言分享成為幼兒教學與課程的一環。此研究由於進行到宅的時間歷程跨越幼稚園大班及國小一年級的學習，若能將研究期程延長，將可針對三位新住民家庭幼兒跨年級的讀寫萌發歷程，有更深入的探究與觀察。

關鍵字：新住民、讀寫萌發、啟蒙計畫（Head Start Program）、幼兒多元文化教育

O39

The Basic Studies of Methamphetamine in addition, Toxicity and Treatment

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Methamphetamine (MA) is a currently abused stimulant in Taiwan and around the world. In addition to addiction, chronic or high dose exposure to MA results in neuronal toxicity, such as psychosis and persistent cognitive deficits, as well as cardiovascular impairment. Thus, this integrated program was performed to investigate MA-induced behavioral dysfunction, neuronal activity and cardiovascular alteration. According to the first year findings, the continuous second year studies are as follows. In component project 1, we determined the restorative effects of calcitriol, a GDNF activator, on MA-induced cognitive dysfunction. Data indicated that calcitriol treatment for 7 days significantly reduced MA-elicited neuronal impairment in NORT. Co-treatment of GDNF antisense oligodeoxynucleotide with calcitriol blocked the restorative action of calcitriol on the cognitive deficits induced by MA. The component project 2 investigated the effects of acute and chronic MA treatment on the neuronal activity in medial prefrontal cortex (mPFC) and amygdala (AMY) in conscious rat. Results showed that the locomotor activity was significantly enhanced as rats were administrated with MA. Meanwhile, the cross correlations between neuron pairs within mPFC or AMY were reduced by MA. Under chronic application of MA, the ratio of the units with excitatory response was decreased and the ratio was further declined after MA withdrawal. In component project 3, restraint prior MA challenge induced more robust increase of Fos-immunoreactive (ir) cells in nucleus accumbens, CeA, infralimbic, and prelimbic cortices. Central administration of corticotropin-releasing factor (CRF), mimicking stress induction, also facilitated MA-induced neuronal activation. Importantly, the enhancing effect of restraint was eliminated by CRF receptor antagonist, α-CRF9-41. The component project 4 determined the central mechanisms of cardiovascular toxicity elicited by MA. Results demonstrated that intracerebroventricular administration of MA induced significant pressor effects and increased the expression of the phosphoserine 896 protein on NR1 subunit in the RVLM in anesthetized rats. The selective protein kinase C (PKC) inhibitor bisindolymaleimide dose-dependently blocked MA-induced pressor effects.

Taken together, our present studies further demonstrate the mechanisms of MA-induced neuronal and cardiovascular toxicity. MA provoked cardiovascular toxicity may be associated with the phosphorylation of NMDA receptor subunit NR1 by PKC in the RVLM. The stimulatory action of MA is potentiated by restraint through CRF signaling. The psychotic symptoms induced by MA may underlie the decrease of neuronal activities in mPFC and AMY. Furthermore, calcitriol could be the therapeutic agent for treatment of MA psychosis-related cognitive deficits through increasing the expression of GDNF. Based on these valuable findings and animal models, our future goal is going to figure out the relationships between MA-induced neuronal and cardiovascular toxicity and to develop other novel therapeutic compounds for MA-elicited neuropsychological and cardiovascular dysfunctions.

O40

(計畫名稱：Therapeutic effects of GDNF inducing agents on methamphetamine-induced neuropsychological impairment)

Restorative Effects of Calcitriol on Methamphetamine-induced Cognitive Deficits in Mice: Role of Glial Cell derived Neurotrophic Factor (GDNF)

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Chronic methamphetamine (METH) abuse produces long-term damage to dopaminergic (DA) and serotonergic neurons, and also provokes cognitive deficits. Glial cell derived neurotrophic factor (GDNF) has been shown to provide significant protection against the DA-depleting effects of METH. Calcitriol, the active metabolite of vitamin D, is demonstrated to potently induce GDNF expression. With this GDNF-inducing effect, this commercially-available compound might have restorative effect on METH-induced cognitive deficits. ICR male mice received four injections of METH (4 x 5 mg/kg, s.c.) or saline at 2 h interval, then novel objective recognition test (NORT) was performed 7 days later to confirm the METH-treated animals with apparent cognitive deficits. After subsequent administration of calcitriol (0.3, 1 and 3 μg/kg, i.p.) once a day for 7 days, NORT was conducted again. In order to investigate whether GDNF is involved in the therapeutic effect of calcitriol, GDNF antisense oligodeoxynucleotide (15 and 30 μM, 5μl i.c.v.) was co-administered with calcitriol for continuative 7 days after METH treatment, then the NORT was performed. The results showed that subsequent treatment of calcitriol (1 and 3 μg/kg) significantly ameliorated the METH-induced impairment in NORT. Co-treatment of GDNF antisense oligodeoxynucleotide (30 μM, 5μl, i.c.v.) with calcitriol (3 μg/kg, i.p.) blocked the restorative effect of calcitriol. These findings suggest that calcitriol may have the therapeutic potential for treatment of METH psychosis-related cognitive deficits through increasing the expression of GDNF.

O41

(計畫名稱：Effect of methamphetamine on the neuronal activities of the forebrain nuclei)

Effect of Acute and Chronic Methamphetamine Administration on the Neuronal Activities of Medial Prefrontal Cortex and Amygdala in Conscious Rat

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Methamphetamine (MA) is a psychostimulant and causes the serious social problem of abuse. Behavioral sensitization which is induced by application of MA is an animal model of schizophrenia. However, how the MA affected the neuronal activity of medial prefrontal cortex (mPFC) and amygdale (AMY) in the conscious animals was still unclear. In order to investigate the effect of MA on behavior and neuronal activities of mPFC and AMY, the behaviors and neural activities were recorded simultaneously in conscious rats. Two multiple- channel electrodes were chronically implanted in mPFC and AMY to collect the multiple single-unit activities. Administration of MA (2.5mg/kg) induced the increase of locomotor activity and both excitatory and inhibitory changes of unit activities in mPFC and AMY. The units with inhibitory responses were more than the units with excitatory responses in both areas. The ensemble unit activities showed inhibitory responses in both mPFC and AMY. The cross correlations between neuron pairs within mPFC or AMY were also reduced by the effect of MA significantly. After the chronic application of MA, the ratio of the units with excitatory response was decreased and the ratio was further declined after MA withdrawal but the ratio of the units with inhibitory response was not changed. The unit activities of mPFC and AMY were also further reduced by chronic application and withdrawal of MA. These results suggested that MA-induced psychotic symptoms may underlie the decrease of neuronal activities in mPFC and AMY.

O42

(計畫名稱：The mechanism of stress-promoted response to methamphetamine)

Effects of CRF and Restraint on MA-induced Neuronal Activation

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Among abused psychostimulants, methamphetamine (MA) with complicated pharmacological properties has become a serious worldwide public health problem. It is known that the reward circuit, the mesolimbic and mesocortical dopaminergic systems, is the main place MA manifests its addictive characteristics. Stress is one of the high-risk factors engaging in initiation of drug use, promotion of current drug abuse and relapse to drug-taking behaviors. In the nervous system, there is an interconnected set of cell groups, including central nucleus of amygadala (CeA), locus ceruleus (LC) and paraventricular nucleus in the hypothalamus, termed the central autonomic system (CAS), which is invariably activated by acute stressors. Corticotropin-releasing factor (CRF) acts as a neurotransmitter/neuromodulator to integrate stress responses. So, it is reasonable to infer that stress may promote addictive effects of MA trough activating CRF signaling. First, we clarified if stressor, restraint (RST, 30min), potentiates MA-induced neuronal activation in cell groups of mesolimbic system. Either RST or MA (2.5 mg/Kg BW, ip) induced significant increase of Fos-immunoreactive (-ir) cells in nucleus accumbens (NA), CeA, infralimbic (IL), and prelimbic (PrL) cortices. RST prior to MA challenge induced more robust increase of Fos-ir cells. Central administration of CRF, mimic stress induction, showed similar facilitative effect on MA-induced neuronal activation. The promotive effect of RST was eliminated by prior central injection of CRF receptor antagonist, α-CRF_{9-41}. For tracing the origins of CRF, injection of retrograde tracer, fluro-gold (FG), in the IL had been done. Additional to the ventral tegmental area and substantial nigra, IL receives innervations from limbic cortex, thalamus, subcortical nuclei and midbrain, including the basolateral amygdale (BLA) and LC which do not use CRF as neurotransmitter, but receive CRF-ir innervation. So, we proposed that RST may promote stimulatory effect of MA by CRF stimulation through LC or BLA indirectly.

O43

The Central Mechanisms of Cardiovascular Toxicity induced by Methamphetamine

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Methamphetamine (MA) is a strongly addictive psychostimulant that not only affects the central neurobehaviour but also cause cardiovascular dysfunctions, including tachycardia, myocardial ischemia and hypertension. MA produces increase in blood pressure and the response of heart rate mainly via sympathetic nervous system. The rostral ventrolateral medulla (RVLM), a central sympathoactivating nucleus, has been well known to be critical to the tonic and reflexive sympathetic regulation of arterial blood pressure. Our preliminary study showed that intraperitoneal administration of MA induced significant increases in the mean arterial pressure and the expression of the phosphoserine 896 protein (regulated by PKC) on NR1 subunit in the RVLM in conscious rats in a dose-dependent manner. Likewise, intracerebroventricular administration of MA also induced significant pressor effects and increased the expression of the phosphoserine 896 protein on NR1 subunit in the RVLM in anesthetized rats. Microinjection of the selective protein kinase C (PKC) inhibitor bisindolymaleimide (40 pmol or 4 nmol) into the RVLM dose-dependently blocked MA-induced pressor effects in anesthetized rats. Our results suggest that MA induced cardiovascular toxicity, especially acute hypertension, is associated with the phosphorylation of NMDA receptor subunit NR1 by PKC in the RVLM, which leads to an increase in central sympathetic outflow causing acute hypertension.

O44

(計畫名稱：登革非結構性蛋白 NS1 及抗 NS1 蛋白所誘發之交叉反應性抗體的病理作用)

Pathogenesis of Dengue Nonstructural Protein NS1 and NS1-elicited Cross-reactive Antibody

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Severe dengue virus (DENV) infections can cause life-threatening dengue hemorrhage fever and dengue shock syndrome (DHF/DSS). Because clinical studies revealed that DHF/DSS only associated with secondary DENV infections, abnormal immune responses were suggested to be vital on the elicitation of DHF/DSS. Commonly accepted antibody dependent enhancement (ADE) model describes the phenomenon that DENV-anti-viral antibody complexes enhance the infectivity of DENV to those Fc receptor positive phagocytes and likely whence increase the host burden and viral load in the DHF patients. After decades of research, it is shown that dozens of viruses have ADE phenomenon, however, only DENV induces a severer disease during the secondary infection. These data suggested that ADE might not completely explain why secondary DENV infection cause life-threatening DHF. Recently, studies demonstrated that DENV nonstructural protein NS1 could elicit pathogenic autoantibodies against surface antigens on platelets, endothelial cells and matrix proteins provide an alternative immune pathogenic hypothesis. As a result, here we want to investigate the mechanism underlining DENV nonstructural protein 1 (NS1) and anti-NS1 antibody induced pathogenic responses. Potential developments on the therapeutic strategies using both cellular and animal models will be discussed.

O45

(計畫名稱：Dengue virus non-structural protein 1 (NS1), anti-NS1 antibody and the immuocomplex-induced pathogenic response)

Ameliorative Role of Intravenous Immunoglobulin on Anti-NS1 Ig mediated Pathogenesis

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Dengue virus (DENV) is an emerging infectious agent in Taiwan. Severe DENV infection can cause life-threatening dengue hemorrhage fever and dengue shock syndrome (DHF/DSS). Since DHF/DSS usually occurred during secondary DENV infection, immune responses were suggested to be part of the pathophysiology. Antibody dependent enhancement (ADE) theory describes the phenomenon that DENV/anti-DENV antibody complexes bound to and internalized by Ig Fc receptors on the cell membrane of leukocytes, and thus enhance the infectivity toward immune cells especially macrophages. However, ADE theory can not fully explain the severe hemorrhage symptoms found in DHF/DSS patients. An alternative autoimmune model has also been postulated. DENV nonstructural protein 1 (NS1) was shown to elicit autoantibody against endothelial cells and platelets. These autoantibodies induced platelet abnormalities as demonstrated in both cellular and animal models, and could be lethal when experimental mice were experiencing a hypercoagulable state. As a result, here we would like to use cell and mouse models to further investigate the mechanism underlining anti-NS1 Ig-mediated pathological response. Here we found that intravenous immunoglobulin treatments have an ameliorative role on anti-NS1 Ig induced pathogenesis in mice. Potential therapeutic strategies to overcome the disease are discussed.

O46

Protective Roles of Chinese Herbs in TNFα/DV-NS1 Antibodies-mediated Damages of Endothelial Cells

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Dengue virus (DV) can cause mild dengue fever and severe dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS). Lots of evidence indicates that the host immune responses play a central role in DV-induced vascular endothelium dysfunction and apoptosis that resulting in DHF/DSS. TNFα secreted by macrophages, the major infiltrating leukocytes in the hemorrhagic tissue, and circulating antibodies against DV-nonstructural protein 1 (NS1) are important mediators attributing to the endothelial damages. The frequency of severe dengue disease is increasing in the recent years, while there is no effective treatment available. Researches indicate that Chinese herbs can down-regulate the TNFα-induced endothelial changes and decrease the TNFα levels released by activated macrophages. They may protect endothelial cells from the TNFα or DV-NS1 antibodies-mediated apoptosis and dysfunction. Based on our previous studies, we selected 10 from 288 Chinese herbs to investigate their protective role in the cultured human endothelial cells, HMEC-1. Subconfluent HMEC-1 cells treated with TNFα for 24 h showed a significant decrease in viability and cell number by 10-20% and 20-40%, respectively. Such decrease was partially reversed by coincubation with certain Chinese herbal extracts. Similar results were observed in the H_2O_2-treated HMEC-1 cells. It is known that superoxide and its derivative H_2O_2 are involved in the process of TNFα-mediated cytotoxicity, our results suggest that Chinese herbs may have potential to protect endothelial cells from death under pathologic stimulus. The Chinese herbs-mediated mechanisms are being studied.

O47

Mechanisms of Immune Suppression Post Immunization of Dengue Virus Non-structural Protein One

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Dengue viruses (DENs) infection causes serious mortality in most tropical and subtropical areas of the world. DEN infection can cause self limiting dengue fever (DF) or a severe, life-threatening illness known as “dengue hemorrhagic fever/dengue shock syndrome” (DHF/DSS) that involves several well-defined hemostatic abnormalities, including plasma leakage, thrombocytopenia, and bleeding. The pathogenic mechanism of DHF/DSS is unclear. The current model of “immune enhancement” is based on the observation that patients experiencing a second infection with a heterologous DEN serotype have a significantly higher risk of developing DHF/DSS. However, the immune-enhancement theory cannot fully explain the pathogenesis of DHF/DSS, especially the hemorrhagic abnormalities, thrombocytopenia, and high mortality. More recently, antibody to dengus virus nonstructural protein 1 was showed to cross-react to many cell host targets, including platelets and endothelial cells, and leads to thrombcytopenia and endotheilial cell apoptosis. These finding give rise to the hypothesis that dengue virus-elicited autoantibodies may involve in the pathogenesis of DHF/DSS.

Since anti-NS1 antibody has been showed to cross-react to many host targets, we thought to investigate its effect on immune responses in general. Preliminary experiments indicated that mice immunized with NS1 twice induced an immune suppressive status and produced less anti-BSA antibody at subsequent challenge with BSA. In this proposal, we will further characterize the nature this NS1 post-immunization suppression and explore its mechanisms. We will first test whether NS1 induces suppression non-specifically to broad range of antigens, the duration of suppression and whether T cell responses are also suppressed. Next, we will check that which component of immune system is responsible for this suppression, antibody or T cells. We will also investigate the role of Treg in this suppression.

We are now in the process of purification of large amount of NS1 and control protein for subsequent experiments.

O48

(計畫名稱：The signal mechnisms for pathological effect of platelet and endothelial cell triggered by NS1 Ab and NS1 protein)

The Signal Mechanisms for Dengue NS1- and NS1Ab-triggered Pathological Effects on Endothelial Cell and Platelet

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Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the most severe diseases caused by Dengue virus. The syndromes observed in DHF/DSS are rather complicated, which includes hemorrhagic manifestation, thrombocytopenia, coagulopathy and vasculopathy. One of the proposed pathological mechanisms responsible for DHF/DSS is the detrimental cellular effects triggered by the Abs derived from dengue nonstructural proteins 1 (NS1). NS1Ab and/or NS1 may attack endothelia cell and platelet leading to vascular damage and coagulopathy, respectively. In this 3-year project, we propose to delineate the signal pathway mediating the pathological cellular effects induced by NS1Ab and/or NS1.

In the beginning, whether NS-1 Ab/ NS1 protein may induce pathological effects such as apoptosis on endothelial cell and platelet will be investigated. If any effects are observed, the relevant signal transduction pathway will be delineated. Firstly, the cell surface receptor cross reactive with NS1 Ab/NS1 will be identified. If any well known conventional receptors, such as PDGFR and EGFR, are found to be involved, the well established signal cascade down stream of these receptors may be examined. On the other hand, several candidate signal molecules such as protein kinase C, reactive oxygen species and MAPK, known to be critical for mediating autoAb reactions, will be examined. In case that the candidate molecule approaches are not validated, protein arrays will be employed for large scale screening of the intracellular signal molecules activated by NS1 Ab/NS1 protein.

After the signal pathways induced by NS1Ab are established, whether inactivation of the critical signal molecules may prevent NS1 and /or NS1Ab-induced pathological effects on its target cells will be investigated both *in vitro* and *in vivo* (established in project I).

In the mean time, the materials required for future experiment are being prepared. The NS1 protein has been purified from BL21 transformed with NS1 expressing plasmid. We will further immunize the rabbit with the purified NS1 for generation of NS1 Ab. Also, the cultured system for endothelial cell is established and will be used for characterizing the cellular effects of NS1/NS1Ab.

