慈 濟 大 學

98 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：98 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：98 年 5 月 11 日~98 年 5 月 15 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 98 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 (二)	12:20~ 12:25	陳俊堯 (總)	TCIRP 96003	Physiological Adaptation and Gene Regulation of *Virbrio* spp. in Response to Environmental Fluctuations	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
5/6 (三)	12:20~ 12:25	張銘一 (總)	TCIRP 95002	Hepatitis C Virus: Molecular Pathogenesis, Cellular and Immune Responses, and Antiviral Therapy	第二教學研討室	免疫學科
	12:25~ 12:45	羅時燕	TCIRP 95002-01	Role of signal peptide peptidase on hepatitis C virus infection		醫學檢驗生物技術學系
	12:45~ 13:05	張新侯	TCIRP 95002-02	Characterizations of the association among viral hepatitis, anti-platelet autoantibody and thrombocytopenia		分子生物暨人類遺傳學研究所
	13:05~ 13:25	張銘一	TCIRP 95002-03	Hepatitis C virus and Sjögren's syndrome: linking infection and autoimmunity		免疫學科
	13:25~ 13:45	曾英傑	TCIRP 95002-04	Construction of in vivo NS5A/NS5B-expressing systems for biological effect study and anti-HCV drug assay		分子生物暨人類遺傳學研究所
5/7 (四)	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	陳怡成	TCIRP 96004-03	Relationship between assembled mechanism and structure of HCV core protein		醫學檢驗生物技術學系

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
	13:25~13:45	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
	13:45~14:05	羅時燕	TCIRP 96004-05	Study on the morphogenesis of hepatitis C virus		醫學檢驗生物技術學系
5/8 (五)	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
5/12 (二)	12:20~12:25	李茹萍 (總)	TCIRP 95008	失血性休克之整合性醫療與護理：從基礎研究到臨床應用	第二教學研討室	護理學系
	12:25~12:45	張芙美	TCIRP 95008-01	探討蜆萃取物對失血性休克下肝臟的保健作用		護理學系
	12:45~13:05	李茹萍	TCIRP 95008-02	探討規律運動對失血性休克的影響與護理監測指標		護理學系
	13:05~13:25	徐邦治	TCIRP 95008-03	探討急性失血性休克下腎損傷的分子機轉與藥物治療趨勢		內科--慈院
	13:25~13:45	怡懋・蘇米	TCIRP 95008-04	急性失血性休克下輸液速度及輸液加溫措施的影響		護理學系

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/13 (三)	12:20~ 12:25	曾國藩 (總)	TCIRP 95003	The Effect of Compression on Cerebral Cortex: Structural Plasticity and Associated Mechanisms	第二教學研討室	解剖學科
	12:25~ 12:45	曾國藩	TCIRP 95003-01	The remodeling of the dendritic arbors of cortical output neurons following compression: phenomena and mechanisms involved		解剖學科
	12:45~ 13:05	何翰蓁	TCIRP 95003-02	Ultrastructural studies on plasmalemma, organelles, and cytoskeleton involved in the compression-induced dendritic plasticity		解剖學科
	13:05~ 13:25	王曰然	TCIRP 95003-03	The regulation of cholinergic innervation and trophic factor on the remodeling of cortical dendritic spines		解剖學科
	13:25~ 13:45	劉培新	TCIRP 95003-04	An investigation of the compression-induced plasticity of cortical receiving neurons and thalamocortical inputs		解剖學科
5/15 (五)	14:00~ 14:05	許木柱 (總)	TCIRP 95001	印尼紅溪河整治效應之科際整合研究	第二教學研討室	人類發展研究所
	14:05~ 14:25	尹立銘	TCIRP 95001-01	紅溪河整治方案之公共衛生影響評估		公共衛生學系
	14:25~ 14:45	盧蕙馨	TCIRP 95001-02	宗教會遇經驗		宗教與文化研究所
	14:45~ 15:05	何縕琪	TCIRP 95001-03	跨文化能力、學習投入與利他表現		教育研究所
	15:05~ 15:25	許木柱	TCIRP 95001-05	族群關係與文化發展		人類發展研究所
5/15 (五)	15:30~ 15:35	張景媛 (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	教育研究所
	15:35~ 15:55	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
	15:55~ 16:15	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
	16:15~ 16:35	陳畹蘭	TCIRP 96001-03	正向情緒在認知、壓力後的生理復原及適應力所扮演的角色		人類發展研究所
	16:35~ 16:55	許木柱	TCIRP 96001-04	慈濟志工之正向心理研究		人類發展研究所

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/18 (一)	12:20~ 12:25	鄭敬楓 (總)	TCIRP 95007	Inflammation and Thrombosis in Cardiovascular and Hepatic Diseases: An Integrative Study from Cell Biology, Animal Models, to Clinical Diseases	第二教學研討室	小兒科--慈院(新店)
	12:25~ 12:45	鄭敬楓	TCIRP 95007-01	G-CSF induce inflammatory-dependent cardiac thrombosis in iron overload heart in mice		小兒科--慈院(新店)
	12:45~ 13:05	余俊賢	TCIRP 95007-02	*In vitro* and *in vivo* studies on the molecular mechanisms of adiponectin in the protection of liver from iron overload and its possible therapeutic implication		小兒科--慈院(新店)
	13:05~ 13:25	林恆	TCIRP 95007-03	The role of adiponectin in ROS-related cardiomyopathy induced by doxorubicin or iron overloading		藥理暨毒理學研究所
	13:25~ 13:45	柯毓麟	TCIRP 95007-05	Acute ischemic syndrome: chest pain center concept with research on genomic, biomarkers, proteomic and cell markers		心臟血管科--慈院(新店)
5/19 (二)	12:20~ 12:25	謝坤叡 (總)	TCIRP 95006	第一型與第二型糖尿病病程與併發症之生物醫學整合研究	第二教學研討室	神經科學研究所
	12:25~ 12:45	謝坤叡	TCIRP 95006-01	Relationships between rhythm-related genes and type I and II diabetes mellitus		神經科學研究所
	12:45~ 13:05	陳宗鷹	TCIRP 95006-02	To evaluate the effects of different inhalation anesthetics on cardiovascular neural regulation of autonomic nervous system in the streptozotocin induced type I and type II diabetic Rat		麻醉科--慈院
	13:05~ 13:25	孫宗伯	TCIRP 95006-03	effects of hyperbaric oxygen on the dysfunctions of cardiovascular neural regulation and cutaneous collateral circulation in type I and II diabetic rats		外科--慈院
5/20 (三)	12:20~ 12:25	林念璁 (總)	TCIRP 95009	台灣原住民幽門桿菌感染與胃癌發生之關係-整合分子流行病學、致病機轉與臨床研究	第二教學研討室	微生物學科
	12:25~ 12:45	胡志棠	TCIRP 95009-01	Relationship between susceptible genetic polymorphisms of the aborigine in Taiwan and *Helicobacter pylori* infection on gastric carcinogenesis		腸胃肝膽科--慈院
	12:45~ 13:05	張凱誌	TCIRP 95009-02	Isolation of virulence genes in *Helicobacter pylori* from eastern Taiwan Aboringines by systemic approach		醫學檢驗生物技術學系