O49

(計畫名稱：Role of dengue viral NS1 protein and anti-NS1 antibody on the differentiation and cellular function of the progenitor cells of platelet and monocyte)

Role of Dengue Viral NS1 Protein and Anti-NS1 Antibody on the Differentiation and Cellular Function of the Progenitor Cells of Platelet

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Dengue virus is a mosquito-born virus. As influenced by the global warming, it becomes an emerging threat of tropical and sub-tropical regions world wild. Dengue virus infections cause wild spectrum diseases ranging from self-limited dengue fever (DF) to life-threatening dengue hemorrhage and dengue shock syndrome (DHF/DSS). The clinical features of DHD/DSS are plasma leakage, thrombocytopenia and hemorrhage. The pathogenic mechanism underlining DHF/DSS is not yet clear and no vaccine available. Because DHF/DSS is associated with secondary dengue virus infections, abnormal immune responses were suggested to be part of the pathophysiology. It is shown that dengue viral nonstructural protein NS1 could elicit autoantibody against platelets and endothelial cells. Treatment of anti-NS1 Ig to endothelial cells could induce apoptosis. Treatments of anti-NS1 Ig to hypercoagulable mice could result in coagulopathy and death. At the mean time, both high level of soluble circulating NS1 and high titer anti-NS1 Ig were associated with the severity of the diseases. These results might suggest that the elicitation of anti-NS1 Ig is part of the immune pathogenesis. Since severe dengue viral infection associated with bone marrow suppression, leucopenia and thrombocytopenia, it might be indicated that these pathological response is associated with the suppression on hematopoietic stem/progenitor cells. Our preliminary *in vitro* HEL cell (a megakaryocyte like cell) differentiation experiments likely support this hypothesis. As a result, here we would like to further investigate the role anti-NS1 Ig/soluble NS1 on the suppression of megakaryocyte and monocyte precursor's function to reveal the mechanism of anti-NS1 Ig/soluble NS1-mediated pathogenesis.

O50

運動引發生理病理變化之機轉探討

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相較於國人傳統保健觀念的吃藥與吃補以外，運動醫學在近幾年來已經漸漸受到各國研究人員的重視，主要在於長期規律運動已經普遍被認為能促進健康、預防心血管疾病、降低糖尿病等疾病之發生率，但運動在對生理功能影響及疾病治療的研究仍十分有限，因為運動對於生理的影響為一複雜的機制，包括心血管系統、呼吸系統、神經系統、消化系統，彼此互相協調，也互相影響。本研究計畫採用強制性跑步機運動之動物模式，藉由各個系統的角度來深入探討運動對於正常情況及各種疾病的影響。

本研究計畫從 98 年 11 月 1 日開始執行，而動物跑步機於 2 月 24 日採購完成，因此在短暫的時間內我們盡速建立大鼠運動訓練模式。目前所建立的中強度運動訓練模式為速度每分鐘 30 公尺，每天給予一小時運動訓練；並依照各子計劃的研究需求給予不同持續時間的運動訓練。首先子計畫一在運動對於心肌細胞保護作用的研究成果中發現，連續給予大鼠五天的運動訓練，可以降低大鼠對於缺血再灌流造成的心肌細胞死亡的面積及脂質過氧化的程度，達到保護心肌的作用，而此保護作用似乎與運動訓練增加心肌細胞熱休克蛋白的表現量有關；進一步利用游離單一心肌細胞研究發現，運動訓練可以降低過氧化氫造成的心肌細胞內氫氧自由基及鈣離子的上升，避免心肌細胞因自由基導致的鈣離子過度負荷及細胞死亡。運動訓練除了可以對心臟產生保護的作用，也可能透過改變自主神經系統對於心血管的調控而降低糖尿病鼠的併發症，因此子計畫二以藥物誘發糖尿病鼠並給予持續一週的運動訓練，發現運動訓練可以明顯地抑制大鼠之血壓變異性中低頻成份(為血管交感神經活性指標) 與心率變異性中低頻與高頻比值(為心臟交感活性指標)；此外，亦發現可同步提高此動物之心率變異性之高頻成份(為副交感神經活性指標)。

運動雖可降低高血壓、糖尿病或肥胖等慢性疾病；然而卻有許多運動員於運動過程中或運動後經常會產生多種不適的呼吸症狀，而此不適之症狀具推測可能與運動過程引發肺 C 纖維的敏感化，所導致引發一連串之呼吸道反射作用有關。子計畫四以大鼠為實驗動物，實驗結果發現連續三天的跑步機運動後，可擴大化學性刺激物所引發的肺 C 纖維參與之肺化學反射，此結果可進一步推測此擴大之呼吸反射可能是因肺 C 纖維敏感化所致。除了保護心臟、改變自主神經系統調控心血管功能及造成呼吸的不適之外，子計畫四探討運動對於跨時區（時差）所造成腸胃道之生物時鐘基因表現的影響，目前也初步發現在小鼠及大鼠的結腸其生物時鐘基因，包括 *Per1*、*Per2*、*Per3*、*Bmal1*、*Cry1* 及 *Cry2* 基因有節律表現；下一步的研究則欲結合運動的訓練，來了解運動對於時差的腸胃不適應其機轉及作用機制。目前各子計畫的研究皆有初步的成果，但其詳細的機轉及其他可能之影響因素仍有待我們進一步探討。

O51

(計畫名稱：Exercise alters $Ca2^{+}$ and H^{+} handling in rat cardiomyocytes)

Exercise Training Reduces Ischaemia-reperfusion Injury in Cardiomyocytes

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Past studies have shown that suitable and regular exercise can enhance cardiovascular functions. But huge free radical will be induced by acute exercise and increasing damage in cardiomyocyte. It also has been reported that intracellular Ca^{2+} and H^{+} will effect cardiac contraction and cell death. But the mechanism is unclear. To better understand the mechanism that exercise regulate $Ca^{2+}$and H^{+} in cardiomyocyte, this proposed study is aimed to investigate the mechanism from cellular levels to a physiological system in details.

During the first year, an animal model of exercise will be established, and it will be used to compare with animals under normal conditions. For example, the rats will be subjected to different days of exercise, and their single cell from the hearts will be isolated by Langendorff, and use microspectrofluorometry to study the intracellular calcium concentration. We wish to determine whether the exercise induces ragulative changes of intracellular Ca^{2+} concentration in cardiomyocyte, and we will further investigate the mechanism of what exchangers participate exercise-induced ragulative changes intracellular Ca^{2+} .

To verify the cardioprotective effect of our exercise training, we measured myocardial infarct size by TTC staining, Infarct area was significantly reduced in the hearts of exercise trained rats compared with control. To further verify exercise-induced protection in the heart, we also measured lipid peroxidation in ventricular myocytes. Our results revealed that myocardial levels of lipid peroxidation were decreased in the exercise-trained rats. Numerous studies have shown that training-induced improvements in myocardial antioxidant capacity contribute to exercise-mediated cardioprotection. We further determined the expression of MnSOD, Zn/CuSOD and heat shock protein 70 (Hsp70) in cardiomyocyte. The expression level of Hsp70 was found to increase significantly after exercise training. Finally, we found that exercise training could decrease the H_2O_2-induced OH・ generation and Ca^{2+} influx in cardiomyocyte.

O52

(計畫名稱：The combined effects of exercise and hyperbaric oxygenation on cardiovascular neural regulation in diabetic individuals)

The Combined Effects of Short Term Exercise and Hyperbaric Oxygenation on Cardiovascular Neural Regulation in Diabetic Individuals

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Exercise is one of the first-line therapies for diabetes mellitus. It is recommended for its beneficial effects on glucose control as well as its abilities to retard the progression of other comorbidities common in patients with diabetes, such as cardiovascular disease. Our previous report had demonstrated that hyperbaric oxygen therapy (HBO) has a significant vagotonic effect, which is beneficial in improving cardiac neural regulation in patients with diabetic autonomic dysfunction. The specific aim of this study is to verify the combined effect of short term exercise and hyperbaric oxygenation on cardiovascular neural regulation on diabetic rats.

We developed diabetic rats by using streptozotocin. Telemetric monitoring of blood pressure and pulse-pulse interval on conscious, free-moving Sprague Dawley rats provides the basic signal acquisition for heart rate and blood pressure variability (HRV and BPV). The effects of 1-week exercise on the low frequency power of BPV (BLF, vascular sympathetic modulation), low frequency power of HRV (HLF, total autonomic modulation), high frequency power of HRV (HHF, vagal modulation) are analyzed.

The laboratory treadmill was not available until February 24, 2010. However, we developed training protocol for exercise model in diabetic rats successfully. Our preliminary result suggested that HLF did not change in diabetic rats during 1-week short tem exercise of 290 rpm, 60 minutes per day. The BLF and HLF/HHF were decreased and the HHF was increased after short term exercise. We are studying the combined effect of HBO and short tem exercise now.

O53

Effects of Exercise on Hypersensitivity of Pulmonary C Fibers in Rats

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Exercise is known to attenuate chronic disease such as hypertension, diabetes, and obesity; however, many athletes have discomforting respiratory symptoms during or after exercise. For examples, chest tightness, cough, dyspnea, and airway hyperresponsiveness, also occur frequently in athletes. Additionally, airway inflammation is known to exist in athletes with respiratory symptoms during or after exercise. Recent evidence suggests that exercise-induced respiratory responses likely involve a remarkable hypersensitivity of lung afferents during airway inflammation. Pulmonary C-fiber afferents, a major type of lung vagal sensory receptors, are known to be sensitive to chemical stimuli. Exercise can cause increased release of various chemical mediators, many of which have been shown to be able to sensitize pulmonary C fibers (PCFs) and further eliciting respiratory reflexes. The first-year study was carried out to determine whether prior 3 consecutive days of running exercise augments the sensitizing effects of chemical stimulants on pulmonary chemoreflexes; and if so, whether these effects are mediated through superoxide anion generation or arachidonate metabolites. Rats were allowed to run on the motor-driven leveled treadmill at a speed of 30 m/min for 1 h/day during dark phase for 3 consecutive days. Subsequently, on the *Day 4*, the pulmonary chemoreflexes to chemical stimulants was recorded in anesthetized and spontaneously breathing rats. Our results showed that exercise significantly amplified the capsaicin-induced pulmonary chemoreflex responses, compared with those of control rats. Similarly, exercise also induced significant increases in the pulmonary chemoreflexes to right atrial injections of adenosine and lactic acid. The enhanced responses to chemical stimulants were prevented by perineural capsaicin treatment of both cervical vagi or bilateral vagotomy, suggesting the involvement of PCF afferents. The results of this study suggest that 3 consecutive days of running exercise possess sensitized PCFs, which may participate in the development of the exercise-induced respiratory responses. However, more evidence will be required to set the final conclusions.

O54

(計畫名稱：Effects of exercise on circadian-clock genes expression in digestive systems during time zone travel (jet lag))

Circadian Patterns of Circadian-clock Gene Expression in Colons of Mice and Rats

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The most common rhythm of organisms at earth is the circadian rhythm. Jet lag is highly prevalent all over the world after using jet airplane as the long distance travel. Jet lag reflects a mismatch between local and circadian time following rapid time zone travel. Because jet lag produces a misalignment between the internal clock and the external light/dark cues, resulting in a serial of symptoms such as sleepiness, decreased alertness, impaired performance, difficulty initiating and maintaining sleep, irritability, and gastrointestinal distress. A number of strategies have developed to overcome the symptoms of jet lags, including the stimulants, hypnotics and exercise. However, those studies did not focus on the digestive distress after jet lag. Recent studies showed that circadian patterns of circadian-clock genes exist in the gastrointestinal tracts and implied that these genes might play an important role in the homeostasis of gastrointestinal functions. Because exercise may be the more harmless way to prevent the problems after jet lag, whether exercise improves the digestive distress by jet lag through circadian-clock genes is the main focus of this study.

The main objective in this first-year study is to build up the patterns of circadian-clock genes expression in the digestive systems in mice. Using the real-time quantitative PCR as the tool to detect gene expression, we found that the circadian-clock genes, *Per1*, *Per2*, *Per3*, *Bmal1*, *Cry1* and *Cry2*, showed the circadian patterns in male mice colons. Unfortunately my co-laboratory, Dr. Chang, who planed to achieve part of goals in this integrated proposal, left our team. This situation forced us to change our subjects from mice to rats and re-join the other teams in our integrated group. Providentially the circadian patterns of circadian-clock genes, *Per1*, *Per2*, *Per3*, *Bmal1*, *Cry1* and *Cry2*, also showed in the colons of male rats. Taken together, the data in this first year proposal indicate that the expression of circadian-clock genes exist circadian patterns in the digestive systems in both mice and rats. In the next step we test whether exercise is able to influence the expression of circadian-clock genes in the digestive systems after jet lag as our expectation.

O55

由生理與心理議題探討影響酒精中毒之預防與治療趨勢：從生理表現到分子機轉

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花蓮地區的高事故傷害發生率與機動車交通事故有極大的關係，根據台灣地區的統計資料顯示，花蓮地區的機動車交通事故粗死亡率高出台灣地區整體平均值的兩倍，肇事原因與酒後駕駛有密切相關，在回溯性的研究中也看到外傷情境中有 40%的患者血中可以測出酒精成分，其中高達 35%患者血中酒精濃度大於 100mg/dL，已達到概念性定義中的酒精中毒程度。酒精中毒若合併其他疾病(共病，co-morbidities)或創傷會導致病人對加護醫療的需求增高及死亡率增高，其後續相關合併症如敗血症與多重器官衰竭等情形也會上升。目前國內針對酒精濫用的研究多數圍繞在胎兒酒癮症候群的議題，還有一些研究是對於酒癮患者進行治療的探討。但在臨床上看到的急診實際狀況，來到急診求治的酒精中毒個案並非只有單一的飲酒問題，多數是因為大量飲酒後合併事故傷害的創傷失血與器官的加成損傷、或合併服用了鎮靜抗鬱藥物造成深度昏迷，結果因酒精中毒又合併了這些狀況後危及生命而住院，然而，在國內研究中卻見不到對於酒精中毒合併上列不同狀況的預防或治療趨勢之研究。因此，本整合性研究子計畫包括：由急慢性酒精中毒者合併情緒障礙的議題為主軸，探討使用抗憂鬱劑合併急性與慢性大量飲酒下對個體生理與炎症作用的影響(子計畫一)，另外也由預防及治療觀點，探討急慢性酒精中毒合併大失血或敗血症等共病因素所造成的腎衰竭狀況(子計畫二)，及急慢性酒精中毒合併大失血時要如何給予適當的輸液治療(子計畫三)，以及當急慢性酒精中毒合併大失血加護治療時，使用鎮靜藥物下的作用及影響(子計畫四)；藉由這四個子計畫的探究，可以描繪出急慢性酒精中毒合併心理與生理之共病下，其預防與治療上必須了解的多面向醫療構面。希望達成的總體目標是：從臨床上這類個案常見的心理議題(子計畫一)與生理議題(子計畫二)切入，並加上治療的觀點著手(子計畫三、四)，進行從生理層面到分子機轉的研究，探討急慢性酒精中毒在共病下的相關預防與治療趨勢。

O56

以清醒鼠模式探討抗憂鬱劑合併酒精中毒的生理作用與炎症反應

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憂鬱症是目前開發中國家及已開發國家中常見的心理疾病，不僅在發生率上有逐漸上升的趨勢，其發生的年齡層也逐漸擴大。在憂鬱症的臨床治療上常給予抗憂鬱劑使用，目前臨床上處方給予的抗憂鬱劑選擇上，最為普遍使用的是 SSRI (Selective Serotonin Reuptake Inhibitor)類藥物，而酒精是常被憂鬱症病人合併使用的物質，臨床上不乏聽聞以酒類合併服用抗憂鬱劑的急診病例。無論是急性酒精中毒或慢性酒精成癮，對人體生理上已經會造成不同的功能改變及炎症反應，許多報告也指出血清素(serotonin)對炎症反應有密切的影響，若個案同時合併使用 SSRI 類抗憂鬱劑，會造成器官與生理功能上的何種影響則不得而知。因此，本研究將探討的主軸放在抗憂鬱藥物合併急性與慢性酒精中毒的生理作用及機轉。在有限的研究經費下，本研究中的藥物選擇將會以臨床最常用的 SSRI 類抗憂鬱劑為主要實驗用藥。第一年研究主要在探討服用抗憂鬱劑合併急性酒精中毒下各器官功能與炎症反應的表現，實驗中將三組大鼠分別給予不同處置：急性酒精中毒組(Alc)-給予空膠囊餵食之後輸注 5gm/kg/3hrs 酒精引發急性中毒；SSRI 組-給予 SSRI 藥物餵食之後靜脈輸注無菌蒸餾水；SSRI+Alc 組-給予 SSRI 藥物餵食之後輸注 5gm/kg/3hrs。在給 SSRI 之前、給酒精之前、給酒精之後，以及給完酒精後 1、3、6、9、18、18、24、48 小時抽血檢測，並於 48 小時犧牲動物做組織病理變化檢測。目前已完成初步的血液酒精濃度、血球計量與血液生化數值檢測，結果顯示：目前的急性酒精中毒模式，血中濃度在給完酒精後可達到 400 mg/dl；Alc 組在給予酒精後出現 WBC、platelet、lymphocyte 下降，Glucose、GOT、GPT、CPK、Amylase 上升的現象；SSRI 組在給藥後出現 platelet、lymphocyte 上升，GOT、CPK、LDH 上升的現象；SSRI+Alc 組在給完酒精後，platelet 與 lymphocyte 下降幅度較 Alc 組大，Glucose、GOT、GPT、CPK、Amylase 上升的現象則較 Alc 組嚴重。根據以上結果，初步推論 SSRI 在急性酒精中毒下會加重器官的損傷。血清發炎細胞激素與組織病理學染色尚在進行檢測中。

O57

清醒鼠模式下探討酒精在不同模式急性腎衰竭的作用

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酒精是常見的濫用物質，對身體也會造成發炎及免疫反應。在大劑量酒精服用會對身體產生傷害。酒精會影響免疫細胞，改變免疫細胞的功能。酒精也影響抗原細胞表現及細胞內訊息傳遞，包含活化 MAPK、增加 NF-κB、影響 Toll-like receptors，進而改變發炎反應。此機轉跟不同劑量酒精造成細胞細胞膜結構變異有關。酒精也可產生氧化壓力，主要為酒精在細胞質及粒腺體代謝所產生的 ROS 及 RNS 有關。急性酒精中毒臨床上為服用大量酒精造成身體上有害反應，包含行為、心臟、腸胃道、肺臟、神經、腎臟、肌肉及代謝傷害。

本研究第一年為建立不同程度酒精中毒模式(低濃度酒精中毒給予: 2.5 g/kg 酒精，中濃度酒精中毒: 給予 5.0 g/kg 酒精，高濃度酒精中毒: 給予 7.5 g/kg 酒精)，並探討不同程度酒精中毒後再 40%出血性休克對器官的影響，及探討不同程度酒精中毒後再 40%出血性休克引起急性腎衰竭腎臟生理、病理、分子生物層面的表現影響。實驗發現給予 7.5 g/kg 高濃度酒精中毒後再 40%出血性休克，老鼠於 40%出血性休克後 1 小時全部死亡。給予中濃度 5.0 g/kg 酒精中毒後再 40%出血性休克，老鼠於 40%出血性休克後 9 小時死亡一半。給予低濃度 2.5 g/kg 酒精中毒後再 40%出血性休克，老鼠於 40%出血性休克後 48 小時均無死亡。故實驗主要以中濃度 5.0 g/kg 酒精中毒後再 40%出血性休克，低濃度 2.5 g/kg 酒精中毒後再 40%出血性休克及 40%出血性休克為對照。中濃度 5.0 g/kg 酒精中毒後再 40%出血性休克對老鼠 GOT、GPT、BUN、Cre、CPK、LDH、Lactate 值相對於低濃度 2.5 g/kg 酒精中毒後再 40%出血性休克及 40%出血性休克更高，更易造成器官損傷。而低濃度 2.5 g/kg 酒精中毒後再 40%出血性休克對老鼠 GOT、GPT、BUN、Cre、CPK、LDH、Lactate 值相對於 40%出血性休克於少數時間點較高，較多時間點下是無差異的。目前對不同程度酒精中毒後再 40%出血性休克對器官病理切片、血清發炎細胞激素及腎臟免疫組織染色，腎臟分子生物層面的表現影響仍持續進行中。

O58

(計畫名稱：探討急慢性酒精中毒合併大失血情況下之合理輸液策略)

探討酒精中毒合併大失血情況下之合理輸液策略

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過去研究指出急性酒精中毒所引起的創傷常會導致高死亡率，而急診單位的外傷患者又有 40%，其血液中含有不等量濃度的酒精。目前已知合適的輸液復甦能改善失血性休克後的器官損傷程度，然而，對於急慢性酒精中毒後大出血，其輸液的影響性為何目前尚無定論。因此，本研究計畫的目的為期望能藉由實驗動物模式，探討酒精中毒合併大失血性後，給予不同比例(1:1 與 1:3)輸液復甦對後續發炎指數與器官損傷之相關性。本研究使用 WKY 大鼠，體重介於 280~320gm 之間，研究中將大鼠分為三組:急性酒精中毒組 (Acute alcohol intoxication; AAI) 、急性酒精中毒併失血性休克組 (AAI+Hemorrhagic shock; HS) 組以及急性酒精中毒併大失血等量輸液復甦組 (AAI+HS+Fluid resuscitation; FR)組，於大鼠急性酒精中毒半小時後，運用失血性休克模式進行大量失血 40%，失血畢 30 分鐘後，立即輸入與失血同等量的晶質輸液(1:1)，在急性酒精中毒前、中毒後 1、3、6、9、12、18、24 與 48 小時各採集血液 0.8ml，檢測血中全血球數值、GOT、GPT、Glucose、BUN、Cr.、CPK、Amylase、Lipase、Lactic acid，以及 ethanol level 等，在酒精中毒 48 小時後，給予大劑量的 pentobarbital 犧牲，進行肺部灌洗，監測 LDH 及預做發炎反應，並留取肺、肝、腸等器官做病理學檢測。初步研究結果發現，三組的酒精濃度於輸注酒精一小時後，皆達到急性酒精中毒的標準，而急性酒精中毒後再失血，其死亡率高達 50%。酒精中毒併失血後初期血液中 GPT、BUN、CPK、LDH、Lipase，以及晚期 Amylase 以及肺部灌洗液 LDH 的濃度，AAL+HS+FR 組皆明顯低於 AAL+HS 組。因此，本研究初步的結論為急性酒精中毒併失血後，給予等量輸液復甦能減少肝臟、腎臟以及胰臟等器官損傷的情形。由於本研究為前半年的初步研究結果，下半年預計進行不同輸液比例(1:3)下，給予急性酒精中毒併大出血後，其各器官表現情形。本計劃也期望第二年能以不同溫度的晶質輸液治療為主軸，瞭解輸液溫度對急性酒精中毒併大出血後大鼠生理病理的影響，以提供急診醫護人員面臨此類患者時，合適輸液介入時機與策略之省思。

O59

Dexmedetomidine 影響急性酒精中毒合併失血性休克的反應機制

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急性酒精中毒為急症病患常見的現象，患者處於嚴重的情況下需住進加護病房以接受密切的醫療照護，而照護過程中為了減輕患者生理上的不適與使用危急生命必須賴以維生的系統，患者需接受麻醉鎮靜治療。Dexmedetomidine 為一種新的加護單位鎮靜劑，較沒有呼吸抑制的作用且容易警醒，為目前麻醉鎮靜劑較理想的選擇之一。過去的研究發現 Dexmedetomidine 對於心臟血管及炎症反應等，有不同程度的影響與保護作用。因此，本研目的亟欲探討 Dexmedetomidine 作用於急性酒精中毒後之生理機制與炎症反應。本研究使用 WKY 大鼠，體重介於 280~320gm 之間，研究中將大鼠分為三組:急性酒精中毒組(Acute alcohol intoxication; AAI)、酒精中毒後低劑量 Dexmedetomidine 組(AAI+Low dose Dexmedetomidine; LDD)組以及酒精中毒後高劑量 Dexmedetomidine 組(AAI+High dose Dexmedetomidine; HDD)組，於大鼠急性酒精中毒半小時後，給予麻醉鎮靜，並連續依所需之劑量，給予連續滴注 24 小時。在急性酒精中毒前、中毒後 1、3、6、9、12、18、24、36 與 48 小時各採集血液 0.8ml，檢測血中全血球數值、GOT、GPT、血液尿素氮、肌酸酐、Amylase、Lipase，以及 ethanol level 等，在酒精中毒 48 小時後，給予大劑量的 pentobarbital 犧牲，進行肺部灌洗，以監測 LDH 及預做發炎反應，並留取肺、肝、腸等器官做病理學檢測。初步研究結果發現：三組的酒精濃度於介入酒精中毒一小時後，皆達到急性酒精中毒之標準， 血液中白血球、GPT、Lipase、以及肺部灌洗液中 LDH 的濃度，AAL+LDD 組低於其他兩組。而 AAL+HDD 組在腎功能 BUN 明顯高於其他兩組，但 amylase 較低。根據以上結果，本研究初步的結論為：低劑量 Dexmedetomidine 的鎮定麻醉能改善急性酒精中毒後肺部及肝臟等損傷。反之，倘若給予高濃度 Dexmedetomidine 反而會導致腎功能的異常。由於本研究為初步之結果，下個階段預計進行 AAL 後給予大失血介入，觀察高與低劑量的 Dexmedetomidine 所造成之生理與炎症反應，進而提供臨床加護醫學作為重症病人麻醉鎮靜時之參考。

O60

脂肪酸與神經血管功能

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The main purpose of this proposed program project is to develop a core research group in the area of neuro-vascular function at Tzu Chi University. This program project consists of 5 component projects in order to comprehensively examine the role of palmitic acid methyl ester (PAME) and related saturated fatty acids in regulating vascular functions directly by acting on vascular smooth muscle cells and/or indirectly via the CNS and the glia.

Project #1 entitled "**Palmitic acid methyl ester (PAME) is the perivascular adipose tissue-derived relaxing factor (PVATRF)**" (PI: TJF Lee, Ph.D), will test the hypothesis that PAME is released from perivascular adipose tissue to cause relaxation of the vascular smooth muscle cells. **Project #2** entitled "**The mechanism of methyl palmitate as a retinal-derived relaxing factor**".(PI: YC Lee, MD) will test if PAME is the retina-derived relaxing factor. **Project #3** entitled "**Role of fatty acids in central sympathetic control of cardiovascular function**" (PI: CC Lai, PhD) is designed to examine the role of PAME in regulating RVLM neurons and SPNs.. **Project #4 is** entitled "**Role of fatty acids in the regulation of the hippocampal and amygdala function**" (PI: CH Lin, PhD). **Project #5 is entitled "Investigation of the PAME formation by Mass Spectrometry**" (PI: A Lai, PhD).

Interactions among 5 projects are active. Bioassay of fatty acids has been carried out by projects #1 and #2, while biochemical measurements of fatty acids have been assisted by project #5. A lab assistant for projects #3 and 4 has been trained for conducting bioassay technique in project #1's lab. Accordingly, each component project has almost completed the platform for carrying out the proposed studies. In fact, all projects have obtained interesting data with one paper accepted for publication from projects #1 and 2. Accordingly, the overall progress of the project is very good.

Publications: 1. Yuan-Chieh Lee, Hsi-Hsien Chang, Chin-Hung Liu, Mei-Fang Chen, Po-Yi Chen, Jon-Son Kuo, Tony J.-F. Lee (in press) Methyl palmitate: a potent vasodilator released in the retina. Investigative Ophthalmology & Visual Science (SCI). 2010 Mar 31. [Epub ahead of print]PMID: 20357193 [PubMed - as supplied by publisher]

O61

(計畫名稱：Palmitic acid methyl ester (PAME) is the perivascular adipose tissue-derived relaxing factor (PVATRF))

Perivascular Adipocytes Release a Potent Vasodilator Methyl Palmitate

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We determined if palmitic acid methyl ester (PAME) or methyl palmitate was the perivascular adipose tissue-derived relaxing factor (PVATRF), and if its release diminished in established hypertension. A superfusion bioassay cascade technique was used with rat isolated PVAT as donor tissue and rat aortic ring as detector tissue. The superfusate was collected and analyzed with gas chromatography/mass spectrometry (GC/MS). The relaxing activity and concentrations of PAME in the medium of cultured NIH 3T3 cells (fibroblasts) and that of converted adipocytes were analyzed. The vasorelaxing activity induced by PVATRF and exogenous PAME of aortic rings of Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) was examined. The aortic PVAT upon superfusion with Krebs' solution spontaneously released PAME and PVATRF as indicated by aortic relaxation. The release of both was Ca2+-dependent. The aortic relaxations induced by PVATRF and exogenous PAME were inhibited by 4-aminopyridine (2 mM) and tetraethylammonium (TEA, 5 mM and 10 mM), but were not affected by TEA at 1 mM or 3 mM, glibenclamide (3 μM), or iberiotoxin (100 nM). Furthermore, aortic relaxations induced by PVATRF and exogenous PAME were not affected by heating their solutions (70°C), suggesting that both are heat stable. The vasorelaxing activity of Krebs' solution containing PVATRF or exogenous PAME was significantly attenuated following hexane extraction. Medium from cultured adipocytes but not that from fibroblasts caused aortic relaxation. Release of PAME from SHR aortic PVAT and sensitivity of SHR aortic ring to PAME were significantly diminished compared to those of WKY rats. It is concluded that PVATRF and PAME share similar biochemical and pharmacological properties. Both act primarily on the voltage-dependent K^+ (Kv) channel of aortic smooth muscle cells, causing vasorelaxation. The cellular origin of PVATRF or PAME is likely adipocytes. Both release of PAME in PVAT and the vascular response to PAME were decreased in the SHR.

O62

(計畫名稱：The mechanisms of action of methyl palmitate as a retina-derived relaxing factor)

Methyl Palmitate: A Potent Vasodilator Released in the Retina

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Purpose: To determine if palmitic acid methyl ester (PAME) or methyl palmitate is the retina-derived relaxing factor (RRF).

Methods: A superfusion bioassay cascade technique was used with rat isolated retina as donor tissue and rat aortic ring as detector tissue. The superfusate was analyzed with gas chromatography/mass spectrometry (GC/MS). The biochemical and pharmacological characteristics of RRF and PAME were compared.

Results: We demonstrated that the retina upon superfusion with Krebs' solution spontaneously released RRF (indicated by aortic ring relaxation) and PAME (measured by GC/MS). The release of RRF and PAME was calcium-dependent, since the release was abolished when the retinas were superfused with calcium-free Krebs' solution. Furthermore, aortic relaxations induced by RRF and PAME were not affected after heating their solutions at 70°C for 1 hr, suggesting that both are heat stable. Exogenous PAME concentration-dependently induced aortic relaxation with EC_{50} of 0.82±0.75 pmol/L. The aortic relaxations induced by RRF and exogenous PAME were inhibited by 4-aminopyridine (4-AP, 2 mmol/L) and tetraethylammonium (TEA, 10 mmol/L), but were not affected by TEA at 1 mmol/L or 3 mmol/L, glibenclamide (3 μmol/L), or iberiotoxin (100 nmol/L). The vasodilator activity of Krebs' solution containing RRF or exogenous PAME was greatly attenuated following hexane extraction.

Conclusions: RRF and PAME share similar biochemical properties and react similarly to all pharmacological inhibitors examined. Both act primarily on the voltage-dependent K^+ (Kv) channel of aortic smooth muscle cells, causing aortic relaxation. These results suggest that PAME is the hydrophobic RRF.

O63

Role of Fatty Acids in Central Sympathetic Control of Cardiovascular Function

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Several studies have shown that palmitic acid and stearic acid, common saturated fatty acids found in body lipids, may participate in several physiological and pathophysiological processes. A recent study demonstrated that palmitic acid methyl ester (PAME) and stearic acid methyl ester (SAME) were released from the superior cervical ganglion (SCG) upon field electrical stimulation (FES) in the presence of nitric oxide synthase (NOS) inhibitor in rats. SCG is synapsed with the fibers of sympathtic ganglionic neurons (SPNs) located in the lateral horn of the spinal cord and the activity of SPNs is regulated by pre-sympathetic neurons in the rostral ventrolateral medulla (RVLM). The current study was carried out to examine the role of free acids and their methyl ester derivatives on central sympathetic control of cardiovascular function.

By using a technique with rat isolated RVLM areas as donor tissue and rat endothelium-denuded aortic ring as detector tissue, our preliminary results showed that RVLM areas upon field electrical stimulation may release vasodilating substances resulting in dilation of aortic ring precontracted with L-phenylephrine (0.1 μM). To determine the role of fatty acids and their methyl esters on central control of cardiovascular function, blood pressure responses were recorded in anesthetized rats. Intracerebroventricular (i.c.v.) or intrathecal injection of high dose of PAME (500 nmol; 100 mM, 5 μl) dissolved in ethanol and mixed with bovine serum albumin (BSA, 10 mg/ml) caused a rapid decrease in blood pressure. I.C.V. injection of lower doses of PAME (<1 mM, 5 μl) had little effects on blood pressure response. A slight decrease in blood pressure was noticed following microinjection of low dose of PAME (200 pmol; 1 mM, 0.2 μl in 0.1% ethanol and 1mg/ml BSA) into the RVLM area. Microinjection of GW9508 (0.5 mM, 0.2 μl), an agonist of fatty acid receptor 1, into the RVLM area also caused a slight decrease in blood pressure. The effects of the other fatty acids and their methyl esters on changes in blood pressure will be examined.

O64

Role of Fatty Acid Methyl Esters in the Regulation of the Hippocampus and Amygdala Functions

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Various studies have shown that saturated free fatty acids, such as stearic acid and palmitic acid may participate in several physiological and pathophysiological processes. A recent research indicated that SAME (stearic acid methyl ester) and PAME (palmitic acid methyl ester) were released from superior cervical ganglion (SCG) upon field electrical stimulation or activation of nicotinic receptors by nicotine in rat. PAME has been demonstrated to be a very potent vasodilator and played important role in modulation of the autonomic ganglionic transmission. However, the physiological or pathological role of PAME in the central nervous system remains unknown. In the present study, we have examined the effects of PAME and stearic acid on synaptic transmission in hippocampus and amygdale in rat.

By using a superfusion bioassay cascade technique with rat isolated hippocampus and amygdale areas as donor tissue and rat endothelium-denuded aortic ring as detector tissue, our preliminary results showed that hippocampus and amygdale areas upon field electrical stimulation may release vasodilating substances resulting in dilation of aortic ring precontracted with L-phenylephrine (0.1 μM). To investigate the role of fatty acids and their methyl esters on CNS synaptic function, electrophysiological experiments were performed and synaptic responses were recorded in hippocampal CA1 and lateral amygala. The results were shown that application of PAME (2 μM) and stearic acid (2 μM) for 30 min resulted in a significantly inhibition in synaptic input-output responses and induced long-term depression of excitatory synaptic transmission in hippocampal CA1 area. To examine the effects of fatty acid on glial function such as extracellular glutamate reuptake, astrocytic primary cultures were exposed to PAME (2 μM) and stearic acid (2 μM) for 3 hr and the extracellular glutamate reuptake assay was performed. A significantly enhancement in extracellular glutamate reuptake was observed in fatty acid-treated astrocyte. The effects of the other fatty acids and their methyl esters on excitatory synaptic function and glial function will be examined.

O65

Investigation of the PAME Formation by Mass Spectrometry

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The potent vasodilator, palmitic acid methyl ester (PAME) is secreted in rat superior cervical ganglion (SCG) upon electrical stimulation. But, the crucial lipid source and biosynthetic pathway of PAME is still unknown. Therefore, we aim to identify primarily the type of lipid source based on GC and LC-mass spectrometry techniques.

The major objectives of this proposal are twofold. The first is to serve as the core facility for helping other projects to identify the active ingredients in their respective research and the second is to investigate the synthetic pathway of PAME.

In this study, we applied mass spectrometric analytical methods to identify lipids from various rat brain tissue homogenate specimens which were incubated and extracted by core facility. In particular, the fatty acids PAME, SAME and other phospholipids were analyzed by GC-MS.

Following superfusing the retina preparation with normal Krebs' solution (or calcium-free Krebs' solution), the superfusate is mixed with Krebs' solution containing phenylephrine (10 μmol/L) at different flow rate to obtain a final concentrations of 0.01~0.1 μmol/L provided by a separate line #1. Phenylephrine induces active muscle tone of the detector aortic ring without endothelial cells (EC) and perivascular adipose tissue (PVAT). Aortic relaxation, indicative of release of retina-derived relaxing factor (RRF), is estimated in the presence of active muscle tone, and PAME content in the perfusates collected below aortic ring is analyzed by GC/MS. As required, Ca2+ can be added to Ca2+-free Krebs' via line #2 (*italic*), which allows the aortic ring to constrict in response to phenylephrine in the presence of calcium (2.5 mmol/L).

The perfusates from the superfusion bioassay cascade system were extracted with methanol to solubilize the organic compounds. The sample was vortexed, sonicated, and finally pelleted via centrifugation at 1500 rpm for 5 min at 20°C. The supernatant was transferred to screw-cap tubes with polytetrafluoroethylene/silicone septa in the caps. Samples were analyzed by using a Agilent 6890 GC/5973 MSD. The GC was equipped with a HP-5MS capillary column (12 m×0.2 mm I.D.; 0.32 μm film thickness). Helium, at a flow rate of 0.6 mL/min, was the carrier gas. Temperatures for the GC injection port and interface were maintained at 250°C and 300°C, respectively. The GC temperature started at 90°C, increased 15°C/min to 240°C, 10°C/min to 300°C. The mass spectrum was obtained by scanning from *m*/*z* 50 to 550 within EI ionization source. Splitless injection mode was used with an injection volume of 2 μL. Agilent G1701AA version 0.300 ChemStation Software in the drug analysis mode was used for data acquisition and analysis. PAME is spontaneously released from the isolated retina preparations. The perfusates, following superfusing the retinas and causing relaxation of aortic rings, were collected for GC/MS analysis of PAME. Two peaks of the GC/MS were identical with those of PAME (retention time, 12.39 min) with *M*r of 270 and SAME (retention time, 13.75 min) with *M*r of 298. Two peaks of the mass spectrometry analysis matched the library ID of PAME and SAME, respectively. This was accompanied by the diminished release of endogenous PAME (determined by GC/MS) from the retina preparations.