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
	13:05~13:25	李茹萍	TCIRP 95009-03	Development and application of *Helicobacter pylori*-infected Rat Model		護理學系
	13:25~13:45	林念璁	TCIRP 95009-04	Effects of *Helicobacter pylori* infection on mucin expression in gastric tissues of aborigines in Taiwan		微生物學科
	13:45~14:05	伍超群	TCIRP 95009-05	The association between *Helicobacter pylori* and specific antigen express in gastric cancer		外科--慈院
5/22(五)	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*		家醫科—慈院
	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
5/25(一)	12:20~12:25	李哲夫(總)	TCIRP 95005	Nicotinic Acetylcholine Receptor and Neurovascular Function	第二教學研討室	神經科學研究所
	12:25~12:45	李哲夫	TCIRP 95005-01	Sympathetic nAChR and cerebral nitrergic neurogenic vasodilation		神經科學研究所
	12:45~13:05	郭重雄	TCIRP 95005-02	Control of common carotid arterial blood flow by nicotinic, glutamatergic, and nitrergic actions in the medulla of cats		通識教育中心
	13:05~13:25	賴志嘉	TCIRP 95005-03	The effects of amyloid beta-peptides on the function of nicotinic and glutamatergic receptors in central sympathetic neurons of rats		藥理學科
	13:25~13:45	許婷婷	TCIRP 95005-04	Effects of nAChR, Aβ and statins on glia cell function		免疫學科
5/26(二)	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		神經科學研究所
	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
5/27 (三)	12:20~ 12:25	賴靜蓉 (總)	TCIRP 95004	間歇性低氧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	賴靜蓉	TCIRP 95004-02	自由基對於間歇性低氧引發正常血壓大鼠與自發性高血壓大鼠之化學反射、自主神經功能及血壓變化之影響		生理學科
	12:45~ 13:05	林恂恂	TCIRP 95004-03	間歇性低氧引發之高血壓：大鼠前腹外側延腦中麩胺酸神經傳導與活性氧種之角色		生理學科
	13:05~ 13:25	劉朝榮	TCIRP 95004-04	間歇性低氧對於凝血功能以及動脈血管的影響		藥理學科
	13:25~ 13:45	楊昆達	TCIRP 95004-05	間歇性低氧造成心肌細胞死亡機制之探討		生理學科

【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
5/15 (五)	13:40~ 14:00	林子晴	TCMRC-P-96012	當代佛教音樂記憶－慈濟歌曲的民族音樂學研究	第三教學研討室	通識教育中心
	14:00~ 14:20	胡安仁	TCMRC-P-96004	Separation and analysis of biological particles		醫學檢驗生物技術學系
	14:20~ 14:40	胡正恒	TCMRC-P-96013	地景記憶再創造－藏彝走廊離散人群的環境永續策略		人類發展學系
	14:40~ 15:00	陳國詩	TCMRC-P-96007	「精確」與「解構」的對話－李立揚英詩中的文化對立.迷惘和整合		英美語文學系
	15:00~ 15:20	漢明遠	TCMRC-P-96009	The Learner’s Response: an investigation into the suitability of a non-EFL textbook for integrated English skills courses for English majors in Taiwan		英美語文學系
	15:20~ 15:40	李文茹	TCMRC-P-96011	戰後日本文學記憶的「殖民地台灣」－以坂口ネ零子「蕃地」作品為始		東方語文學系

【醫學研究所博士生研究成果發表】

註：底線為論文指導教授

日期	時間	姓名	題目	地點	論文輔導委員
5/12 (二)	08:30~ 09:05	廖家信	Pharmacological enhanced ^{18}F-FDG PET imaging for evaluation of Parkinson's disease in rats	E717	郭重雄 馮清榮 韓鴻志 邱紫文 廖光文
	09:05~ 09:40	謝佳恆	Pathophysiology of urothelial dysfunction in patients with interstitial cystitis/painful bladder pain syndrome – increased apoptosis and decreased junctional protein expression of urothelium due to suburothelial inflammation	E717	郭漢崇 許永祥 邱鐵雄 林銘德
	09:40~ 10:15	李明哲	In vitro study of human and guinea-pig gallbladder smooth muscle function	E717	黃士哲 許永祥 李威震 張子明
	10:30~ 11:05	徐聖曜	Analysis of retinal nerve fiber layer and macular thickness measurements in Taiwanese individuals	E717	蔡榮坤
	11:05~ 11:40	楊久滕	AAV mediated immune regulatory gene therapies for malignant tumors	E717	胡勝川 韓鴻志 魏秋偉
5/13 (三)	08:30~ 09:05	李慧超	Sympathetic α3β2-nAChRs mediate cerebral neurogenic nitrergic vasodilation	E717	李哲夫 陳嘉祥 林恆 顏瑞鴻
	09:05~ 9:40	王瑛杰	The influence of antipsychotic agnent to heart eart rate variability: evaluation in schizophrenic patients switched from typical antipsychotic agents to amisuliride and olanzapine	E717	賴靜蓉 楊靜修 郭博昭 蔡世仁
	09:40~ 10:15	李原傑	Methyl palmitate is a retinal relaxing factor	E717	李哲夫 郭重雄 張新侯 胡芳蓉
	10:30~ 11:05	耿念慈	Interaction of ethanol with nmda receptor antagonists on spinal nmda-induced pressor responses in rats	E717	賴志嘉 邱鐵雄 李哲夫 陳炯東 蔡明正
	11:05~ 11:40	許智偉	Caffeine induces reinforcing behaviour via inhibition of adenosine A_{2A} receptor associated with phosphorylation of DARPP-32 in mice	E717	邱鐵雄 賴志嘉 劉怡均

日期	時間	姓名	題目	地點	論文輔導委員
	11:40~12:15	陳聰毅	Exercise training protects against cardiomyocytes death in irreversible ischemia-reperfusion injury	E717	楊昆達
	13:30~14:05	曾子玲	Promising role of a plant extract (TChi-2) in the post-treatment of LPS-induced acute lung injury in the rat	E717	李哲夫 劉朝榮 郭重雄 趙瑞益
	14:05~14:40	曾國烈	The molecular mechanisms for Tanshinone IIA to inhibit the proliferation of human lung cancer H292 cells	E717	賴靜蓉 楊靜修 石明煌
	14:40~15:15	黃昱閔	Involvement of sympathetic function in the sleep-related change of gastric myoelectrical activity in rats	E717	賴靜蓉 賴賢勇 張耀仁 胡明燦 郭博昭
	15:30~16:05	賴奕菁	Immediate impacts of electromagnetic treatments on cardiac autonomic function in schizophrenia patients	E717	謝坤叡 賴賢勇 楊靜修
5/14(四)	13:30~14:05	陳星助	The impacts of prospective payment system implementation on health insurance in Taiwan—The influences and relative factors analysis on Diagnosis related groups	E717	朱正一 徐祥明 陳筱華 溫信財
	14:05~14:40	謝美玲	Validation of three different nutrition screening tools in hospitalized patients for primary nutrition assessment	E717	石明煌 高瑞和 黃士哲
5/15(五)	08:30~09:05	諶伯綱	Identification of genes that play potential roles in megakaryocytic differentiation	第二教學研討室	孫德珊 劉朝榮 葉日弋 劉德模
	09:05~09:40	林碧芬	An animal model of adolescent toluene exposure	第二教學研討室	賴滄海 吳文陞 蘇宏基 黃建華 林棟樑
	09:40~10:15	黃玄舜	Intravenous immunoglobulin ameliorates thrombocytopenia through modulating the selectin pathways	第二教學研討室	張新侯 張銘一 彭國証
	10:30~11:05	蔡夙美	Expression and function of FGF7 during liver regeneration	第二教學研討室	王文柄 翁慶豐 沈家寧 鄒安平 羅時成