O66

Nursing Guide for Parkinson's Disease: Developing and Testing Multimedia VCD and Printed Booklet

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Bacground: Along with aging process, the incidence of Parkinson's disease has been increased. As the disease progresses, individuals experience additional symptoms such as freezing gait, difficulty swallowing, drooling, and voice softening. Such fluctuations in symptoms are likely to be accompanied by decline in functioning, psychosocials well-being, and quality of life. And because of the increasing medical expenses and various change in health insurance system, the length of hospitalization shortens. Thus, providing patients with proper education has become more important to improve self-efficacy to regain health and facilitate the self-care.

Purpose: The aims of the project are to develop and test multimedia VCD of exercise and printed nursing guides for patients with Parkinson's disease.

Method: Design and development of the multimedia videodisc system and pamphlet included the following phases: editorial reviewing of all material available regarding the related educational program, creating initial sketches, content editing to provide words, figures, images, sound, production, reviewing and testing. The program's content validity was achieved through a formal consensus process of the consultants who are experts in the areas of parkinson's disease. During the period of testing, the VCD and nursing guides are used on Parkinson patients from neurosurgical unit at a medical center in eastern Taiwan.

Results：Incoporating the previous expert's feedbacks were used to produce progressively refined versions. Then a total of 9 subjects with Parkinson disease watched educational VCD and nursing guides, and subjects then received appraisal of satisfaction of VCD and nursing guides. The results indicated 77.7%(n=7) of patients felt satisfaction regarding these interventions.

Conclusions：The multimedia and printed nursing guides can be inergrated in clinical setting and used as an educational resources and may be fessible for home use as well. These interventions are expected to improve self-care of patients and their families and better health status for patients with Parkinson's disease.

O67

台灣歸化植物的風險評估

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外來植物種類總共有 63 科 137 屬 159 種，這其中蕨類植物有 1 種，裸子植物有 5 種，雙子葉植物 116 種，單子葉植物有 37 種。植物的習性方面，喬木有 28 種，灌木有 45 種，藤本有 14 種，草本有 72 種，其中栽培的種類有 95 種，歸化種有 33 種，入侵種有 31 種。風險評估中以以菊科、禾本科與豆科為主。

入侵植物的物候資料可以發現，大部分的植物全年可以開花結果，這可說明這些入侵植物的生殖策略相當成功,繁殖能力很強整年都可以繁衍下一代。其播遷機制為風力、動物傳播和蒴果開裂為主，有的會以無性繁殖為主來大面積拓展其領域。入侵植物的原產地主要以熱帶美洲或南美洲為主。這些植物的分佈，主要以全區低海拔開闊地、破壞地、河川地為主如藿香薊、大花咸豐草等。槭葉牽牛、銳葉牽牛、紅花野牽牛、百香果、毛西番蓮、三角葉西番蓮等藤本植物分佈於低海拔林緣地，另外咸豐草，野塘蒿、大扁雀麥、椒草主要分佈在中高海拔開闊地。非洲鳳仙花、紫花酢醬草、吊竹草分布於低海拔林下。大部分的植物全年可以開花結果,這可說明這些入侵植物的生殖策略相當成功，繁殖能力很強整年都可以繁衍下一代。另外小花蔓澤蘭及銀合歡雖然不是全年開花結果，但其種子產量多且繁殖力強，所以也造成嚴重的危害。不過其開花期較為固定可以提供人工砍除或防治的時間表。

O68

(計畫名稱：Mechanisms of bladder cancer inhibition by thalidomide and TrkB siRNA)

Suppression of Bladder Cancer Cells by Thalidomide and TrkB shRNA

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Due to its nonspecific symptoms and signs, the diagnoses and treatments of bladder cancer are often delayed. Once distant metastasis occurs, the median survival is approximately one year despite aggressive chemotherapy. New strategy for bladder cancer therapy should be explored.

Thalidomide is emerging as a treatment for hematologic cancers and solid neoplasms, such as multiple myeloma and prostate cancer. However, there is no report investigating thalidomide as an agent for bladder cancer therapy. In this study, we found that thalidomide inhibited the viability of BFTC905 cells (a cell line derived from a grade III bladder papillary transitional cell carcinoma) in a dose-dependent manner, exhibiting maximum inhibitory activity at 200 μM, which caused DNA fragmentation, suppressed TNFα-induced cell invasion associated with the inhibition of MMP-9 and ICAM expression, and inhibited p65 nuclear translocation. Concomitant or post-treatment with 5 μg thalidomide 3 times weekly suppressed or delayed the growth of xenograft BFTC905 cells in SCID mice. These results suggest that thalidomide may be a potential therapeutic agent for bladder cancer therapy.

Trk family receptors and their ligands have been implicated in several cancers. We have found the expression of BDNF (brain-derived neurotrophic factor) and its receptor, Trk B, in bladder cancer cells and human bladder cancer specimens. Due to the lack of specific TrkB inhibitors, TrkB siRNA may be more specific and more effective accompanied by less toxicity for the treatment of bladder cancer. We have designed a lentiviral vector #46 TrkB shRNA that reduced the expression of TrkB protein in BFTC905 cells. The combination of TrkB shRNA and thalidomide may become a new strategy for bladder cancer therapy.

O69

(計畫名稱：The longitudinal studies of cardiovascular disease in end-stage renal disease （ESRD）undergoing long-term dialysis)

Heart Rate Variability in Patients with Cardiovascular Disease and Chronic Hemodialysis

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Introduction - In end-stage renal disease (ESRD) patients, the leading cause of mortality is cardiovascular disease (CVD) as it accounts for almost 50 percent of deaths. In the United States Renal Data System database, the single largest specific cause of death is attributed to arrhythmic mechanisms or sudden cardiac arrest (SCA). Heart rate variability (HRV) has been claimed to be related to disturbance of autonomic nervous system and therefore to be a predictor for arrthymia occurrence. We demonstrate the HRV difference between chronic hemodialysis patients with and without CVD.

Method –Patients under chronic hemodialysis over six months were recruited from the hemodialysis unit at Hualien Tzu-Chi General hospital. The MP35 (BioPack Inc.) with the Biopac Student Lab PRO was applied for data acquisition in this study. MATLAB R2007a (The MathWorks Inc., Natick, MA) and SPSS 12.0 for Windows (Copyright c SPSS Inc., 1989-2003) were used for signal processing and statistic data analysis. Their ECG signals have been recorded for 5 minutes at supine position for heart rate variability (HRV) analysis. Differences in quantitative and categorical data at baseline between the study groups were compared, respectively, by the Student's t-test.

Results - In the frequency-domain analysis, features are obtained from the power spectrum of the RR series. In general, fast Fourier transform (FFT) was applied on RR tachogram with 4Hz resample rate to obtain the frequency power spectra. The frequency bands are divided into very low frequency (VLF), low frequency (LF), and high frequency (HF) bands. Dialysis patient with CVD is significant older then the patient without CVD. The LF and LF/HF are significant smaller in CVD group but the HF is less in non-CVD group.

Table1 . HRV measures in patients grouped by CVD status

	with CVD(n=60)	without CVD (n=30)	*p* value
Age	59.7±8.31	51.2±11.65	0.001
LF (ms^2)	28.87±9.86	35.48±12.61	0.014
HF (ms^2)	50.76±7.71	45.49±10.42	0.016
LF/HF	0.61±0.30	0.87±0.48	0.007

Data represent mean±SD

Discussions & Conclusions - Dialysis patients with CVD are older and have a cardiac autonomic dysfunction as manifest by decreased HRV. Hence, HRV is potentially a risk indicator for dialysis patients to predict CVD.

O70

中國現代小說的「寫實」與「擬寫實」問題研究—以 1918 至 1949 年間的小說為研究對象

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根據本人的研究，西方寫實主義的文論主張共三大點：

1. 真實的要求
2. 如實的觀察，精確的記敘
3. 客觀的態度：（1）無我；（2）中立

在此文論主張之下，寫實主義大致包括幾個基本創作觀點：

1. 主張創造時代的藝術，與作家同時代的各種人物均可成為寫作的對象；
2. 人物描寫要貼近生活的真實，因此寫實主義重視環境的作用，要讓人物在真實的環境中活動；
3. 沒有神話，沒有傳奇，沒有誇張的想像，情節不重迭宕曲折，而趨於淡化；
4. 觀察生活，蒐集資料，然後如實的描寫，在事件、人物諸細節的描繪當中，使讀者彷彿身歷其境；
5. 作者秉持客觀的態度，不參與個人主觀見解。

根據以上，中國現代小說史上，符合西方流派意義的寫實主義小說，數量可說並不是很大，由於數量上的有限，在中國恐怕說不上能夠形成一個「傳統」。然而中國到底因為社會、政治、國情的需要，從一開始的認識、誤讀、選擇、偏離，到將寫實主義改造成一種非寫實的「現實主義」，在這個過程中，形成了中國式的「現實主義傳統」。這個「現實主義傳統」其內容含納甚廣，既包含諳合西方寫實主義之作，也含納了熔象徵主義、浪漫主義等流派於一身的作品，甚至包括受革命文學影響而態度無法客觀的小說。中國「現實主義」這個詞，雖與「寫實主義」同作為 realism 的對等翻譯詞，其實早已遠離西方 realism 在學術術語上的流派意義，而形成一種中國式的內涵，而且據不同人的認識，又產生不同層次的見解，可說是眾說紛紜，而又包含甚廣。所以，如果這個「傳統」一定要以 realism 為名，那我們我們只能名之為 pseudo-realism，意即「擬寫實主義」，或「偽寫實主義」傳統。

O71

被壓抑的記憶——卡內提自傳中的猶太認同問題

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從卡內提的傳記中可以看出，他的前半生非常猶太式地四處遷徙，彷彿具體而微地呈現出猶太人共同的命運。但他雖然身為猶太人，作品中卻不常涉及猶太人與德國人之間愛恨情仇的議題，這是因為他終生堅持以德語寫作，對德國文化有特殊情感的緣故。在他的自傳《被拯救的舌頭》中，記載了他如何會接觸德語的始末。他的父親猝死後，年輕的母親極為懷念父親，由於兩人是在維也納求學時相識，德語是他們的戀愛語言，因此母親遂於翌年將全家遷至維也納，並強迫小卡內提學習德語，替代父親的位置。對母親的愛慕在卡內提心靈深處與德語緊緊結合一起，即使納粹對猶太人的迫害屠殺，顛沛流離之餘，依然堅持以德語寫作，德語成為他的「第二母語」，母親是最重要的因素。

這本自傳中另外記載中學裡一件侮辱猶太人的事件。學校高層介入處理，聰明地將整件事無聲無息解決。卡內提後來在寫給母親的信中說，這個事件雖然曾經令他憎恨過人類，但現在他已經和解了，不再想著要復仇。這似乎是針對著二次大戰後受創的猶太人而說的，他們應該迎接新的世界，不該沉湎於過去的仇恨。

第二本自傳《耳中的火炬》中的主要人物為卡爾・克勞斯，他也是猶太人，身兼出版家、批評家與作家，擅長諷刺散文、抒情詩、戲劇、箴言等等，但是克勞斯的猶太特質並不明顯，甚至還有反猶太傾向。卡內提被克勞斯的道德勇氣所震攝，後者也因此成為影響他最鉅的人，卡內提自述其終生都在克勞斯的影子下。

第三本自傳《眼眸遊戲》評論許多當時的人物，其中最吸引注意的卻並非負有名氣的人士，而是一位猶太長者，是與《耳中的火炬》中的克勞斯完全相反的另一種典型。卡內提沒有在書中直接寫出他的名字，而是以太陽博士（Dr. Sonne）稱呼他。他類似於隱士一般，口中從未出現過「猶太」的字眼，他不將自己的出身背景當作是仇恨或是炫耀的憑藉，雖然他本身即是猶太文化最精緻深厚的具體化身。但太陽博士對於卡內提而言，卻成為他心中永遠的典範。

在遊記《馬拉克施的聲音》中，卡內提記述了他參訪這個城市老城猶太區的經過，這是他首次正視自己猶太人身分的問題，並以自身的猶太性作為主題的作品。卡內提藉著在這本遊記中所描述的異域，從中東回教的現實中，塑造了一個與當代社會迥異的世界，這個世界被歐洲強勢文明逼到角落，卡內提的描述給予了它發聲的機會。

O72

(計畫名稱：經典閱讀讀書會提升大學生閱讀理解及批判思考之研究)

慈濟大學生經典認知與閱讀習慣之調查研究：以學院為研究類別

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本調查研究的定位是進行大學生經典閱讀的一項前置計劃，透過社會調查方式獲得大學生對於經典閱讀的相關認知和習慣之若干數據和結論，以便作為大學及教師們設計經典課程方向和實施教學計畫方案的參考依據。

本研究之研究對象是慈濟大學的學生，進行學院和系別班級的集叢抽樣，有效樣本為 593 人，進行封閉性的問卷調查，並以 SPSS 進行資料的統計分析，主要是以學院為類別的交叉表分析（次數分配、百分比）。

研究結果顯示，受試學生均相當肯定經典讀的功能，認為其有助於批判思考和人文素養等能力的培養，但主動閱讀或擁有經典的人數比例卻不高，閱讀的次數、量及時間也偏低，而且在閱讀也覺得有所困難，但對學校或教師的協助要求，卻大多只是推薦書目而已。由此可見得受訪學生多認為閱讀經典不具必要性，且受限於時間的應用，只能在空暇時間閱讀，其無法真正理解經典閱讀所能習得的智慧和經驗是可以應用在生活上和提昇精神的層次的。因此，鑑於學生自主性學習的不足，教師更有必要以融入式或正式課程帶領其進入經典世界，藉以養成其閱讀經典的習慣，並學習到活用經典知識的重要性，亦即大學經典教育有推廣和提昇之必要。

關鍵字：經典、經典閱讀、經典教育

O73

多元閱讀策略教學提升大學生英文閱讀理解成就及批判思考能力之研究

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在學習的歷程中閱讀能力佔有不容忽視的關鍵地位，閱讀能力越強的人，在各個領域的學習成就也就越高。對以英文為第二外語的大學生而言，英文閱讀能力是一項基本且重要的技能。因為精熟英文的閱讀技巧，可以幫助學生在使用英文教科書的學科學習上，達成有效率的學習。英文閱讀能力的提升，除了勤於閱讀外，更重要的是採用有效的閱讀理解策略。知識來自閱讀及思考，有謂「思而不學則罔」。當此知識經濟的時代，有形資產已不再成為絕對優勢，取而代之的是思維能力。因此大學教育的重要目標之一是培養學生獨立思考的能力，而透過閱讀來實現思維能力的培養，是最有效的方法之一。在大學的英文閱讀課程教學中，教師常礙於學生的英文閱讀能力有限，多數的教學重心都只放在認知、理解兩個最基礎的層次上，而忽略了分析、綜合、評鑑的高階批判思考能力的培養。

本研究欲藉由在慈濟大學英語教學中心開設「英文閱讀與寫作」的課程，選用適合進行批判辯證的英文文章，以「小組提問討論閱讀策略法」教導學生運用合宜的英文閱讀策略，提升學生英文閱讀理解的成效，並對文章進行分析批判與綜合評鑑，以培養學生批判思考的能力。

本研究採用「不等組前後測」的準實驗研究設計，於研究者任教之「英文閱讀與寫作」課程中進行之。實驗組接受「小組提問討論閱讀策略法」，控制組接受「一般講述式教學法」。於實驗前施測「中級全民英檢模擬測驗之閱讀測驗」及「批判思考量表」，以了解實驗進行前兩班學生的英文閱讀能力，及批判思考能力。然後進行十八週的實驗教學，以「單元英文閱讀理解測驗」對每一篇指定閱讀文章進行理解測驗，並以期中考與期末考瞭解學生階段性英文閱讀學習成果；期末再次施測「批判思考量表」，以瞭解學生批判思考能力的變化。同時期末以「英文閱讀學習滿意度問卷」瞭解學生對本課程的看法。

研究結果發現接受「小組提問討論學習法」教學的實驗組學生，其英文閱讀理解的測驗成績，顯著高於控制組。由此顯示「小組提問討論學習法」的教學，有助於提升學生英文閱讀理解能力。小組提問學習法對高成就學生的閱讀理解成效最彰著，對中等程度學生效果亦明顯，惟對低成就學生的效果相當有限。實驗組學生肯定小組提問討論學習，對其英文閱讀理解幫助大，特別是在閱讀主題不熟悉或偏難的文章時，尤其有效。但是接受「小組提問討論學習法」教學的實驗組學生在「批判思考量表」的表現與控制組並無明顯差異。

兩組皆有超過 69%的學生對教師的教學感到滿意，兩組皆有超過 60%的學生對肯定所選用之英文閱讀文章，主題多元，兼顧深度及廣度，確實增廣其見聞，增長其英文閱讀能力。兩組學生對課程的滿意度，並無顯著差異。但實驗組對閱讀英文文章的興趣則顯著高於控制組。顯示「小組提問討論閱讀策略」對提升學生英文閱讀興趣有助益。

O74

Connecting Principled Information with Worked Examples: Can't Do or Don't Do?

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This research addressed the issue of creating connections between pieces of information. In particular, it investigated whether students have problem relating principled information to worked examples or vice versa, and if so, what may be the causes for their difficulty. A topic selected from mathematics curriculum was used for designing the experimental materials.

It was found that even though about 60% of the participants thought about the principle when studying the example, many did not do so. When asked to find the similarities and differences between the principle and example, on average, the participants could find two or more, depending on the experimental condition. However, the correct rates of such comparisons ranged from 40%-something to 60%-something. Further results showed that the participants were more likely to find similarities than differences between the principle and example. In addition, they were more likely to compare elements that were explicitly mentioned in both the principle and example. This is probably because the former has more obvious correspondences to the example and has greater resemblance as the example than the latter. These findings suggest that one of the difficulties of connecting the principle and example is the non-occurrence (non-overlap) of the elements to be related. That is, the elements that connect the principle and example do not show explicitly in both the principle and example.