日期	時間	姓名	題目	地點	論文輔導委員
	11:05~11:40	鄭敏志	Changes in Egr family proteins in mice hippocampus after chronic treating with methamphetamine	第二教學研討室	陳嘉祥 陳慧誠 陳炯東
	11:40~12:15	阮振維	CXCR2(IL-8Rβ) was upregulated by hSecurin through direct transactivation	第二教學研討室	陳紀雄 林銘德 陳怡成
5/15(五)	13:30~14:05	邱佩瑜	The role of Min system in *Helicobacter pylori*	E717	林念璁 林光慧 余美萱
	14:05~14:40	黃寒裕	Active Tuberculosis due to double strains in eastern Taiwan	E717	林念璁 張凱誌 蔡佩珍
	14:40~15:15	廖碧虹	Studies of blood-brain barrier permeability change during rabies infection and therapeutic achievement after passive immunity delivering into CNS	E717	陳立光 王明升 徐偉成 羅時燕
	15:30~16:05	謝翺合	Immunization of vaccine with complement (C3dg) and CpG-B ODN induce SLE-like syndromes in Balb/c mice	E717	張銘一 莊育裡 王士廉
	16:05~16:40	羅政弘	Characterization of the *Helicobacter pylori* bacteriophage, ϕHP1	E717	林念璁 劉哲文 張凱誌

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】

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【大學生　看板論文摘要】

【畢業生及其他　看板論文摘要】

O01

Physiological Adaptation and Gene Regulation of *Vibrio* spp. in Response to Environmental Fluctuations

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Vibrios are distributed in natural environments worldwide. Members of this genus are capable of thriving in aquatic environments as well as invading animal bodies, suggests the existence of quick and efficient adaptation mechanisms to diverse habitats. This year we continued to examine response of vibrios to various types of stress. Cross protection experiments have been performed, and combination of stress in general induced higher mortality in *V. parahaemolyticus* and *V. vulnificus*. Vibrios survived starvation for extended period of time. We found evidence of starvation-induced mutagenesis and selection, which started as early as 2 days in starvation.

Regulation by stress-related sigma factor RpoS and oxidative stress has been assumed to be important in the physiological response against various stresses. The role of *katE* and *katG* in response to oxidative stress has been characterized in both *V. parahaemolyticus* and *V. vulnificus*. The results show a unique regulation compared to the well-studied *Escherichia coli* model. Examination of *V. parahaemolyticus rpoS* mutant indicates that *rpoS* is required for survival in starvation, but may not be required in cold stress. One band demonstrating catalase activity on zymogram was missing in the *rpoS* mutant preparation, suggestingthe involvement of *rpoS* in protection of oxidative stress. Proteomic approach and expression dynamics of *oxyR, ompR* and *rpoS* in bacterial cells under acid stress have been examined.

We have started the screening of mutant library to identify genes involved in response to various stresses, and so far isolated *V. parahaemolyticus* mutants with reduced viability under cold stress. RT-PCR evaluation of stress-related gene expression is currently underway to confirm the importance of each gene. By comparison the Vibrio patterns with the *E. coli* system, we expect to provide a novel view on the diversity of bacterial stress response.

O02

(計畫名稱：Physiological Adaptation and Gene Regulation of *Vibrio* spp. to Chemical and Nutritional Changes)

Response of *Vibrio vulnificus* to environmental stresses: the role of osmotic and nutritional stresses in intraspecific diversity

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Vibrio vulnificus ubiquitously distributed in warm brackish waters, and can adopt life styles as either animal pathogens or environmental commensals. Prompt adaptation to fluctuating environment ensures the survival of this species in the environments. A local tilapia-pathogenic *V. vulnificus* strain 93U204 was selected to study the stress response of this species, in light of its ability to survive in aquatic environment and inside host body.

We measure the survival of 93U204 receiving two-stress serial treatment of combination of acid, cold, hyperosmotic, oxidative stresses or starvation. Generally the stress combination caused higher mortality compared to single stress. Cross protection was observed only in hyperosmolarity pretreatment to acid stress, and starvation pretreatment to acidification. Starvation consistently induced high mortality when applied as the second stress despite what the first stress was. However starvation did not exacerbate the bacterial survival when applied as the first stress.

Inter-strain variation in physiology and morphology in response to hyperosmotic stress was found among the tested strains. Increased phenotypic heterogeneity was observed within in starvation-treated population. Replicate experiments did not yield populations with similar phenotypic composition, suggesting the observed heterogeneity was a result of increased mutation rate and not due to proliferation of selected existing members. Strains isolated from 33-d starvation population demonstrate increase in competitiveness with wild type strain but not increase in resistance to starvation. Using competition experiments, we demonstrate that the osmotic and nutritional stress duo may act as a driving force of niche differentiation of *V. vulnificus* strains.

O03

(計畫名稱：Physiological Adaptation and Gene regulation of *Vibrio* spp. to Oxidative Stress and Oxygen Deprivation)

Response of *Vibrio parahaemolyticus* to environmental stresses: Two catalase genes in *V. parahaemolyticus* are differentially expressed in response to stressful conditions

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Surveillance of the genes involved in hydrogen peroxide detoxification showed the presence of two copies of bifunctional catalase (KatG) and monofunctional catalase (KatE) in *Vibrio parahaemolyticus* genome. In order to understand the underlying molecular mechanism governing the function of catalase genes, we first examined the expression pattern of catalases in *V. parahaemolyticus* at various growth stages by zymogram. Catalase activity was estimated at each stage by measuring rate of hydrogen peroxide decomposition. The result demonstrated that one of two bifunctional catalases plays a significant role in response to oxidative disturbance at stationary phase as well as nutrient starvation. On the contrary, a heat-stable catalase (corresponding to *katE* in *Escherichia coli*) was strongly expressed during exponential phase, and the expression gradually decreased after cells enter stationary phase.