Further results showed that, on average, comparing two examples was easier than doing so for a principle and an example. This suggests that the abstractness of principles is another difficulty for finding connections, consistent with other research. The results also showed that if the participants compared two examples first and then the principle and example, then the latter performance would be improved, compared to no such event occurred first. However, the two examples to be compared may not be any two examples. As the results indicated, such facilitating effect occurred when one of the examples was different from the other. This finding implies that when the students try to connect two concrete examples, the concreteness help them see the correspondences between the two in terms of entities, relations, and so on. Therefore, later when they try to relate the principle to an example, they have clearer ideas of how to relate them.

O75

Interaction of Ethanol with NMDA Receptor Antagonists on Spinal NMDA-induced Pressor Responses in Rats

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NMDA (N-methyl-D-aspartate) receptor has been shown to be a pivotal target for both ethanol and ketamine. The present study examined the interaction between ethanol and ketamine on NMDA receptor activation. Repeated intrathecal injection of NMDA (2 nmol) into T7-T9 segment of spinal cord every 30 min caused reproducible increases in blood pressure in urethane-anesthetized rats weighing 250-275g. Intravenous injection of ethanol (0.16, 0.32g).or ketamine (2, 4mg/kg) inhibited NMDA-induced pressor effects in a blood concentration-dependent and reversible manner. In the following experiments, the rats were pretreated with intravenous ethanol (0.16g) 0, 10 or 30 min prior to administration of ketamine. Intravenous ketamine at 0 or 30 min after administration of ethanol produced synergetic effects on the inhibition of NMDA-induced pressor effects, i.e. the combined inhibition is greater than the sum of individual inhibition. However, the synergistic effects were not observed at 10 min after intravenous ethanol. The results indicated that, while co-administration of ethanol and ketamine elicited a synergistic effect on inhibition of NMDA receptor activation.

O76

Cocaine- and Amphetamine-regulated Transcript (CART) Peptide Activates ERK Pathways via NMDA Receptors in Rat Spinal Cord Dorsal Horn in an Age-dependent Manner

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Activation of extracellular signal-regulated kinase (ERK) cascade in the spinal cord dorsal horn may contribute to pain hypersensitivity. Our recent study showed that cocaine and amphetamine-regulated transcript peptide fragment 55-102 (CARTp) increased the levels of phosphoserine 896 and phosphoserine 897 on the N-methyl-D-aspartate (NMDA) receptor NR1 subunit (pNR1-ser896 and pNR1-ser897) via protein kinase A (PKA) and protein kinase C (PKC) signaling pathways leading to increases in NMDA receptor function in spinal cord dorsal horn neurons. Because NMDA receptor, PKC, and PKA signaling pathways may participate in ERK activation, we examined the effects of CARTp on ERK activation in spinal cord dorsal horn neurons *in vitro*. Western blot analysis showed a significant increase in the level of phosphorylated (activated) ERK (pERK) in the dorsal part of the spinal cord slices after incubation of the slices with CARTp (300 nM). Co-administration of CARTp with an NMDA receptor antagonist, MK801 or AP5, or an ERK inhibitor PD98059 blocked the increase in the level of pERK. Interestingly, the increase in the level of pERK by CARTp was observed in postnatal week 3 (W3) and postnatal week 4 (W4), but not in postnatal week 2 (W2) rats. The age-related responses were also found in CARTp-induced increases in the levels of pNR1-ser896 and pNR1-ser897. In the *in vitro* electrophysiological study, CARTp increased the amplitude of NMDA-mediated depolarizations in substantia gelatinosa neurons of W3 and W4 rats, but not W2 rats. The results suggested that CARTp activated ERK signals via NMDA receptor in spinal cord dorsal horn in an age-dependent manner.

O77

Changes of Circadian-clock and Cytokine Genes Expression in the Liver and Spleen by Streptozotocin-induced Diabetic Rats

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From bacteria to humans, these organisms exhibit a variety of physiological and behavioral rhythmicity in circadian patterns. The circadian system is responsible for the generation of about 24-hour rhythm in organismic and cellular levels. The circadian-clock genes show not only in the central nervous system but also in the peripheral organs. More and more studies found that circadian-clock genes play important roles in regulating circadian rhythms and energy balance. Due to diabetes mellitus is related to energy imbalance and excessive activities of inflammatory cytokines in peripheral tissues, whether the expression of circadian-clock and cytokine genes in livers and spleens will be influenced by progress of diabetes mellitus was the main focus in this study. Adult male Sprague-Dawley rats were used in the study and diabetes mellitus was induced by a single intraperitoneal injection of the β-cell toxin streptozotocin (STZ; 60 mg/kg in citrate buffer, pH4.2) after an overnight fast. After 3 days of STZ injection rats were measured the plasma glucose and significantly hyperglycemic animals (plasma glucose >300 mg/dl) were as the diabetic groups. Animals were sacrificed by decapitation for 7, 14, 28 days after STZ injection and genes expression determined by real-time quantitative PCR. We found that the light-on food intake was significantly increased in the diabetic groups and diabetes-induced decrease in body weight was also observed. The expression of circadian-clock, circadian clock-controlled and cytokine genes, including *Per1-3, Bmal1, Dbp, E4BP4, PEPCK, PDK4, IL6, IL1β* and *TNF-α* were altered in the liver and spleen. In conclusion, the circadian-clock genes expression in peripheral tissues may be related to the function or daily pattern of metabolic processes and inflammatory responses.

O78

Post-treatment with an Active Component of Scutellariae Radix (TChi-2) Reduces LPS-induced Acute Lung Injuries and Fatality

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Acute respiratory distress syndrome (ARDS) is a devastating clinical problem. It is caused by excessive secretion of proinflammatory and inflammatory mediators, resulting in diffuse alveolar damage, disruption of alveolar epithelium, and capillary injury. The aim of this study was to assess possible role of a purified plant extract (TChi-2) in the treatment of lipopolysaccharide (LPS)-induced acute lung injury in male Sprague-Dawley rats and lethality in mice. 24 hrs after its application, LPS (10 mg/kg, either iv or ip) significantly elevated plasma tumor necrosis factor-α (TNF-α) and nitric oxide (NO), increased pulmonary edema, and thickened interalveolar septa in lung tissues. These changes were prevented by posttreatment of TChi-2 (15 mg/kg, iv or 30 mg/kg, ip), administered one or six hrs after LPS-challenge. These treatments also significantly attenuated LPS-induced release of late cytokine high mobility group box 1 (HMGB1), and activation of NF-κB in lung tissues. Treatment with TChi-2 also significantly increased survival rate in LPS-treated mice. These results suggest a promising role of TChi-2 in treating LPS-induced acute lung injury.

O79

Bubbles and Bowel Sounds

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Bowel sounds represent a physical examination that can be carried out easily, conveniently, painlessly and at low cost. However, drawbacks existed as objective and quantitative measurements of bowel sounds were largely lacking. Although gas has been speculated to play a major role, the exact role and the mechanism through which gas participated have not been elucidated. With the use of a combined real-time ultrasonography and videofluoroscopy, we have been able to demonstrate certain bubbling processes whereby bowel sounds were generated. The acoustic signals were studied through simultaneous recording and were coupled to the image parameters. We have studied sequentially the "bubbling bowel sounds" (BBS's) when single or chains of bubbles were produced, the "loop sounds" generated by running bubbly flows, and a specific bubbling sound originated in the stomach called the "gurgling sounds".

In the BBS study, we have shown that 1) bubble oscillations are responsible for the bubbling bowel sounds; and 2) the oscillation frequency of a free rising bubble correlates inversely with its size. A BBS should be categorized as a pathological bowel sound because of the presence of increased gas and liquid content in a context of decreased contractility of the bowel.

For the loop sounds, frequencies were linked to the mechanism of "collective oscillation" of bubble clouds, which may account for their marked reduction. The presence of loop sounds suggested underlying increased contractility, bowel capacitance and gas content. The presence of these components was sufficient to preclude the possibility of underlying ileus or small bowel obstruction on a clinical ground.

The gurgling sounds, obtaining their name from the word "gurgle", represent the gastric counterpart of BBS's except rupturing into a half-filled cavity rather than a fluid-filled loop was the rule. The gravity-dependent nature has made it vulnerable to change of body position. The frequencies of the gurgling sounds were speculated to depend on the difference in height between liquid levels across the contraction ring: when over 1.5cm, the frequencies of sound were determined by the size of the bubbles. On the other hand, when the height difference was less than 1.5cm, no intact BBS's were allowed to form and the frequencies of sound were determined by the resonating cavities.

Our future work may thus include: 1)to recruit as many patients as possible to make more advanced studies and setting up of a magazine of bowel sounds; 2) to analyze bowel sounds more efficiently; 3)to correlate a specific bowel sound to a certain illness or symptom, and finally, 4) to establish the uniquenss of bowel sound analysis.

O80

The Molecular Mechanism of Amiodarone, an Anti-arrhythmia Drug, to Cause the Defective Formation of Cardiac Valves During Zebrafish Embryogenesis

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Amiodarone, a type III anti-arrhythmia drug, has been classified as category D in FDA pregnancy drug, which is commonly used to treat arrhythmia during emergency cases. In order to know whether Amiodarone affects on the heart development, we used zebrafish as an experimental animal due to the available transgenic lines and the transparent embryos which make *in vivo* dynamical observation be possible. After zebrafish embryos were treated with 15 μM Amiodarone from 10 hours post-fertilization (hpf) to 72 hpf, an apparent defect in heart values was observed to cause blood regurgitation. Specific genes such as *versican* and *has2*, which are restrictively expressed in the atrioventricular canal (AVC) where the valves develop, were found to be ectopically over-expressed in the myocardium of whole heart of Amiodarone-treated embryos at 72 hpf. Moreover, VE-cadherin (*cdh5*), which is normally down-regulated at the endocardium during invagination, was also over-expressed at the AVC endothelium in the Amiodarone-treated embryos. However, knockdown of *cdh5* by *cdh5*-morpholino (MO) could rescue the valve defect in the Amiodarone-treated embryos, indicating that Amiodarone inhibits invagination by over-expression of *cdh5*. To verify the molecular mechanism of modulation among these gene during valve development, we knockdown *versican* by injection 16 ng of *versican*-MO, the expression of *cdh5* was downregulated at 72 hpf, suggesting that *versican* may act upstream of the *cdh5* expression. Taken together, our findings conclude that Amiodarone induces the ectopical over-expression of *versican*, resulting in ectopical over-expression of *cdh5*, which, in turn, causes the failure to form heart valves during zebrafish embryogenesis.

O81

Pharmacologically Enhanced Imaging of ^{18}F-FDG PET for Evaluation of Parkinson's Disease in Rats.

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After methamphetamine enhancement, ^{18}F-flurodeoxyglucose (^{18}F-FDG) PET (positron emission tomography) imaging was able to apply for the evaluation of Parkinson's disease (PD) in rats. Six rats were subjected to imaging analysis, three with 6-OHDA unilateral lesion and the rests as control group. The pharmacological challenge with methamphetamine induced significant difference in the metabolic activity of striatum between normal rats and lesioned group. Drug enhanced rotation behavior and immunohistochemical staining for tyrosine hydroxylase (TH) confirmed the loss of dopamine cells in the striatum of the ipsilateral side in the affected animals. ^{18}F-FDG, a PET tracer advantageous in convenience and low cost, was successfully applied for grading of PD progression in these rats. Besides, we also find the activity of motor cortex is related to the connection between dopamine system and behavioral performance. Our study support a new noninvasive imaging approach that could help scientists better utilize PD rat model for their researches in understanding the progression of PD, and may potentially lead to the realization for the active cerebral area in PD patients.

O82

The Induction of Endoplasmic Reticulum Stress Protein GADD153/CHOP Expression by *n*-butylidenephthalide as Pharmaceuticals on Prostate Cancer Therapy

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The nature compound *n*-butylidenephthalide (BP), which is isolated from the chloroform extract of *Angelica sinensis*, has been investigated for its antitumoral effects in a variety of carcinoma cell lines *in vitro* and *in vivo*. This study is the first to investigate the anticancer effect of *n*-butylidenephthalide (BP) on prostate cancer.

We performed a microarray study to identify alterations in gene expression induced by treatment with the BP in the prostate cancer LNCaP cell line. This analysis identified several BP-inducible genes, including the GADD153/CHOP, an endoplasmic reticulum stress-regulated gene. Activation of GADD153/CHOP protein was corroborated by immunofluorescence and western blot. We then tested the contribution of GADD153/CHOP to protection against BP-induced cell death using RNA interference. Blockage of GADD153/CHOP expression by si-GADD153 siRNA significantly reduced BP-induced cell death in LNCaP cells. Taken together, these results suggested that BP induces the antiproliferative effect through a mechanism facilitated by ER stress in prostate cancer LNCaP cells. Administration of BP showed similar antitumoral effects in LNCaP xenograft tumors. This study demonstrates that *n*-butylidenephthalide may be a novel anticancer agent for the treatment of prostate cancer.

O83

Molecular Mechanisms Underlying Urocortin-induced Restriction of Proliferation in Neural Stem Cells

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Urocortin (UCN), a 40 amino acid neuropeptide, is a member of corticotrophin releasing hormone, which can mediate the hypothalamic-pituitary-adrenal (HPA) axis and coordinates the endocrine response to stress. Previous documents indicate that stress suppresses stem cell proliferation and increases expression of UCN in mammalian brain, but whether neural stem cells (NSCs) response to UCN remains unclear. We observed that UCN is expressed in the developing brain cortex and its receptors CRHR1 and CRHR2 are present in NSCs in ventricular zone in the same region. Treatment of cultured NSCs with UCN reduced cell proliferation. This decrease was not caused by apoptosis but due to prolongation of cell cycle progression. Cell cycle analysis revealed that UCN induced more NSCs arrested in G_0/G_1 phases. Bulk colony formation assay showed that UCN could maintaine pool of NSCs in a quiescent state. Mechanistic studies showed that UCN directly inhibited histone deacetylase (HDAC) activity leading to histone hyperacetylation which transactivated expression of krüppel-like factor 4 (Klf4) and p21. Knockdown of Klf4 resulted in downregulation of p21-mediated cell-cycling restriction caused by UCN. These results suggest that UCN might contribute to regulate NSCs homeostasis during development and stress.

O84

FGF Signaling in Intestinal Cell Differentiation

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There are four cell lineages, which are derived from intestinal stem cells, locate at the the crypt and villus in the mammalian intestine: the non-secretory absorptive enterocytes, and secretory cells including mucous-secreting goblet cells, regulatory peptide-secreting enteroendocrine cells and antimicrobial peptide-secreting Paneth cells. Many signaling molecules regulate stem cell self-renewal, cell proliferation, and cell differentiation in the intestine, including Wnt, BMP and Notch pathways. Although Fgf signaling is important for cell proliferation and differentiation in various tissues, its role in intestinal differentiation is less discussed. Previously, abnormal differentiation of goblet cells was observed when Fgf signaling was inhibited in SU5402-treated embryos, *hsp70:dnfgfr1* transgenic line, and *fgfr2* morphants. Among them, *fgfr2c* morphants had the most severe defect in goblet cell differentiation. Furthermore, the number of enteroendocrine cell was also reduced in *fgfr2c* morphants. We also identified several Fgf ligands expressed in the 5 dpf intestine of zebrafish. We found differentiation of goblet cells was reduced in *dae*, an *fgf10* mutant fish. However, the phenotypes were less severe compared to *fgfr2c* morphants. We further found the differentiation of goblet cell was affected in *fgf24* morphants. Additionally, there was a severe defect for the differentiation of goblet cell in *fgf10/fgf24* double morphants. This phenotype was similar to *fgfr2c* morphants, but with a difference in enteroendocrine cell differentiation which was normal in the double morphants. We conclude that the Fgf signaling can regulate the intestinal cell differentiation in zebrafish.

O85

The Role of Parkin in Cell Progression

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Chromosome instability and turmorigenesis have been correlated with defects of mitotic or spindle assembly check point (SAC), which is a key mechanism that ensure accurate chromosome segregation during mitosis. Here, we report that Parkin is a centrosomal protein, and C-terminal truncation of Parkin results in a defect of SAC when overexpressed. This Parkin truncation does not alter it's localization to centrosome but fail to self-interact with endogenous Parkin. C-terminus truncation of Parkin also results in formation of multinucleated cells when overexpressed. These results indicate that malfunctional Parkin proteins may interfere with signaling cascade of the SAC, which in turn leads to chromosome instability.

O86

No Need, No Demand, No Cost?

– Establishing Individual Accountability to Contain Medical Expenditures

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The core value of a public health insurance system is to provide necessary medical services at affordable prices. This, however, leads people to demand more services and results in escalating health insurance expenses. Constant reforms hence become a norm in order to contain health costs. Reform attempts normally focus on the supply side like limiting the number of physicians or the capacity of hospitals, setting sector or global budgets, applying prospective payment mechanisms. Traditional demand side interventions have been centering on reducing the package of public services and increasing the private contributions of beneficiaries. The strategy is to increase the cost awareness of the individuals for the utilization of medical services. Studies show that health behavior such as over-consumption of food, lack of exercise, smoking, and stress accounts for approximately 30-50 percent of morbidity and morality in the U.S. One of the latest developments, as advocated by WHO and adopted by European countries such as Germany, the Netherlands, and U.K., is to make individuals more responsible of their own health. The rationale behind this personal accountability is quite straightforward: healthy people have no need for healthcare and hence no demand for medical services. We intend to use the datasets from Taiwan National Health Insurance System to explore if significant cost differences exist between people who pay more attention to prevention activities, such as vaccinations, disease screenings, medical checkups, etc., and people who do not. The findings will then be used to advocate that promoting individual accountability should be incorporated in the coming national health system reforms.

O87

Factors Associated with the Avoidance Behavior of Household Environmental Tobacco Smoke among Pre-school Children's Mothers

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Objective. This study aimed to identify the factors associated with adoption of environmental tobacco smoke (ETS) avoidance behaviors among pre-school children's mothers.

Methods. A cross-sectional study was used to obtain a sample of pre-school child's mothers (n=1,020) in 30 registered kindergartens in eastern Taiwan. Overall, 919 (response rate 90%) completed the questionnaires. Regression models were used to identify the related factors on avoidance behavior of ETS.

Results. The prevalence of exposure to household ETS was 70 % and 50 % for mother and their children, respectively. After adjustment for other variables, mothers who were current smokers (β=-0.259, $p<0.001$), had spouses smoked (β=-.058, $p<0.05$), and household ETS exposure (β=-.136, $p<0.001$) were less likely to avoid ETS, whereas mothers who have a high knowledge score of ETS (β=0.082, $p<0.05$), positive attitudes toward avoidance behavior of ETS (β=0.275, $p<0.001$) and high self-efficacy of avoiding ETS (β=0.398, $p<0.001$) were more likely to avoid ETS. Hierarchical regression analyses confirmed that the best predictors for avoidance behavior of ETS were self-efficacy, current smoker, and attitude with 55.5% of total variance explained ($p<0.001$).

Conclusions. The high prevalence rate of exposure to household ETS in mothers and their child suggests the government should implement the tobacco control education on pre-school child's mothers to reduce their child's exposure to ETS in the home, in particularly to strengthen knowledge, enhance self-efficacy and positive attitude among mothers.

O88

Experiencing the Body of Hemodialysis Patients

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This article discusses the partial results revealed in an ongoing study on the illness behavior of the patients with hemodialysis in northern Taiwan. Patients with chronic kidney disease were mostly unaware of the body impairment until the CKD Stage 5. After dialysis, part of discomfort sensation was eased, albeit not eliminated, followed by many unpleasant body experiences. Our interviews and field study indicated that, even though the patients have accepted the western medication for years, they demonstrated rather different perception and interpretation about their body experiences, a difference related to their illness behavior. We start with the body experience of the dialysis patients, the way they feel their bodies, and then discuss how they interpret them.

P01

The APCCdh1 Form of Anaphase-promoting Complex is Required for Proteolysis of the S-phase Cyclin Clb6 during the Transition from G1 to S phase

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The cell cycle progression can be divided into four phases: G1, S, G2 and M. Cyclin-dependent kinase complex is a key regulator for cell cycle progression. Cyclin proteolysis was important for regulation for CDK activity. In *Saccharomyces cerevisiae*, two major ubiquitin ligase complexes, SCF and APC/C, are required to promote cyclin protoelysis. SCF complex is required for proteolysis of Cln1-3 and Clb6 whereas APC/C complex required for proteolysis of Clb1-5.

We have found that APC^{Cdh1} was also required for Clb6 proteolysis during G1 transition to S. Mutations in both KEN box and destruction box could enhance Clb6 stability in early and late G1. Also, expression of $Clb6_{mkb,mdb}$ could result in an earlier entry into S-phase. Therefore, our observations suggest that in addition to the SCF^{Cdc4} ubiquitin ligase complex, the APCCdh1 ubiquitin ligase complex should also regulate Clb6 turnover at the G1/S transition.

P02

A Yeast Two Hybrid Screening to Identify the HCMV pp65aa336-439 Motif Targeted Human Proteins

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease. Due to break down of tolerance by either B or T cells, SLE patients often develop autoantibodies against different cellular components, such as anti-double strand DNA (anti-dsDNA Ab), anti-Smith antibody (anti-Sm Ab), anti-small nuclear ribouncleoprotein antibody (anti-snRNP) and ect. Previous investigations showed that mechanisms that initiate SLE include genetics (such as human leukocyte antigen (HLA), complement and cytokines), sex hormone and environmental factors (such as drug, virus infection, ultraviolet light and stress). Prior studies also demonstrated that human cytomegalovirus (HCMV) infection could increase the risk in the development of SLE. Our study showed that more than 82% of SLE patients' sera reacted to HCMV pp65 antigen (65 kDa phosphoprotein). In animal model, female NZB/W F1 and BALB/c mice immunized with plasmids encoding HCMV pp65 open reading frame (pcDNApp65) developed anti-dsDNA activity. Western blotting analysis revealed that peptide of pp65-aa336-439 is recognized by SLE sera at a rate significantly higher than healthy infected sera. This finding indicated that B cell epitopes may locate on pp65-aa336-439 and play a important role in promoting SLE. In this study, we found that the pp65-aa336-439 protein can interact with Hela proteins via western blotting analysis. Current yeast two hybrid screening support this finding and identified human proteins that HCMV pp65aa336-439 motif targeted. This finding may not only assist future understanding on development of autoantibodies, but also provide link to infection induced tolerance break.

P03

Role of GroESL Involved in Biofilm Formation of *Thermus aquaticus* NTU103

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Thermus aquaticus NTU103 is a gram-negative, nonsporulating, nonmobile, aerobic, rod-shaped filamentous eubacteria that grows naturally at 70℃ in hot springs. It able to grow as matrix-enclosed multicellular communities called biofilms. *T.aquaticus* NTU103 were analyzed by proteomic analysis in order to identify proteins involved in biofilm formation. Two-dimensional gel electrophoresis revealed distinct and reproducible different protein expression pattern between sessile and planktonic cells. According to previous results, I have selected chaperonin (GroE) for further study. GroE can be divided into GroES and GroEL; it was ubiquitous and essential for protein folding. In genetic approach, *groEL* mutants were generated by disrupting the gene via the double crossing over from *T.aquaticus* NTU103. It was unable to get correct *groEL* mutant. In addition, we were constructed GroESL overexpression strain of *T.thermophilus* HB27. The result of biofilm assay and scanning electron microscope showed that biofilm formation ability was increased when GroESL overexpression. In addition, growth curve result showed that GroESL overexpression strain had lower rate of growth. In biochemical approach, *groES* and *groEL* were cloned to pET-30a and pQE-30 plasmid, respectively then purified by nicol-chelate affinity chromatography and ready for antibody preparation. Western blot showed that GroESL overexpression was not influenced GalE, TTC1138 and TTC1483 expression. To analyze the function of GroESL, we used citrate synthase as substrate protein. Result showed that GroESL protein were able to suppress citrate synthase from aggregation and able to reactivate denatured citrate synthase. In addition, analysis of the relationship between GroESL and biofilm formation, we used GalE and Glyceraldehyde-3-phosphate dehydrogenase(G3PDH) as substrate protein. Result revealed that GroESL protein were not able to reactivate denatured GalE and G3PDH. In previously study, GroESL from *Thermus thermophilus* that control refolding of several enzymes for macromolecule synthesis, including isopropylmalate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase. Taken together, these result suggest that GroESL increased biofilm formation by regulated macromolecule synthesis.

P04

Characterization of Acid Resistance Genes Involved in Biofilm Formation of *Vibrio parahaemolyticus*93

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Vibrio parahaemolyticus is a major, world-wide cause of gastroenteritis, they caused human diarrhea and fatal septicemia. The aim of this research was to known why *Vibrio spp*. can resistant stomach acid and reach to small intestines furthermore cause human disease. In this research, acid resistant mutants from transposon mutant library (construct by Mei-Shiuan Yu lab), were screened using TSB3S medium containing pH5 hydrogen chloride, and also used arbitrary PCR to amplified the genes which disrupt by Mini-Tn*10*. We used BlasN program, and found three acid related genes in *Vibrio parahaemolyticus* after sequencing. The function of individual mutant were putative multidrug resistance protein (11F8)、hyperosmotically inducible periplasmic protein (E12)、transcriptional activator RfaH (B8), respectively. Comparing wild type and mutant growth curve there are no significant difference suggesting that transposon were not influence the survival of mutant. Results also revealed that no significant difference between wild type and mutants while under acidic or osmotic stress. Interesting, biofilm forming ability of 11F8 was increase and which of it were reduced in B8 and E12 in compare with WT when bacteria culture for 48 hours. In contrast to previous study, the data of RfaH biofilm formation was opposite to *E.coli* research, indicated the regulation of *Vibrio parahaemolyticus* biofilm was different with *E.coli.* In the meantime, the rugose colony morphology of 11F8 was observed, suggested that was the reason of biofilm higher than wild type.

P05

The Effects of Androgen and Androgen Receptor (AR) signaling on D1 cell Adipogenic Differentiation

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Androgens (such as testosterone and 5α-dihydrotestosterone (DHT)) are the main male sex steroid hormones. Androgens not only affect the development of male phenotypes during embryogenesis and the sexual maturation at puberty, but also influence the functions of skin, bone, muscle, and brain. Androgen receptor (AR) belongs to the nuclear hormone receptor superfamily and mediates the action of androgen as a transcription factor to regulate it's target gene expression. Studies have showed the regulatory role of A/AR signaling on adipogenic differentiation, but the molecular basis of androgen-related adipogenesis decrease is still unclear. In the present study, we examined the role of A/AR signaling and its molecular mechanism on adipocyte differentiation at the cellular and molecular level using D1 cell line, a multipotent mouse bone marrow stromal precursor cell line, as a model. We determined the expression and function of AR on the adipogenic differentiation of D1 cells by observing the molecular and cellular changes between undifferentiated and differentiated Dl cells. Our data showed that hormonal induction cocktail with dexamethasone (10^{-6}M), insulin (0.01mg/ml), and rosiglitazone (20μM) treatment (added EtOH as control) induced D1 cells to adipocytes, demonstrated by Oil-red O staining, as well as the enhanced expression of adipogenic marker genes such as aP2 and PPAR-γ by semi-quantitative RT-PCR. Further experiments showed that DHT (10^{-8}M) treatment in cocktail differentiation medium caused less lipid storage and decreasing adipogenic marker genes expression level during the adipocytic differentiation of D1 cells. And the XTT data indicated that the induced D1 cells treated with cocktail differentiation medium with EtOH as a vehicle control, had lower cell proliferation than both undifferentiated control and induced D1 cells with DHT treatment in differentiation medium. These results indicated that DHT suppresses D1 cells into adipocytes. Currently, we further explored the possible molecules involved in the regulation of A/AR signaling on adipogenic differentiation with proteomic approaches. The 2D-electrophoresis data indicated that patterns of proteins expression in D1 cells with DHT treatment or not were different; therefore we will further identify these proteins by MS. We hope our study will delineate the molecular mechanism, by which androgen/AR signaling exerts on adipogenic differentiation.

P06

The Transcriptional Mechanism for Snail to Mediate Gene Expression of $p^{15INK4b}$ and Matrix Metalloproteinase 9 Induced by 12-O-tetra-decanoylphorbol-13-acetate

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Transcription factor Snail plays multiple roles in development and tumor metastasis by positively or negatively regulating a lot of target genes. However, the transcriptional mechanism for Snail to upregulate gene expression was still unclear. Here, we use hepatoma cell HepG2 as a model to investigate the detail transcriptional mechanisms by which Snail upregulates gene expression of $p15^{INK4b}$ and matrix metalloproteinase 9 (MMP9), induced by tumor promoter 12-O-tetra-decanoylphorbol-13-acetate (TPA). Snail can bind on a proposed target region upstream of a putative EGR-1/SP-1 overlapping site to associate with EGR-1 and SP1 for activation of $p15^{INK4b}$ promoter. The similar mechanism can also be observed in Snail-mediated activation of MMP9 promoter. Thus we proposed a novel mechanism by which Snail plays as positive regulator of transcription.

P07

Ethambutol Induces PKC Isozymes Activity for Cytotoxic Effects on Human Retinal Pigment Epithelial Cells (RPE)

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Ethambutol (EMB) is one of the first line anti-tuberculosis drug and one of its adverse effects that usually be reported is ocular toxicity. Even some cases have been reported that patients who receive EMB treatment became blind. However, the pathogenesis is not addressed clearly so far. It has been reported that EMB may damage the ocular nerve. Recently, we also observed that EMB had toxic effect on retinal pigment epithelium (RPE) cells, including intracellular vacuoles formation and reduction of phagocytic ability. Human RPE cells locate on the most inner layer of retina to keep choroid off retina. They can absorb and metabolize toxic substances, the poisonous waste from Cone photoreceptor and Rod photoreceptor, to protect ocular nerves. The molecular mechanism for regulation of the phenotypes of RPE cell has been well studied. One of the critical signal pathway that regulates RPE growth and pathogenesis is mediated by protein kinase C (PKC). We also found that the toxicity of EMB on RPE cell is via activation of PCK pathway. It has been known that there are at least 12 kind of PCK isozyme with different physiological functions. Therefore, we want to investigate what kind of PKC isozymes are involved in the pathway for EMB-induced toxicity on RPE cells. In the begining, we examined whether vary kind of isozyme inhibitors and shRNA of PKC affect EMB toxicity on RPE cells. We observed that inhibitor of PKCδ (rottlerin), shRNA of PKCβII and PKCδ all had preventive effects on vacuoles formation and reduction of phagocytic ability of RPE induced by EMB. In Western blotting analysis, we observed that PKCβII translocated from cytosol to cell membrane in RPE cells after treatment of 8mM EMB for 3 hours. Membrane translocation of the other PKC isozyme PKCδ was not induced by EMB, however, induction of gene expression of PKCδ was observed. These results indicated that the toxicity of EMB to RPE cells is mediated by activation of PKCβII and induction of PKCδ gene expression.

P08

The Effect of Glucocorticoid /Glucorticoid Receptor on Erythroid Differentiation of K562 Cells

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Glucocorticoid receptor (GR) belongs to the nuclear receptor family which comprises ligand-dependent transcription factors involved in regulating the expression of genes critical for cell growth, differentiation, and metabolism. Glucocorticoid receptor regulates gene expression by binding to a specific DNA sequence called glucocorticoid responsive element (GRE). Glucocorticoid and their cognate receptors have a direct effect on the differentiation of erythroid precursor cells from animal or human bone marrow. However, their mechanisms of intracellular action in differentiating cells are not clearly defined. In the present study, we examined the role of GR and its molecular mechanism on erythroid cell differentiation using K562 cells, an erythroleukemia cell line. We used a GR agonist, dexamethasone in our study to determine the role of GR in K562 cell erythroid differentiation. First, we examined the change of the of GR expression level during by Western blot assay. The effect of glucocorticoid-GR signaling on K562 cell erythroid differentiation was examined by benzidine staining for the change in their hemoglobin and glycophorin expression by flowcytometry. Moreover, the expression level of genes involved in erythroid differentiation such as GATA-1, c-Myc or other transcription factors was compared between the differentiated cells with or without dexamethasone treatment by RT-PCR. The effect of dexamethasone on cell proliferation was determined by XTT assay. Reporter gene assay was used to demonstrate the transcriptional regulation of GR during erythropoiesis of k562. This study will find the roles of GR in erythropoiesis and its molecular regulatory mechanism on erythroid cell differentiation.

P09

Evaluation of Matrix Effects in Pesticide Residue Analysis

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Previous publication pointed out that the matrix extracted the analyte, may coelute with the anslyte during chromatographic separation . When the analytes are determined with LC-MS, matrix may affect the target signal relative to the pure solvent signal by enhancement or suppression.

The laboratory pesticide residue analysis use two methods: 1.Standard addition method: adding reference standards (at least two different concentration) in the samples, and teated with the uncpibed sample to determine pesticides concentration. 2. Calibration curve method: using a series of standard solutions of different concentrations to obtain the relationship between the response vs concentration. In this research, a variety of matrixs were teated for their effects on 230 pesticides using liquid chromatography-tandem mass spectrometry and gas chromatography mass spectrometry to determine matrix effects. Different metrixs of blank sample (blank) after liquid / liquid extraction, will be add with the pesticide standard solution and compared with pesticide standard solution. The purpose of this study is to determine whether it is possible to use one matrix for calibration curve, and to quantify of pesticide residues in other matrix.

P10

Isolation of Novel Bacteriophage Endolysins with Lethal Activity against Multidrug-resistant Bacteria

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In recent years, the problem of antibiotics abuse had become more and more serious, which made many bacteria to develop the resistance to many antibiotics, including the *Acinetobacter baumannii*. *Acinetobacter baumannii* is an opportunistic pathogen which often caused nosocomial infection in recent years. In order to solve the problem that there was no optimal drug for treatment of patients with multiple drug resistant bacteria infection, we attempted to find some protein that could be used on cure of infection with multiple drug resistant *Acinetobacter baumannii*. Previous studies have shown that the endolysin that bacteriophage manufactured after infecting bacteria can degrade the peptidoglycan layer of bacterial cell wall. It may be an effective antibacterial agent. In the study, we found the gene predicted endolysin with the bioinformatic tool in the chromosome of *Acinetobacter baumannii* ATCC17978. In the same time, we also found the second endolysin gene in *Acinetobacter baumannii* bacteriophage. To characterize antibacterial activity of two kind of endolysin encoded by *Acinetobacter baumannii* and its phage respectively, were cloned into the expression plasmid. The resultant plasmid was used to transform *Escherichia coli*, and then expressed and purified them. Analysis of the biological activity, we observed that our protein inhibited both gram positive and negative bacterial survival. To three different bacteria, it caused the cell wall destruction by the purified protein with the scanning electron microscope. Furthermore, in order to find out whether the peptidoglycan was the substrate of the protein or not, we performed the zymogram assay. The results showed that our protein could degrade the peptidoglycan and inhibit bacterial survival.

P11

Study on Cellular Factor(s) Facilitating Influenza A Virus Replication

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To search for cellular factors affecting influenza A virus replication, cellular genes differentially expressed between mock-infected and virus-infected A549 cells were screened by microarray analysis and ddPT-PCR. In microarray analysis, three genes (DHFR, RRM1, RRM2) down-regulated and two genes (DEAD box and IFIH1) up-regulated 23 hrs after virus infection were selected for further analysis. In ddPT-PCR, three genes (PRPF8 , RPL35, DBI) up-regulated after virus infection were selected for further analysis. Cellular genes up-regulated after virus infection could be factors facilitating virus propagation. If it is the case, knockdown of these genes using siRNA technology should inhibit virus production. On the other hand, cellular genes down-regulated after virus infection could be factors suppressing virus propagation. In this case, over-expression of these genes should also inhibit virus production. At present, two cellular factors possibly affecting influenza A virus propagation were identified.

P12

Heparin Cofactor II Represses HCV Replication

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Infection with hepatitis C virus (HCV) will cause chronic infection, liver cirrhosis, and even hepatocellular carcinoma (HCC). At present, neither an effective treatment for chronic HCV infection nor a vaccine to prevent HCV infection is available. Identification of host factors involved in viral replication is critical for understanding the molecular mechanism of virus replication. Identification of host factors involved in viral replication also facilitates the development of anti-viral agents. Genes differentially expressed (over-expressed or down-regulated) in HuH7 cells with or without HCV sub-genomic replicon were identified by dd-RT-PCR. Genes over-expressed in HCV replicon cells could be the factors facilitating HCV replication while down-regulated genes could be the factors repressing the HCV replication. Heparin cofactor II (HCII) identified by ddRT-PCR was down-regulated in HCV replicon cells. To determine whether HCII represses HCV replication in the HCV replicon systems, both loss-of-function (knockdown of HC II) and gain-of-function (over-expression of HC II) approaches were used. Our results showed HC II could indeed inhibit HCV replication. Furthermore, heparin could reverse the suppressive effect of HC II on HCV replication. HCII represses the HCV replication possibly through interacting with HCV NS3 protein. The binding domains of HCII and HCV NS3 proteins were also determined by yeast two-hybrid system.

In conclusion, our studies suggested HC II could interact with HCV NS3 protein and in turn represses HCV replication. Further studies to characterize the mechanisms how HCV down-regulates the expression of HC II are needed.