In *V. vulnificus*, genomic survey identified one copy of *katE* and one copy of *katG*, but only KatG showed detectable catalase activity in zymogram analysis, suggesting a different paradigm. Taken together, we hypothesize that vibrios, and perhaps other marine bacteria, apply controlling scheme different from that used by *E. coli*, in response to oxidative stress.

We constructed *V. parahaemolyticus* RpoS mutant and KatG mutant in an attempt to differentiate the temporal expression pattern of the two *kat* genes. We constructed reporter plasmid for the two *katG* promoters, in preparation to the study of catalase gene expression under stressful condition. We are currently conducting a screening effort for mutant with reduced tolerance to oxidative stress, as an effort to identify other genes involved in catalase regulation.

O04

(計畫名稱：Physiological Adaptation and Gene Regulation of *Vibrio* spp. to Temperature)

Response of *Vibrio parahaemolyticus* to environmental stresses: role of RpoS in stress survival and identification of cold tolerance genes using a mini-*Tn*10 mutant library

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Vibrios are Gram-negative curved rods that are widespread in the coastal and estuarine waters. Some species, e.g. *Vibrio cholera*, *V. parahemolyticus*, *V. vulnificus*, may cause diseases in both humans and aquatic animals. Many studies reported that global climate change may have contributed to those vibriosis emergence. In order to cope with particular environmental fluctuations, bacteria need to modulate gene expression by alternative sigma factors. The alternative sigma factor RpoS (sigma S) is considered as a general stress response regulator that controls many stress response genes.

To clarify the role of RpoS in environmental fitness of *V. parahaemolyticus*, a leading cause of seafood-borne illness worldwide, we constructed a *rpoS* mutant by allelic exchange. The mutant demonstrated a similar growth rate as the wild type strain during the exponential phase under normal growth conditions. The *rpoS* mutant was subjected to various environmental stresses, and its survival was compared to that of the parental strain. Although there was no significant difference under cold stress between the two strains, the *rpoS* mutant was more sensitive to starvation than the parental strain. Zymogram analysis of catalase activities in native gel electrophoresis revealed three distinct bands in the parental strain. However, the lowest band was not detected in *rpoS* mutant. In order to identify genes required for *V. parahaemolyticus* growth under cold stress, we started the screening for cold-intolerant clones in a mini-*Tn*10-based mutant library. We have found one mutant with reduced viability after 5 days of 4^{o}C incubation, and many mutants exhibiting growth defects at 4^{o}C. We expect to elucidate the sequence and identity of the loci disrupted in these mutants, by arbitrary PCR and the following sequencing efforts.

O05

(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. Mutant library of *Vibrio* species were constructed for mutant gene cloning, using a *Tn*10-dervied transposon containing *E. coli*. replication origin. At least 3,000 transposon insertion mutants of *V. parahaemolyticus* and *V. vulnificus* have been created and properly stored, and all of the pH and low temperature intolerant mutants will be characterized in detail in this laboratory in the future. We have isolated at least 6 mutants of *V. paraheamolyticus* that failed to survive the first few days of cold treatment. We also screened the library for mutants failed to survive sub-lethal pH. With these approaches, we expect to find pH shift-responding genes as well as pH shift-responding promoters. Results of this study might provide further insight into the interaction of *Vibrio* species to environmental fluctuation.

O06

(計畫名稱：Hepatitis C Virus: Molecular Pathogenesis, Cellular and Immune Responses, and Antiviral Therapy)

Hepatitis C Virus: Molecular Pathogenesis and Cellular and Immune Responses

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Hepatitis C virus (HCV) infections often associate with acute and chronic liver diseases and hepatocellular carcinoma. To study the molecular and cellular mechanisms underlying the viral hepatitis, a Program project was organized to elucidate the molecular principle for viral replication, molecular pathogenesis and cellular immune responses. To achieve these goals, we design a three-year Program Project, which represents an integrated effort involving four laboratories collaboratively working on a central theme. These four faculty members from three graduate institutes are housed in the same research building in Tzu Chi University. The greatest strengths of this Program Project are four individuals combine their expertise as a task force working together on the daily basis. For the molecular pathogenesis, Dr. H.H. Chang's laboratory revealed that anti-platelet antibody might involve in hepatic damage and thrombocytopenia in acute viral hepatitis. In addition, Dr. Tzeng's lab has successfully established NS5A expressing cell lines for future studies on viral mediated pathogenesis. The study on viral replication by Dr. Lo's team found that the replication of HCV requires KCNJ8, PLCG2, ELOVL4, PLA1A, LGALS2. And finally, for the cellular immune responses, Dr. Chang's laboratory uncovered some novel infection-induced autoantibodies which show chronic reactivity to cell-cycle related components after successful treatment.

O07

Role of signal peptide peptidase on hepatitis C virus infection

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Production of hepatitis C virus (HCV) core protein requires the cleavages of polyprotein by signal peptidase and signal peptide peptidase (SPP). Cleavage of signal peptide at the C-terminus of HCV core protein by SPP was characterized in this study. The spko mutant (mutate a.a. 189-193 from ASAYQ to PPFPF) is more efficient than the A/F mutant (mutate a.a. 189 and a.a. 191 from A to F) in blocking the cleavage of signal peptide by signal peptidase. The cleavage efficiency of SPP is inversely proportional to the length of C-terminal extension of the signal peptide: the longer the extension, the less efficiency the cleavage is. Thus, reducing the length of C-terminal extension of signal peptide by signal peptidase cleavage could facilitate further cleavage by SPP. Our results further suggest that both sequences of the signal peptide and the E.R.-associated domain are important for the signal peptide cleavage of HCV core protein by SPP.

Genes differentially expressed (over-expressed or down-regulated) in HuH7 cells with or without HCV sub-genomic replicon were identified by microarray and dd-RT-PCR. Expression of the top 30 up-regulated genes in the HCV replicon cells was confirmed by quantitative 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. Knockdown of the over-expressed genes in HCV replicon cells by shRNA knockdown technology should repress HCV replication if they do facilitate HCV replication. Using this approach, KCNJ8, PLCG2, ELOVL4, PLA1A, LGALS2 genes were demonstrated to facilitate HCV replication. Furthermore, inhibitors to KCNJ8 or PLCG2 could also inhibit HCV replication in HCV replicon cells. We are going to confirm these results in the system using JFH-1 infectious clone and the *ex vivo* system using liver biopsy sample.

O08

Characterizations of the association among viral hepatitis, anti-platelet autoantibody and thrombocytopenia

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The mechanism underlining thrombocytopenia in acute viral hepatitis remains unclear. We randomly screened thrombocytopenia patients with platelet counts lower than 10^4/μl, from hospitals at Taiwan and found that a higher percentage of viral hepatitis patients compared to randomly selected patients (40% vs. 25%) had thrombocytopenia. We found that thrombocytopenia in viral hepatitis patients was specifically occurred during acute phase in association with elevated aspartate aminotransferase and alanine aminotransferase (AST/ALT) levels and a higher anti-platelet titer. These features are also unique when compared to other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis. Immunizations of experimental rabbits by human platelets not only elicited the anti-platelet antibody but also exacerbated thrombocytopenia and elevated ASL/ALT levels, indicating liver damages. In addition, chemical treatments to induce liver damages tended to induce a higher anti-platelet Ig level in experimental mice. Our data suggested that anti-platelet antibody might involve in hepatic damage and thrombocytopenia in acute viral hepatitis.

O09

(計畫名稱：Hepatitis C Virus and Sjögren's Syndrome: Linking Infection and Autoimmunity)

A Novel Rheumatic Autoimmune-like Pattern with Ethnic Predominance by HCV Infection

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Infection could aggravate or initiate autoimmunity through different mechanisms. Viral infection induced chronic inflammation is a possible link to IL17 activation, which is a component in several autoimmune diseases. Genetics, on the other hand, plays a “hard-to-verified-essential factor” on most autoimmunity. Studies showed that HCV infected individuals frequently develop autoimmune-like symptoms that resemble Sjogren’s syndrome (SS). Nevertheless, many studies also suggested that HCV induced SS (HCV-SS) are different from primary SS and other rheumatic autoimmunity in several ways, including targeted autoantigens and duration of autoimmunity. In this study we revealed that HCV induced autoimmunity is not only influenced by genetics (ethnicity), but also shares striking similarities with rheumatic autoimmunity. Sera of HCV infected patients from two ethnic groups, Hakka（客家） and Min-nan（閩南）, were collected for this study. Regardless the ethnic origins, most patients developed equivalent humoral reactivity to viral core antigen. Surprisingly, patients of Hakka background exhibited unequivocal elevated reactivity to viral NS5 antigen than Min-nan patients. Standard treatment of HCV infection involved cocktail of interferon-alpha/ribavirin, which has the potential to elevate antibody responses to nuclear components as observed from both ethnic groups. Further analysis revealed novel ethnic predominated autoantigen reactivity. Immunofluorescent stains further demonstrated that a significant proportion of patient posses’ antibodies to midbody, chromosome associated antigens and other cell cycle related components. Antibodies to both cell cycle and chromosome associated antigens have been reported on patients of rheumatic diseases and few other hematoma patients but not HCV infected persons. It is noteworthy that HCV-SS associated landmark autoantibodies, including cryoglobulinemia and autoantibodies to mitochondria, often subsided following treatment and recovery, while most rheumatic associated autoantibody do not. Those antibodies to cell cycle and chromosome associated antigens from HCV infected patients, however, were not diminished regardless the treatment outcome.

O10

Construction of in vivo NS5A/NS5B-expressing systems for biological effect study and anti-HCV drug assay

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HCV, a member of the *Flaviviridae* family, is a positive-sense, single-stranded RNA virus with a genome size of ~9.4 kb. The genome RNA encodes a polyprotein of 3,010 to 3,011 amino acid residues in the order NH2-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH. This polyprotein is processed by host and viral proteases. The nonstructural protein 5B (NS5B) is a virus-encoded RNA-dependent RNA polymerase (RdRp) that is responsible for replication of the viral RNA genome. Another nonstructural protein 5A (NS5A) has multiple functions including modulating NS5B activity. The biological effects of NS5B alone and its co-effects with NS5A in the liver are interesting, however, have not been studied.NS5B is an enzyme corresponding for HCV RNA replication and a functional counterpart of NS5B does not exist in mammalian cells. Therefore, the NS5B enzyme has become a primary target in the search for novel inhibitors of HCV replication. A variety of in vitro assays for NS5B polymerase activity have been developed for antiviral therapy. For this reason, the strategy designed by using an inhibitor of NS5B could serve as an effective and selective agent for treating HCV infection. In practice, generation of culture cells or animals expressing NS5B can be used as simplified model for antiviral treatment.The purpose of this project was to construct in vitro and in vivo models for studying biological effects of NS5B and NS5A respectively or combinatively. To characterize the phenotypes of NS5A, NS5B in vitro, cDNAs of NS5A, NS5B were cloned into mammalian expressing plasmids under the control of albumin promoter (i.e., pAB-NS5A as well as pAB-NS5B), thereafter, transfected into mouse liver cells. Several cell lines are undergoing selection procedure by using G418 treatment. Furthermore, the DNA fragments of pAB-NS5A and pAB-NS5B were micro-injected into pronuclei of mouse one-cell embryos to generate in vivo model. Among 63 pups derived from the injected embryos, tail DNAs from 6 pups were identified to carry AB-NS5A transgene and among other set of 49 pups 6 were detected to be AB-NS5B carrier by using Southern blot analysis. Five transgenic mouse lines expressing NS5A were constructed, while generation of NS5B transgenic founders is still developing. Based on our preliminary, the transgenic animals display shaggy fur macroscopically. Once the mouse models will be generated, they will support for the measurement of NS5B inhibition activity of anti-HCV agent proposed by Dr. Lin (project numbered 5). Additionally the effect of NS5B in the mouse liver of these models will be inspected with microarray and proteomics analysis to study the impacts of this gene in the liver.

O11

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 years, we hold joint laboratory meetings monthly to discuss research information and share 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 RdRp. Through this collaboration, we will understand more regarding structural information of HCV proteins.

O12

(計畫名稱：Atomic Force Microscopy of Hepatitis C Virus Proteins)

Structural Analysis of Hepatitis C Virus Core Auto-assembly using Atomic Force Microscopy

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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. Although enjoy similar degree of resolutions, the AFM have many advantages over the electron microscopy. 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 presentation will demonstrate the results to date regarding to the structural analysis of the HCV core assembly with different core protein lengths (full length and truncations) in different environmental conditions, as well as the importance of the hydrophobic tail of the core protein during the virus core auto-assembly process by applying the atomic force microscopy.

O13

(計畫名稱：Spectroscopic studies of structural proteins of HCV)

Spectroscopic studies of HCV E1 protein

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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 three structural proteins: a capsid protein and two envelope glycoproteins, E1 and E2. E1 and E2 are thought to play pivotal roles at different steps of the HCV replicative cycle. There is now strong evidence that they are essential for host-cell entry, binding to receptor(s), inducing fusion with the host-cell membrane as well as in viral particle assembly.

E1 and E2 are type I transmembrane (TM) glycoproteins, with N-terminal ectodomains and a short C-terminal TM domain. These proteins interact with each other and assemble as noncovalent heterodimers. Like other viral envelope proteins involved in host-cell entry, HCV envelope proteins are thought to induce fusion between the viral envelope and a host-cell membrane. The HCV envelope glycoproteins E1 and E2 are thought to be class II fusion proteins because the putative fusion peptide is supposedly localized in an internal sequence linked by antiparallel β-sheets.

Previously we have shown that only E1 260 fragment can induce liposome fusion under low pH but not pH 7 environment while other E1 212 and 232 fragments can not (Tsai, 2008). Thus, we propose that the segment of 232-260 of E1 is the key element to induce pH-sensitive membrane fusion. Synthesized peptide will be used to test this hypothesis. Also, site-directed labeling will be used to detect possible conformational change.