P13

Structural Analysis of Hepatitis C Virus (HCV) Core Assembly and HCV Core-lipid Interactions

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Hepatitis C virus, one the major causes of liver diseases, consists a positive-strand RNA , a virus core, and an envelope containing envelope proteins (E1, E2) and lipid bilayer obtained from the host. The knowledge of core assembly is important for the true understanding of the virus formation mechanism. The full length core protein consists of 191 amino acids with a highly hydrophobic tail. Because of its highly hydrophobic nature, the full length core protein have been very difficult to be expressed in bacterial systems and purified in aqueous conditions. Facilitated with fermenting technique and urea system, we have successfully over expressed and purified the full length core protein in sufficient quantity for the core assembly study.

The atomic force microscopy (AFM) and transmission electron microscopy (TEM) both showed that the assembled core particles formed by the full length core protein were significantly larger than those assembled by C-terminal truncated proteins indicating that the hydrophobic tail did affect the core assembly in some extent. As the virus core in a mature virus is covered with a layer of lipids gained from the mammalian host cells, the interactions between the lipids and the core protein might also play an important part in correct virus core formation. By applying the synchrotron radiation circular dichroism (SRCD) and protein fluorescence wavelength shifts, the structural dynamic changes of the core protein when interacting with lipid DMPC were analysed. According to the SRCD data, when interacting with DMPC, the α-helix contents of the core protein increased from approximately 7% to 17% and β-sheet contents decreased from approximately 41% to 30% in both solutions and thin films strongly implying the contributions of the lipid-protein interactions in virus core formation which was not been investigated previously.

P14

開發最佳化參數方法用於癌症分類技術的改良

Developing Optimize Parameter Algorithm for the Improvement of Cancer Classification

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微陣列晶片(Microarray)資料研究的高度發展，儼然已成為各種癌症疾病在做臨床診斷和預測時的重要資料，然而微陣列晶片資料卻有著高維度(High-dimension)的問題，因此適合用資料探勘的方法來分析。資料探勘的監督式學習方法是一種機器學習的技巧，可以從輸入的資料訓練一個模組，由這個模組來做分類(Classification)。目前廣泛被使用的分類演算法皆已行之有年，分類準確率的比較常常是這個領域研究者們評判分類器優劣的依據，然而影響分類器準確率的因素很多，分類器參數的設定就是其中一個。

在分類微陣列資料這個領域上，支持向量機(Support vector machine, SVM)總是準確率最高的，但參數設定對結果影響很大；隨機森林(Random forests, RF)是近年來新興的分類器，準確率能保持高水準，但是其發表者說參數設定對結果影響不大。然而以往的研究在分類器參數的設定上並沒有一個標準，多是採用人為設定，如此對分類器準確率的比較存在著不公平的問題。

因此本論文將搜尋參數方法和分類器做結合，開發能夠自動搜尋最佳參數的新型分類器，可以幫助評估人為設定參數及自動搜尋參數的分類器，哪種分類準確率較高。期望最佳化參數能夠提升分類器的分類準確率，並提供一個新的改良癌症分類的方向，能夠讓分類器發揮更大的效能。

關鍵字：微陣列晶片、資料探勘、機器學習、支持向量機、隨機森林

P15

以微型核糖核酸表現為基礎的人類心臟疾病研究

The Study of Human Heart Disease Base on MicroRNA Expression

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近年來的研究發現了一種嶄新的核糖核酸結構，這種核糖核酸不會轉譯成蛋白質，但是對於人體機能有極重大的影響，稱為微型核糖核酸（microRNA）。microRNA 的長度很短，只有大約 19 到 24 個核苷酸（nucleotide），這種微小的核糖核酸序列不會轉譯成蛋白質。在人體內的 microRNA 對於特定基因有抑制的能力，因此 microRNA 在人類疾病中扮演著極為重要的角色，異常表現的 microRNA 會導致致病因子的產生而致病。目前許多研究使用 microRNA 微陣列（microarray）產生的 microRNA 表現資料，研究與分析出疾病中異常表現的 microRNA，進而達到疾病分類和預測的效果。

本論文將使用兩種網路建構技術建立起三種心臟疾病類別的 microRNA 關聯性網路，研究 microRNA 之間在各類別下的相互關聯性。藉由研究建立各類別的關聯性網路，我們可以瞭解其中的網路結構特性、microRNA 的關聯途徑和重要的生物標誌(biomarker)。更進一步地，藉由比較各類別分別加入測試樣本之後，導致網路拓撲結構的改變程度，而達到分類和預測出三種心臟疾病類別的效果。

關鍵字：微型核糖核酸、微陣列、網路拓撲。

P16

Generation and Characterization of Conditional TCTP Mouse Mutants in Nestin-Cre Derived Nervous System

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Translationally controlled tumor protein (TCTP) was originally highly conserved protein that is linked to cell growth, survival, allergy, tumor reversion and anti-apoptosis signaling pathway. Recently, TCTP protein level decreased in Alzheimer's disease and Parkinsonism patients. Hence, TCTP may implicate in the progression of neurodegeneration. Our results showed TCTP expressed high levels mRNA in forebrain and heart at stages of E10.5, little is known regarding its role in nervous system development. The aim of this study is to address the function of TCTP in the center nervous system. We generated Conditional deletion of TCTP in neural progenitor cells mediated by Nestin-Cre resulted in early postnatal lethality, increase apoptotic cell death, impaired corticogenesis, and reduced proliferation of progenitor cells in the ventricular zone. Here, we showed for first time that TCTP is required for mice early postnatal survival and growth, and is important for maintaining the brain morphological development. Loss of TCTP in neuronal progenitors leaded to lost distribution of neurons in ventricular zone of cortex and dentate gyrus. Deletion of TCTP caused the decreased Mcl-1, Bcl-xL, Hax-1 and cyclin D2 protein expressions. Therefore, it might contribute to regulation of apoptosis and G1 phase cell cycle in brain. Take together, our results are first to demonstrate the requirement of TCTP not only for neonatal survival, but also preventing brain morphology defect. TCTP could be proved a novel strategy of gene therapy for study of Alzheimer's disease and Parkinsonism.

Key words: TCTP, Neurodegeneration, Alzheimer's disease

P17

Expression and Characterization of SK△59, a Truncated Form of Streptokinase with NH_2-terminal 1-59 Deletion

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Streptokinase (SK), a currently used antithrombotic agent, was regarded as a fibrin-nonspecific plasminogen activator compared with tissue-type plasminogen activator (t-PA). A recent study found that removal of the N-terminal residues 1-59 allowed the remaining molecule (SK△59) to cause plasminogen activation in a *fibrin-dependent* manner, suggesting that SK△59 harbors the potenetial to become a novel thrombolytic agent. This study aimed to mass-produce SK△59, which allowed us to study further the action mechanism of SK△59 in lysing thrombi.

The DNA segment corresponding to the SK△59 was amplified by PCR and cloned into the expression vector pGEX-2T, which allowed SK△59 to be produced in fusion to the C-terminus of glutathione S-transferase (GST) in *Escherischia coli*. The successful transformant was grown in LB culture medium with additive of ampicillin (100 μg/ml) and glucose (20 mM). As the cells reached mid-log growth, IPTG (0.2 mM) was added to trigger GST-SK△59 expression for 5 hours. After that, cells were harvested, disrupted by sonication, and the GST-SK△59 fusion protein in the soluble fraction was purified with glutathione Sepharose 4B affinity column followed by anion exchange column chromatography. The GST part was then removed by thrombin cleavage.

Purified SK△59 exhibited a high purity when analyzed by SDS-PAGE. Chromogenic substrate assay revealed that SK△59 was less potent than SK in converting glu-plasminogen into plasmin, whereas it was as well as SK in triggering fibrin clot lysis in test tubes. These results, in accordance with previous observations by other investigators, encourage us to investigate further the mechanism underlying the activation of plasminogen by SK△59.

P18

The Mechanism of Curcumin Induced Apoptosis in Hepatoma J5 Cell Line.

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Curcumin has been examined for antitumor effect on many tumors. However, little is known about molecular mechanisms for Curcumin induced pro-apoptosis endoplasmic reticulum stress in hepatoma cell line. J5 cells were treated with increase dose of Curcumin for 24 and 48 h. The cell viability was detected through MTT assay and the observation of cell morphology. The protein expression of Caspase-12, ATF-4, CHOP, Calnexin, Calreticulin, PDI, and Ero1-Lα which attend the endoplasmic reticulum stress and unfolding protein response pathway were examined by western blot. The cell cycle assay was detected by flow cytometry. The protein expression of mitochondria dysfunction, TCTP, Mcl-1, Bcl-2, and Bax were detected by western blot. The locations of protein expression were examined by Immunocytochemistry. Our data suggest that Curcumin induced unfolding protein response through down regulation of Calnexin, PDI, Ero1-Lα and up regulation of Calreticulin, a Ca^{2+} stress important protein. Curcumin induces CHOP expression through cleaving caspase-12, ATF-6 and translocating to nuclear. Curcumin causes mitochondria dysfunction through down regulation of Mcl-1, Bax and Bcl-2. In addition, Curcumin also affects cell cycle through Cdc2 down regulation. In summary, this present study shows that Curcumin induced apoptosis are associated with endoplasmic reticulum stress, cell cycle arrest at G2/M phase and mitochondria dysfunction in J5 cell line.

P19

Acetyl-L-Carnitine Ameliorates Methamphetamine -induced Behavioral Dysfunction in Mice

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Methamphetamine (METH) is a widely abused illicit psychostimulant. METH abusers show behavioral and cognitive deficits and neurodegenerative damage. Acetyl-L-carnitine (ALC), an endogenous quaternary ammonium compound, plays a vital role in the mitochondrial oxidation of fatty acids and shows a protective and regenerative action profile on the nervous tissue after toxic or traumatic injuries. ALC may exert its neuroregenerative effect on the basis of their neurotrophic and neuromodulatory properties. In this study, we investigated the therapeutic effects of ALC on METH-induced behavioral deficits. Male ICR mice were received one day drug treatment with four injections of METH (4 x 5 mg/kg, S.C.) or saline at 2 h interval. Behavioral tests including novel location recognition test (NLRT), novel object recognition test (NORT) and social interaction were performed 7 days later to confirm the METH-treated animals with apparent behavioral deficits. After subsequent administration of ALC (30 and 100 mg/kg, I.P.) once daily for seven consecutive days, behaviors were assessed again. The data show ALC could reverse METH-induced impairment in NLRT, NORT and social interaction. These findings suggest that ALC may be a novel therapeutic strategy for treatment of METH abuse-related behavioral abnormality.

P20

Compare the Brown Adipose Tissue in Mammary Gland of hHB-EGF Transgenic Mice with Wild Type Mice

比較 hHB-EGF 轉殖小鼠乳腺中棕色脂肪與野生型小鼠的差異

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hHB-EGF (Human Heparin-binding epidermal growth factor-like growth factor) is a member of EGF family, whish has strong affinity for heparin. It can be produced as a membrance-anchored form (pro-HB-EGF) and later processed to a soluble form (s-HB-EGF) by ADAM (a disintegrin and metalloproteinases) and MMP (matrix metalloproteinase). Our preliminary discovery that there were hyperplastic brown adipose tissue (BAT) in the mammary mesenchyme in the hHB-EGF transgenic mice at lactation day 10. To understand the role of BAT in the mammary mesenchyme of hHB-EGF transgenic mice, we must first compare the amount of BAT in all lifespan of hHB-EGF transgenic mice with wild type mice. We use whole mount and HE staining of mammary gland to understand the spread of BAT and use RTPCR and Western blotting to detect the RNA and protein expression of BAT. Then we compare RNA expression of BAT between hHB-EGF transgenic mice and WT mice by statistics diagrams.

P21

Identification of Novel Genes Involved in Erythrocytic Differentiation

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Erythrocyte differentiation is a multistep process regulated by cell-type specific genes expression. Our previous studies indicated that *Bacillus anthracis* lethal toxin (LT) could suppress GTP (guanosine 5'-triphosphate) induced erythrocytic differentiation in erythroleukemia cell line K562. To investigate the novel genes regulate erythrocytic differentiation, microarray analysis was performed. Our results revealed that five retinoid related genes-neural proliferation, differentiation and control protein (NPDC1), retinol binding protein (RBP5), B-cell translocation gene family with anti-proliferative properties (BTG2), retinoic acid receptor responder protein 3 (RARRES3) and retinoid X receptor alpha (RXRA) were up-regulated after GTP treatments and down-regulated after LT pretreatments. We hypothesized these genes may play a conceivable role in normal and LT-suppressed erythrocyte differentiation. Flow cytometry was used to study whether the differentiation abilities of K562 cells were blocked by shRNA of candidate genes. According to our data, there were four genes (NPDC1, RBP5, BTG2 and RARRES3) may play putative roles in erythrocytic differentiation.

P22

The Effects of SV40 T-antigen in Transgenic Mice

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Simian Virus 40 (SV40) is a small DNA tumor virus that has been used extensively to study tumorigenesis. The SV40 early region encodes three tumor antigens, large T (LT), small T (ST) and 17KT that contribute to tumor formation. In our previously study, transgenic mice expressing SV40 T/t-antigen driven by mammary gland specific promoter WAP were used as animal model to study its metastatic role in breast cancer. We observed some of transgenic mice expressing SV40 T/t-antigen has shown metastasis into lung, but its molecular basis is largely unknown. To understand the molecular basis, we will use microarray to identify and characterize the genes which involves in the metastasis. We will further examine whether these genes play a role in the invasion of the tumour cells into neighboring tissues through processes such as migration, invasion and EMT (epithelial–mesenchymal transition).

P23

Roles of Dengue Virus Envelope Protein on the Elicitation of Thrombocytopenia, Platelet Activation and Coagulopathy

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Dengue virus (DENV) belongs to the flavivirus family, and it's transmitted to human by Aedes mosquitoes. DENV infection is a major cause of clinical symptoms, the more severe forms are DHF/DSS, which are characterized by thrombocytopenia, hemorrhagic manifestations. However, the mechanism of DHF or DSS development are not clear. In a previous study , we found that purified recombinant soluble dengue envelope protein domain III (rsEIII) can be directly bound to human platelet , and induced platelet activation on cytometry , and rsEIII can induce thrombocytopenia in B6 mice, which were similar to clinical sign observed at early stage of dengue virus infection patients . In this study , We have established an *in tro* model , using ELISA hight thought-put screening rsEIII and platelet inhibitors block the combination , using SEM to observe combines rsEIII on platelet number and morphology , to observe the levels of platelet activation. From past studies we know that the flavivirus virus infection of host cells primarily through cell surface heparin sulfate receptor , as a GAG , so do it in several common (GAG CS/Heparin)screening . The results indicate that , heparin , heparin I , heparin III , heparin I-S , chondroitin sulfate (CS)A , CSB , CSB will reduce rsEIII and platelet combination , heparin I most obvious . In the SEM was found when the treat rsEIII higher the concentration, combined with the greater the number of platelet, and the higher the levels of activation. Will then build *in vivo* model to observe the coagulopathy, and whether it can be used to save ELISA screening to the GAG.

P24

Effects of Granulocyte-colony Stimulating Factor (G-CSF) on Anthrax Lethal Toxin Induced Anemia

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Anthrax, a disease caused by *Bacillus anthracis* infection, induces animal and human death through unknown mechanisms. Lethal toxin (LT), a mitogen-activated protein kinase kinase (MAPKKs) inhibitor, is the major virulence factor of *B. anthracis*. LT treatments cause lethality and certain anthrax-like pathogenesis of experimental mice that including hypoxic tissue damages and anemia. It is suggested that LT-induced hemolysis plays certain roles. Our previous studies showed that treatments of LT inhibited not only the proliferation and guanosin 5'-triphosphate (GTP)-induced erythroid differentiation of a human erythroleukemia cell line K562, but also reduced the number of erythroid colonies in the mouse colony-forming cell (CFC) assay, by the way, both percentage and cell numbers of bone marrow erythroblasts were suppressed. Since granulocyte-colony stimulating factor (G-CSF) is a pleiotropic cytokine playing a major role as regulator of hematopoiesis and has the ability to protect cells from apoptosis. These prompt us to analyze whether treatments of G-CSF could ameliorate LT-induced anemia and lethality. Our data showed that G-CSF treatments reduced LT-mediated mortality is associated with amelioration of reduced anemia response. The underlining mechanisms will be further investigated.

P25

Mechanism Underlies Dengue Viral Envelope Protein Elicited Leukocytopenia

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Dengue virus, a member of the *flavivirus* family, causes an emerging global health threat. Infection of dengue virus can result a range of clinical symptoms, including the severe diseases dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The clinical symptom of DHF/DSS includes plasma leakage、leukocytopenia、thrombocytopenia and increased vascular permeability. However, the molecular mechanism of DHF or DSS is not yet clearly. In this study, we found that intravenous injection of recombinant dengue envelope protein could reduce the leukocyte counts, specifically the monocyte counts, in mice, which is similar to the clinical observations in dengue patients. In addition, an antagonist for TNF-α pathway seems to have ameliorative effect in dengue envelope protein-induced low leukocyte counts. Our data suggested that there may be a TNF-α dependent pathway involved.

P26

Mechanism Underlying Chemical Induced Liver Damage and Thrombocytopenic Response

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Currently, hepatitis is a major cause of death in Taiwan. Hepatitis can lead to liver cirrhosis and hepatoma. In clinical studies, hepatitis was shown to cause thrombocytopenia. Since thrombocytopenia causes bleeding tendency, these patients are unable to use liver transplantation to save their life. The mechanism responsible for hepatitis-induced thrombocytopenic is not yet clear. Thioacetamide (TAA) has been used extensively in the development of animal models of acute liver injury. In this study, TAA (50 mg/kg) is administered intraperitoneally to induce liver damage in mice. After TAA administration, we observed elevated blood levels of aspartate transferase (GOT) andalanine transferase (GPT) and reduced platelet counts in these mice. Flow cytometry data indicated that there are anti-platelet antibody productions. The mechanism responsible for this chemical-induced thrombocytopenia and autoantibody production remains to be further investigated.

P27

Regulation of Borealin Degradation in Mitosis

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It has been proposed that chromosome passenger complex (CPC) which comprises INCENP, Aurora B, surviving and Borealin is important for key mitotic events. The CPC regulates various mitotic functions to maintain genomic stability. Borealin is required for stability of the bipolar mitotic spindle. Borealin protein levels increase during G_2/mitosis, and is decreased during mitotic exit. How Borealin is regulated in this process remains to be determined. The aphase-promoting complex/cyclosome (APC/C) is an *E3 ubiquitin* ligase that targets proteins for degradation by the 26S proteasome and regulates mitotic exit. Here, we show Borealin protein level is regulated by Cdh1, one of the activators of ubiquitin ligase APC/C. Furthermore, Borealin contains the APC/C recognition signals, a N-terminal D box implying for ubiquitin dependent destruction. Our results imply that the D box in Borealin might be required for Cdh1-induced destruction of Borealin.