O14
(計畫名稱: Relationship between Assembled Mechanism and Structure of HCV Core Protein)

pH-dependent Assembly and Disassembly of HCV Core Protein 1-116

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The hepatitis C virus (HCV) core protein is the major component of HCV nucleocapsid and also exhibits multiple functions involving in cellular growth, proliferation and other functions. Following the previous study, we characterized the effect of pH on the assembly of HCV core protein 1-116. Using circular dichroism spectroscopy, the secondary structure of core protein 1-116 contains 23 % α-helix, 34 % β-sheet and 47 % random coil in pH 7.0, 19% α-helix, 22 % β-sheet and 59 % random coil in pH 4.0, and 27% α-helix, 32 % β-sheet and 41 % random coil in pH 9.0. Gel-filtration and sucrose gradient analyses showed that core protein 1-116 could assemble into a virus-like particle with a buoyant density of 1.16g/cm^3-1.19g/cm^3 in neural and basic conditions while in acidic pH, HCV core protein 1-116 failed to form virus-like particles as further verified by the images using atomic force microscopy and transmission electron microscopy. Obviously, the possible reason for this pH-dependent assembly of HCV core protein may arise from the change of conformation, particularly the increase of random coil in acidic pH.

O15

(計畫名稱：A Bioinformatic Approach to Study The Viral Entry and Morphogenesis of HCV)

Starting from the NS5B in HCV --- Analysis of the viral RNA-dependent RNA polymerases

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Hepatitis C virus (HCV) is a positive, single-stranded RNA virus belonging to the *Flaviviridae* family. The viral genome encodes a polyprotein which is subsequently processed into individual structural and non-structural proteins. The key component of the HCV RNA replication machinery is RNA dependent RNA polymerase (RdRp) which resides in non-structural protein 5B (NS5B).

To explore the sequence and structural features of RdRp in RNA viruses as a whole, we systematically collected sequences of RdRp domain from UniRef50 database. The collected RdRp sequences were further separated according to the viral genome type of positive-single, negative-single, and double-stranded RNA. In combination of multiple sequence alignment and clustering techniques, several unreported conserved motifs are identified in some of the clusters. The functional and structural roles of the newly described residues in RdRp are waited to be analysed. Other approaches, such as the prediction of secondary structure and the stochastic sampling searching for conserved patterns will be applied next.

O16

(計畫名稱：Study on The Morphogenesis of Hepatitis C Virus)

Interactions between hepatitis C virus NS3 and cellular dNT-1 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. dNTs are present in most mammalian cells and involving in the regulation of intracellular dNTP pools by substrate cycles. Substrate cycles are relying on the interplay between a deoxynucleoside kinase and a nucleotidase, participating in the regulation of dNTP pools. dNT enzymes attain special importance in cells of the lymphoid system that are low in deoxyribonucleotidase activity, and, in their absence, dATP and dGTP specifically accumulate in B and T cells and cause diseases.

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.

O17

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.

O18

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. Interestingly, we found p53 can down-regulate EBNALP co-activation with EBNA2 but not with p300/CBP, suggesting a selective down-regulation of EBNALP by p53 may depend on the co-activating target of EBNALP. 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.

Taken together, we demonstrate two potential targets can be utilized to develop the therapeutic approaches for EBV associated diseases.

O19

(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 were identified as EBNA1 associated cellular proteins, suggesting they may have a role in EBNA1 mediated transcription. The extensive research work is now conducted to uncover how these cellular factors contribute to EBNA1 mediated transcription that is linked to latent infection by EBV. 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 potential. In addition, an EBV lytic infection reporter plat-form will be also generated in the context of EBV latent infected type I AKATA-neo cells. The anti-EBV effects of EGCG will be further emphasized in this model system. 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 drug targets to treat 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.

O20

(計畫名稱：Mechanistic Insight of Cyclooxygenase-2 Induction by Latent Membrane Protein 1 in EBV Associated Cancers, and Efects 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. We are now trying to investigate whether the formation of autophage is correlated with the functional profile of LMP1. Further more, the scenario of which protein degradation of LMP1 induced by EGCG is under investigation. Our current data suggested that EGCG can target to LMP1 and appears as an anti-EBV drug to treat EBV associated malignancies.

O21

失血性休克之整合性醫療與護理：從基礎研究到臨床應用

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大失血造成的休克是急診與重症病人常見的死亡原因之一，大部分病人的死亡發生在大失血後的 24 小時內，經由急救存活下來的病人常會面臨到後續感染與器官衰竭的問題，也是造成後續死亡的主因。因此，若能預防與治療失血後嚴重的疾病變化，將可有效降低病人的死亡率。本研究從保健到醫療的觀點，探討保健食品與保健行為對大失血狀況是否具保護效果，並找出較佳的治療模式及輸液給予方式，整體目標是希望藉由實驗研究，提供急重症單位醫護人員在面對大失血病患時，給予最佳醫護處置與建議，並提供執行安全又有效輸液措施與治療時的參考指引。整合計畫中子計畫一與子計畫二的動機起源於飲食與運動衛教是臨床出院病人的護理項目，而我國民情上有食療重於醫療的文化觀點，一些食品常被賦予保健的意義，因此本研究以保健的觀點，探討民間的保肝食品蜆萃取物以及規律運動的保健行為，在大失血狀況下是否具有保健效能；若個體經歷大失血而存活下來，最常出現的肝腎衰竭現象，可能導致體內不平衡、代謝異常甚至造成死亡，若能預防或阻止這些現象發生，則可降低病人續發的器官損傷並提高其存活率，因此，本研究中子計畫一、三將就大失血後肝、腎衰竭的發生與治療做相關探討；目前臨床處置上，對於急性期的失血性休克病患，多數是遵循以大量、快速的回溫輸液灌注為導向，但近年來臨床上開始質疑大量灌注可能會造成損傷與後續的器官衰竭有關，也有人提出低溫療法可促進病人存活率，因此，本研究子計畫四在輸液的速度調控與輸液溫度上做了相關探討。整合前兩年與目前進行中第三年的研究結果發現：失血對肝臟與腎臟確實造成嚴重損傷，而無論是以失血前的保健觀點或失血後的治療方式，給予蜆萃取物都能降低失血引發的肝損傷；除此之外以保健的觀點來看，有規律運動下，當面臨大失血時各項器官損傷程度也較無運動組輕，48 小時的存活率也較高；在不同輸液速度與溫度下，有給 Fluvastatin 治療時其失血下的器官損傷也較輕；輸液速度方面，給予慢速輸液下呈現較長的低體溫、對於器官的損傷較輕微，而不同輸液溫度下，則呈現接近室溫的輸液溫度引發的損傷較輕。目前研究結果顯示蜆萃取物、規律運動、Fluvastatin 對嚴重失血下有保護器官的功效，而慢速輸液對肝臟、肺臟、腸道損傷較輕，室溫下的輸液溫度器官損傷情形低於低溫或高溫輸液。本整合型計畫之各子計畫實驗階段已進入尾聲，目前正積極完成染片與統整數據資料，且前兩年的研究結果已有相關研究成果投稿中或已刊登於國外 SCI 期刊。