P28

The Role of *Phytochrome B* and *Poltergeist* in Plant Development

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在 *Arabidopsis* 中存在著多種的光接受器，有接受藍光為主的 cryptochrome、接受 UV-B 的 phototropin，以及主要接受紅光與遠紅光的 phytochrome。在擬南芥中的 phytochrome 有五種(A、B、C、D、E)分成遇光穩定型的 phytochrome B~E，以及遇光快速分解的 phytochrome A。在接受到紅光的刺激之後，Phytochrome 由 Pr 轉變成 Pfr 的活化形態，並進到細胞核中藉由 PIF3、PIF4 等許多的 transactivation factor 調控光型態發生(photomorphogenesis)基因的表現，使幼苗胚軸停止延長、子葉張開等反應。

CLV(CLV1、*CLV2*、*CLV3)*、*WUS* 是一些會影響頂端分生組織的基因，其中 WUS 促進分生組織細胞的細胞分裂，而 *CLV* 基因則促進細胞分化，二者在維持頂端分生組織的形成上都扮演重要的角色，*POL* 可調控 *CLV* 對 *WUS* 的抑制作用，*clv pol-7* 雙重突變株的種筴型態反而會逆轉回像野生株的型態。

之前的結果發現如果先給予黑暗中的幼苗短暫紅光，*pol-7* 的胚軸會比野生株短；已知 PHYB 蛋白和 POL 蛋白之間有交互作用，且 POL 的 C 端具有微弱 phosphatase 2C 的活性，我們推測此活性可能調節 PHYB 引發反應。另外，觀察到 *pol-7* 在短日照時，呈現多個營養葉底座(rosette)的型態而野生株只有一個，且 *phyB-9pol-7* 的雙重突變株有更多營養葉底座的趨勢，由此推測短日照之下多出營養葉底座表示可能跟頂端分生組織發育有關，所以我們進一步探討 POL 和植物頂端分生組織基因 *CLAVATA*(*CLV*)、WUS 之間的關係，希望能得知 POL 是否位於 phytochrome 與 CLAVATA 兩條 pathway 之間作為調控的角色。為了了解 POL 在植物頂端分生主織的發育上所扮演的角色，我們以 *POL*、*WUS*、 *CLV* 的啟動子來表現 gluculondase(*GUS*)基因片段，並轉殖到 *pol-7*、*phyB-9* 和野生株擬南芥中來看 POL、WUS、 CLV 在長短日照之下所表現的位置，進一步來分析之間的關係。

EODFR/EODR 實驗確定 POL 確實會調控 PHYB Pr/Pfr 型態的轉換；**Dark reversion** 實驗顯示在正常情況下 POL 會對 PHYB 的去進行去磷酸化作用，改變 phytochrome Pr/Pfr 的比例而使胚軸縮短；**GUS staining 結果指出**在短日照下， PHYB 促進 POL 表現，PHYB、POL 共同促進 WUS 表現，抑制 CLV1 表現，加上 CLV2 表現量低，推測這樣的結果會使短日照之下開花期的擬南芥頂端分生組織偏向細胞增生，花序分化不全，而呈現多個營養葉底座的型態。可能還有其他的去磷酸化酶和 POL 共同將 PHYB 去磷酸化，接下來將進一步探討 PLL(poltergeist like)基因對 PHYB 去磷酸化的影響。

P29

The Effects Testosterone on Stress-Induced Activation of Medial Preoptic Nucleus in Male Rats

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It is well known that there is sexual difference on stress responses. Studies indicate that testosterone (T) shows an inhibitory effect on stress-induced hypothalamic- pituitary-adrenal (HPA) activation. Androgen receptor (AR) is found throughout the central nervous system, but paraventricular nucleus (PVN), the integrator of HPA activity, expresses little. Evidence shows that medial preoptic neucleus (MPN) expressing robust of AR plays a critical role on modulator effects of T on stress-induced HPA axis activation. The mechanism, however, is not clear. Male SD rats (250-300g) were used in this project. First, we clarified if there was difference on stress-induced neuronal activation at MPN in intact and gonadectomized (GDX) rats. The rats were divided into four groups: sham-GDX (intact) and GDX rats and sampled under basal or restraint stress conditions. The numbers of Fos-immunoreactive (ir) cells were regarded as the indication of neuronal activation. We found that, the numbers of Fos-ir cells at both MPN and PVN in GDX and intact rats were similar under non restraint condition. But GDX rats showed more Fos-ir cells than intact control after restraint challenge. T replacement using subcutaneous Silastic implants (each 35 mm in length, 0.062 mm ID, 1.25 mmOD) that were packed with crystalline testosterone, reversed the effect of GDX on restraint-induced Fos expression at MPN. These results indicated that T might tonically inhibit stress-induced HPA activation through inhibiting MPN neuronal activity.

P30

小鼠感染性心內膜炎動物模型之建立

Establishment of a Mice Model of Infective Endocarditis

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本篇論文研究目標主要是利用金黃色葡萄球菌和聚氨酯細管並且改良手術的方式建立小鼠的心內膜炎動物模型，研究此動物模式下所引發的心內膜炎並造成瓣膜生成贅生物以及其他部分器官金黃色葡萄球菌感染的現象。心內膜炎形成原因在於一開始當心臟內的內皮細胞受損使得血小板會包覆傷口。另外細菌表面會產生一種跟黏附相關的表面蛋白 (microbial surface components recognizing adhesive matrix molecules, MSCRAMMS)，使得金黃色葡萄球菌容易附著在血小板與纖維細胞上。金黃色葡萄球菌也會刺激血小板在細菌周圍凝集，不斷重複這過程會最後會在心臟瓣膜上面形成贅生物。以往心內膜炎的動物模式是在大鼠和兔子比較佔據空間，也因為體積較大使用藥物的劑量也較大。。所以我們改用小鼠可以減少實驗的空間和成本。過往之研究曾有使用一端堵塞的聚氨酯細管插入小鼠之頸動脈，製造主動脈瓣的損傷。此方法雖然有效，但亦有其缺點。例如主動脈瓣損傷的程度在組織切片前無法確實明瞭；此外，有外來物留存於小鼠頸部，也易導致併發症甚或小鼠死亡。

本篇論文我們在此將嘗試改良產生心內膜炎的方法，我們選用的是 ICR 小鼠手術的部份利用 32G 兩端都通的聚氨酯細管整根從左側頸動脈插入一直插入到主動脈瓣並來回插入，並使兩端聚氨酯細管都放入在左側頸動脈裡。使小鼠雖瓣膜受損但是不會影響其生存。再利用小鼠超音波來了解聚氨酯細管在小鼠心臟內的位置以及對心功能的影響，以確定手術對於小鼠的傷害程度。最後從尾部靜脈打入 $1*10^8$ C.F.U/ml 的金黃色葡萄球菌菌液。一天後藉由 H&E、Masson's trichrome 染色，來證實心臟瓣膜上贅生物的生成，以及巨噬細胞和嗜中性白血球細胞浸潤的現象。是故證明說經過改良的手術方式再打入金黃色葡萄球菌液可以造成小鼠心臟的心內膜炎，從對於俾使研究心內膜炎的動物模式對於抗菌藥物的研究可以廣泛利用。由於血栓素 A_2 (Thromboxane A_2)在贅生物的形成扮演很重要的角色。利用金黃色葡萄球菌的分泌的外毒素之一:α-毒素(alpha-toxin)去處理初級培養的心肌細胞跟纖維母細胞，由實驗結果發現 α-毒素可以促進血栓素 A_2 合成酶(Thromboxane A_2 synthase)。因此由以上的實驗結果得知金黃色葡萄球菌產生贅生物的過程是經由 α-毒素的刺激所參與。

P31

Social Influence on the Avoidance Learning in Rats

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This study investigates the role of different brain areas in the social learning. The Long-Evans rats were used in this study. The demonstrators were trained with a hot plate in the step-down inhibitory avoidance task. The observers were restrained in an observing box with a transparent window in front of the hot plate to observe the demonstrator to learn this task.

After the observers experienced the aversive stimulus, they stayed longer on the platform than the base level before encountering aversive stimulus. The staying times of the observers on the platform would be enhanced after they observed the demonstrators to learn the step-down task. If the observers stayed in the observing box without the demonstrators or with demonstrators not learning the task, the time on the platform would not be changed. Even the transparent window was covered with a thick paper, the observers still learned from the demonstrator and increased the staying time on the platform. In the different groups, anterior cingulate cortex (ACC), amygdala (AMY) and insular cortex (IC) lesions blocked the social learning ability of observers from the demonstrators.

These result suggested that rats had social learning ability in the step-down inhibitory avoidance paradigm and the ACC, AMY and IC might play roles in the social learning ability.

P32

Grouping of GBS with RAPD-PCR and Its Correlation with Virulence Factor and Isolation Sites

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Group B streptococcus (GBS) is a significant human pathogen, especially in newborns and pregnant women. It is the major cause of neonates meningitis and sepsis which often leads to fatal. Hence, the relationship between genotype and virulence factor has accentuated the importance of efficient and sensitive typing methods. A rapid and convenient method for detecting the genomic polymorphism of GBS was generated by RAPD-PCR. A 10-mers primer was used in this assay to achieve the polymorphic amplicons based on its ability to differentiate GBS genotypes. The isolates were clustered into four major groups with their respective polymorphic patterns. Intragroupic variations were detected indicating the heterogenous nature of individual GBS strains. Apart from that, we are also interested to investigate the correlation of the virulence factors with the RAPD-PCR groupings. A few putative virulence genes (*spb1*, *rib*, *bca*) which were previously recognized their roles in the epithelial adherence and invasive were selected to observe the distribution of these genes within those isolates. The objective of this study was to determine if DNA polymorphisms generated by RAPD-PCR could be utilized to characterize GBS for epidemiology purposes with the correlation of the virulence genes. From our studies, we have discovered that the virulence genes were mostly distributed in the RAPD-PCR group IV. Most of the isolates from this group were isolated from vaginal and rectal which we suspect they might be virulent strains. Thus, we concluded RAPD-PCR is a useful and reliable assay for the characterization of GBS isolates as it is able to discriminate between the genomic polymorphism of GBS which correlates with virulence factors and its isolation sites.

P33

Physiological Response and Gene Expression of *Vibrio parahaemolyticus* under Multiple Conditions

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Vibrio spp. is the most significance food borne pathogen in Taiwan which causes food poisoning with symptoms such as diarrhea, abdominal cramp, and nausea. This bacterium pathogen is virulence after transmitted to human gastrointestinal. The aim of this study is to identify the mechanism of acid adaptation of *Vibrio* spp. in human gastrointestinal and observe the physiological response and gene expression of *Vp*93 under multiple stress conditions. For physiology approach, we observed the growth condition of *Vibrio* spp. in both log phase and stationery phase after it was given acid adaptation treatment. The results showed that the *Vibrio* spp. in log phase has higher resistance than in stationery phase towards acidic environment after acid adaptation. On the other hand, we are also interested to investigate the effect of cross protection as there are also other stresses from the environment i.e. cold stress、oxidation stress. We found that *Vp*93 has no cross protection in osmotic stress-acid stress, and also acid stress-starvation stress or starvation stress-acid stress. Interestingly, we found that *Vp*93 was more acid resistant under low temperature. We use transcriptional profiling to identify genes that control acid resistance under cold stress, including putative hyperosmotically inducible periplasmic protein, o*xyR*, *RafH*, C-di-GMP phosphodiesterase and *csdA*. However, only *csdA* is involved in cold stress resistance mechanism, while the other four genes are osmotic stress resistance related gene. Thus, we presume that mechanisms involved in acid resistance are regulated by osmotic stress related genes in *Vp*93.

P34

Epiphytic Bacterial Communities on Marine Alga and Anemones

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Coral reef has the highest biodiversity of multicellular organism, among all marine habitats that have been surveyed. Epiphytic microorganisms have significant influence on host health, however, their diversity has not been systematically studied so far. This study was aimed to examined the diversity of epiphytic bacteria on various marine multicellular organisms in a local coral reef intertidal region. The dominant multicellular organisms in the sampling site include red algae, green algae, brown algae, and mat anemone. A total of 43 red algae, 16 green algae, 11 brown algae species were collected and identified in spring of 2010. Various mat anemone morphotypes were also sampled, but identification to species level were not achieved.

Epiphytic bacteria were isolated from algal homogenates and anemone mucus samples. The culturable community composition varied among algal samples. Epiphytic bacterial diversity estimated from culturable community is generally highest in red algae, and lowest in brown algae samples. Composition of culturable bacteria varied significantly among morphotypes of sea anemone. However, the bacterial diversity is lower than that in most algal sample, suggesting some microbial control measures might be present in mat anemones. Many small colored colonies were found in dimethyl sulfide-supplemented agar were recovered after prolonged period of incubation. These colonies also appeared in agar supplemented with red algae homogenate after incubation in the dark. These results suggested these isolates likely members of the newly discovered *Roseobacter* clade. We successfully enriched bacteria that can grow on medium supplemented with brominated organic compound as the only carbon source, from several red algae and brown algae. This suggests that some epiphytic bacteria may use this ability to evade brominated antimicrobials produced by macroalgae.

P35

Physiological Responses and Genetic Variations in *Vibrio vulnificus* Receiving Acute and Chronic Environmental Stress

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Vibrio vulnificus has an ubiquitous distribution in warm brackish waters, but it is also a pathogens and can cause primary septicemia and necrotizing wound infection. Rapid adaptation to various stresses ensures the survival of microbial cells, and this ability may be even important for *V. vulnificus*, which needs to switch between animal host and aquatic environments.

Tilapia-pathogenic *V. vulnificus* strain 93U204 was chosen to study the stress response and stress-induced evolution. We reasoned that if response to one stress can provide cross-protection to another stress, the physiological responding pathways to these two stress must have something in common. We examined the bacterial survival under acute hyperosmotic, cold, starvation, and acid stress. In most cases, when two types of stress were applied sequentially, no cross protection was found, and led to even higher mortality. Continuous sublethal stress may induce stress-induced mutagenesis and facilitate phenotypic change, which may lead to increase in fitness. We cultured *V. vulnificus* in chronic stress including low-nutrient (1% Tryptic Soy Broth), low-salinity (0.5% NaCl) and high-salinity (4.5% NaCl) conditions, and examined their stress tolerance after 8 and 15 days. Bacterial isolates derived from the triplicate samples exhibited both intra- and inter-treatment variation in phenotypic composition (hemolysis, proteolysis, motility). The longterm stress condition can select for certain phenotype and fix it genetically, which allows for further identification or characterization. Despite the type of stress used in selection, these cultures evolved tolerance to other types of stress, suggesting certain mutation might provide protection to both stress. We conclude that chronic stress can effectively improve the survival of *V. vulnificus* in environments with multiple types of stress, possibly through increased mutation.

P36

Construction of a Gateway Plasmid pYES-52-Leu for Functional Expression of Plant Terpenoid Cyclase in Yeast

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Terpenoid exhibits a wide range of structural diversities and biological activities, and have long been recognized as highly important source of medicinal compounds. The biosynthetic pathways of plant and yeast terpenoids share the same upstream steps and diverge at the cyclization of 2, 3-oxidosqualene, which is cyclized to ergosterol in yeast and to cycloartenol in plants. The shared initial metabolic steps between fungi and plants enable the use of transforming sterol-defective yeast mutants to screen the plant cDNA libraries for rescued recombinant terpenoid cyclase.

To clone and characterize novel terpenoid cyclase from plants, a gateway plasmid pYES-52-Leu was constructed for the expression of plant cDNA libraries in yeast. Plasmid pYES-52-Leu is obtained form substituting the URA3 gene of pYES-DEST-52 with the Leu2 gene from pRS-514. Expression of pYES-52-Leu in yeast provided the transformed cells to grow successfully on leucine minus plates.

Gateway plasmid pYES-52-Leu was applied for expression of plant cDNA libraries in yeast. Taking the advantage of the same initial metabolic steps, yeast mutants with sterol defects will be possible to be recombinant through the plant terpenoid cyclase. This system provided a rapid selection tool for screening of plant terpenoid cyclase.

P37

Characterization of *TTC1138* from *Thermus thermophilus* HB27 Involved in Biofilm Formation

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Thermus thermophilus HB27 was isolated from Japan's hot springs. It is viable at 68℃ and with ability to form biofilm. The proteome differences between biofilm cells and planktonic cells were observed by 2-DE. One of the protein is the *TTC1138* encoding protein. According to the data base of NCBI, *TTC1138* is a two component system regulator gene. The aim of this experiment is to characterized *TTC1138* from *T. thermophilus* HB27 involved in biofilm formation. The No.1 mutant of *T. thermophilus* HB8 increased biofilm at 18th hour and 36th hour. Biofilm formation of No.5 mutant of *T. thermophilus* HB8 can not be detected. Biofilm forming ability had no significant difference between mutants of *T. thermophilus* HB27 and wild type. In order to know what gene will be regulated by TTC1138. Western blot was used to check GalE, TTHA1483 and GroES, which are related to biofilm formation. In addition, *TTC1138* was cloned into pWUR112/77-1. *TTC1138* over expression strain will be used to confirm the relationship with those genes which are related with biofilm formation. Result showed that, TTC1138 might not regulate those genes. There were only 6 protein spots in mutant different from *T. thermophilus* HB27 total protein by 2-DE. On the other hand, there were only 8 protein spots in *T. thermophilus* HB27 different from mutant total protein. *T. thermophilus* HB8 and TTC1138 mutant of *T. thermophilus* HB8 have no significant difference in morphology. There was filament like structure around colony of *T. thermophilus* HB27, which was not found in mutant.

P38

Antibiotic Potency of Imidazolium Salt Derivatives

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In recent years antibiotic misuse has created a serious clinical issue , and the development of compounds to substitute the existing antibiotics to be imperative. We use imidazolium salt derivatives to test its antibiotic potency for some of common pathogens in this experiment. The goal of this study is going to find the most potency imidazolium salt for growth inhibition of bacteria. First, we use the disk diffusion method to determine minimum inhibition concentraction (MIC) and 96 microplates were applied to obtain IC_{50}. Transmission electron microscope was used to observe the morphology of bacteria after treating by imidazolium salt derivatives. Results showed that im-009 is the most stable and effective in all of the imidazolium salt derivatives. The morphology of bacteria after treated by im-009 for 12 hours is resemble to the antibiotic which inhibit protein synthesis of bacteria. So far the possible mechanism of im-009 treatment may relate with protein synthesis according to the morphological observation. Further studies will be helpful to elucidate the function of im-009 and it’s possible killing mechanism.

Memo