O22

探討蜆萃取物對失血性休克下肝臟的保健作用

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臨床上接觸到大失血個案的情境，主要為急診創傷病患、各類外科手術後病患與各科加護急症病患等。由於這類病患之病況瞬息萬變，醫護人員常面臨極大的壓力與挑戰。失血性休克之生理病理反應與系統性發炎反應(SIRS)有密切之關係，可能會造成器官功能喪失，最後形成包括肝臟等的器官衰竭，這種現象造成個案的高死亡率。蜆常被多數國人認為是一種有益肝臟的機能性食品，因此肝病喝蜆湯自古相傳不絕，然而蜆在失血狀況下的保健與治療功能仍未完全證實。因此，本研究目的在探討出血性休克引起急性肝衰竭的生理、病理機轉，並觀察蜆萃取物對於失血前後肝臟保護與治療之機制與功效。第一年研究中，主要探討出血性休克下引起急性肝衰竭的生理、病理層面的機轉，接著在第二年研究中，觀察失血前給予蜆萃取物對肝臟之生理表現機制與保護功效。前兩年的研究結果顯示：急性大失血導致平均動脈壓下降，血清 TNF-α、AST、ALT、LDH 確實有升高的現象，而組織 HE 染色明顯顯示肝細胞損傷，若是在失血前給予蜆萃取物能使血壓下降幅度減小，並使 TNF-α 值降低，同時 24 小時後 IL-10 有逐漸升高的趨勢，血清 AST、ALT 與 LDH 濃度亦降低，組織 HE 染色也顯示肝細胞損傷程度減輕。第三年研究主要是失血後給予蜆萃取物，觀察對肝臟保護之生理表現機制與功效，以測試蜆萃取物除了在保健功能外，是否也具有治療的功效。實驗以大鼠模式，進行大失血後給予蜆萃取物，評估 48 小時內血清中發炎前趨物質 tumor necrosis factor-α (TNF-α)、interleukin-10 (IL-10)及血清生化值 AST、ALT、LDH 之變化，並在第 48 小時後犧牲實驗動物留取肝臟，以 HE 染色法及免疫組織化學染色評估肝臟組織之損傷情形。目前結果顯示：急性大失血導致平均動脈壓下降，血清 LDH、CPK、AST、ALT 逐漸升高，若失血後給予蜆萃取物 20 mg/kg，則這些數值明顯降低，顯示蜆萃取物能減輕肝細胞損傷。後續將進行血清中發炎前趨物質 TNF-α、IL-10 與組織 HE 染色，以觀察肝細胞損傷變化程度，作為評估蜆萃取物對治療急性失血所引發之肝損傷的效用。

O23

探討規律運動對失血性休克的影響與護理監測指標

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失血性休克是造成創傷病人常見的死因，失血性休克後造成的炎症反應將導致組織傷害，有研究指出運動訓練與炎症反應有相關，在比較運動和無運動組的傷害時，發現有運動組的感染率會下降，然而規律運動是否對失血引發炎症反應與後續器官損傷有影響，目前則無相關研究。由於運動衛教是臨床病患護理中的必要指導項目，因此，本研究的目的在評估運動是否能降低大失血引發的器官損傷，以作為未來護理衛教的實證參考。實驗方式因考量到失血狀況對人體的傷害，因此本研究使用清醒鼠模式進行全血量40%的失血，使用八週齡的大鼠隨機分配到運動後失血組或無運動失血組，運動組給予每天 30 分鐘 15m/min 的跑步(相當於中等強度)，在運動四週後進行失血實驗，另外以同齡大鼠進行失血組實驗，在執行失血前、失血後 1、3、6、9、12、18、24 與 48 小時，各組採集血液檢體檢測血中各項生化與免疫物質之數值，實驗動物在失血後 48 小時犧牲，擷取組織進行熱休克蛋白之檢測。第一年的研究在探討有無運動對失血性休克之生理病理影響，結果顯示：有運動失血組在血液生化數值、肺泡支氣管灌洗液中的顆粒球比例明顯低於純失血組；第二年比較有無運動下經歷大失血時的體重變化、器官損傷的影響、活動力變化與存活率，結果顯示：有運動組的體重增加較無運動組少，且活動力較高、48 小時的存活率也較高，且肺、肝、腎臟病理學損傷程度較輕；在第三年的研究中，將針對急性炎症反應以及熱休克蛋白進行探討，以了解其保護器官免於損傷的機制趨向，目前的研究結果發現運動組於失血後的血糖與血小板竄升幅度小於無運動組，TNF-alpha、IL-6 較低、IL-10 較高，另外，組織中熱休克蛋白的表現正在進行免疫組織染色中。三年的初步研究結果顯示規律運動對嚴重大失血下確實可降低炎症反應，有減輕器官損傷的功效，而臨床上面對規律運動者，則需注意勿因為其症狀較輕微而低估了其失血量。

O24

探討急性失血性休克下腎損傷的分子機轉與藥物治療趨勢

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因大量出血引起的休克常造成的腎臟損傷，出血後引起的心臟輸出量下降是造成器官血流不足、缺血性傷害，而後續輸液的灌注及組織再灌流又進一步傷害腎臟。出血性休克引起急性腎衰竭的病理生理機轉非常複雜，可能包含腎小管的傷害、血管的傷害及血管內皮細胞的傷害，隨後造成白血球浸潤而引起發炎反應，產生一些細胞激素、化學激素及過氧化物，再進一步產生細胞壞死及細胞凋亡，最後導致急性腎衰竭。急性腎衰竭是出血後引起的身體早期傷害的表現，對於不同狀況下出血性休克引起急性腎衰竭的機轉，值得深入探討，以做為臨床治療的參考。目前在不同出血量、不同輸液補充速度及不同輸液溫度灌注，對於出血性休克引起急性腎衰竭的病理、生理、分子生物層面的機轉仍不明。本研究第一年為探討不同出血量下出血性休克引起急性腎衰竭的病理、生理、分子生物層面的機轉，研究發現：在 20%、40%、60%出血性休克下會引起 BUN、CPK、LDH 的上升，出血量愈多則變化愈大，但 Cre 變化以 40%及 60%出血性休克才有，進一步取 Fluvastatin 以 40%及 60%出血性休克引起急性腎衰竭，也發現有保護的作用。第二年實驗探討在 60%出血使用不同輸液補充速度下 Fluvastatin 對器官衰竭能否有保護作用，研究結果發現：腎臟病理、免疫組織染色 Fluvastatin 於 60%出血後快速輸液可改善因出血造成的腎小管 E-cadherin 流失，及減少因 60%出血性休克造成的腎小管 NF-κB 活性。本研究第三年使用 Fluvastatin 在 40%出血性休克下，探討不同輸液溫度(常溫、高溫 42℃、低溫 34℃)對出血性休克引起器官衰竭能否有保護作用，目前研究結果發現：Fluvastatin 在 40%出血下，高溫與低溫輸液的傷害都較常溫輸液低，Fluvastatin 於出血後低溫輸液比正常溫度輸液於腎臟保護更好。目前 Fluvastatin 於 40%出血性休克後病理切片、血清發炎細胞激素及腎臟免疫組織染色仍持續進行中。

O25

急性失血性休克下輸液速度及輸液加溫措施的影響

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高級創傷救命術已將創傷早期及大量的快速輸液治療及輸液回溫視為一項重要的急救指標，然而，過去的研究發現快速輸液可能造成嚴重的灌流損傷，當創傷後身體呈現輕度至中度的低體溫狀態，反而能抑制炎症過程的進展，此外，臨床目前針對外傷患者傾向於輕至中度低溫的輸液療法，其目的為減少後續性的器官損傷問題。由於輸液治療措施是急重症護理人員常要面對的情境，而目前處置措施仍眾說紛紜，因此，本研究的目的為探討失血後的輸液在不同速度及不同溫度下，是否會影響失血後器官損傷的表現，以作為臨床急重症護理處置之實證參考依據。本研究計畫過去兩年建立了失血性休克後大量輸液速度與體溫之間的相關性，研究結果發現：失血後慢速輸液組，失血後呈現輕微低體溫的時間較長，血液生化數值中肝功能及肺部灌洗液中的發炎因子，明顯低於快速輸液組，病理學損傷程度上也較輕。由此結果顯示溫度在輸液治療過程中佔有重要的意義，因此，計劃的第三年主要為探討失血後輸液治療的溫度對失血性休克後生理表現及器官損傷的影響。研究中將大鼠分為失血組、失血後高溫輸液（40℃）組、失血後常溫輸液（21-22℃）組及失血後低溫輸液（14-15℃）組，在進行全血量40%的失血後，持續監測與紀錄肛溫、血壓與心跳48小時。依照臨床慣例，輸液組在失血後立即給予3倍失血量之輸液灌注，分別在失血前、失血後1、3、6、9、12、18、24與48小時各採集血液0.8 ml，檢測血中全血球數值(CBC, D/C)、GOT、GPT、血液尿素氮、肌酸酐、LDH，及發炎激素TNF-α、IL-6及IL-10。實驗動物於失血後48小時犧牲並留取肺、肝、腸等器官做病理學檢測。目前結果發現：低溫輸液組於失血後給予15℃的低溫輸液，體溫會呈現中度低體溫（34℃）的狀況，而高溫輸液組雖然體溫無明顯下降，但血液及肺部灌洗液的生化數值表現與低溫輸液組無明顯之差異，但這兩組在肝臟、心臟及肺部損傷的生化指標上，皆明顯的較常溫輸液組嚴重，目前病理表現仍在染色中，完成後將於結案前呈現結果。目前結果似乎與臨床所主張的使用高溫與低溫輸液療法之理念有異，從另一觀點而言，這也開啟臨床在輸液溫度處置上之不同省思與參考。

O26

The effect of compression on cerebral cortex: structural plasticity and associated mechanisms

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Trauma and meningioma that compress cerebral cortex are expected to alter the function of underlying cortical neurons. However, the pathophysiology and response of these neurons to decompression remain largely unexplored. This integrative project aims at investigating how cortical neurons remodel when subjected to compression. We approached it with a rat epidural bead implantation model to compress the sensorimotor cortex without causing direct chemical interaction. This allows us to study structural remodeling of cortical neurons in vivo. The thick, straight and uniformly aligned apical dendritic trunks of cortical pyramidal neurons made possible the evaluation of proximal dendritic reconfiguration in detail. The first project of this integrative series studies the molecular changes associated with the shortening of the proximal dendrites of pyramidal neurons. The second project studies the associated ultrastructural changes especially the role of microtubules. The third project addresses whether nerve growth factor regulates the densities of dendritic spines on cortical pyramidal neurons through cholinergic innervation. The last project aims at understanding whether and how cortical receiving neurons, in the layer IV are affected.

As presented in detail in each project, the results obtained demonstrated that kinase and phosphatase activities could be altered within minutes of compression and led apparently to a swift large-scale structural remodeling of dendrites at segments far from terminals. The process appeared to be microtubule depolymerization-dependent. Nerve growth factor appeared to regulate the spine densities of cortical pyramidal neurons through cholinergic fibers. In addition, compression altered the neuronal circuitry of the cortical layer that receives thalamic inputs. The swiftness of the reconfiguration and the fact that changes showed slow and marginal recovery if prolonged argue strongly for early clinical intervention of abnormalities that compress the cortex.

O27

The remodeling of the dendritic arbors of cortical output neurons following compression: phenomena and mechanisms involved

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Using a rat epidural bead implantation model we found that compression distorted the dendrites of underlying cortical pyramidal neurons instantly and the twisted apical dendritic trunks regained a shortened but straight morphology in 3 days. Here we examined the molecular correlates of these events. Compression increased the phosphorylation of microtubule-associated proteins (MAPs) at sites known to destabilize microtubules including MAP2 from 30 minutes to 1 hour and tau from 10 minutes to 12 hours afterward. Immunostaining confirmed phosphorylated MAPs concentrated at the soma and dendrites of compressed neurons. Concomitant alterations of enzymes regulating MAPs' phosphorylation including downregulation of protein phosphatase 2A, but not 2B, activity from 10 minutes to 1 day and transient excitatory phosphorylation of extracellular signal-regulated protein kinase 1/2 and p38/mitogen-activated protein kinase for up to 3 hours and 30 minutes, respectively following compression. The temporal coincidence of these events suggests alterations of phosphatase and kinase activities to underlie MAP2 and tau phosphorylation which in turn rendered the dendritic structural plasticity of cortical neurons subjected to compression. The immediate nature of the scheme disclosed enables neurites to reconfigure swiftly along their length upon external cues.

O28

(計畫名稱：Ultrastructural Studies on Plasmalemma, Organelles, and Cytoskeleton Involved in The Compression-induced Dendritic Plasticity)

Blocking microtubule remodeling by taxol affects dendritic plasticity upon epidural compression

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Previous studies on a rat epidural compression model have shown that compression alone reduced the thicknesses of cerebral cortex with no apparent cell death. Besides, the apical dendritic lengths of pyramidal neurons also decreased significantly following compression. By applying transmission electron microscopy, we have demonstrated the involvement of microtubule remodeling and active endocytosis in apical dendritic trunk remodeling following epidural compression. To further examine the role of microtubule dynamics in dendritic plasticity, animals were treated with taxol to prevent microtubule depolymerization during cortical compression. Without taxol, vehicle (DMSO)-injected animals subjected to compression showed decreased microtubule density within apical dendritic trunks and microtubules arranged slightly irregular. This result is consistent to what we have found in animals subjected to compression alone, indicating lateral ventricle-injection itself has little effects on cellular morphology. On the other hand, in taxol-injected animals, the randomness of microtubule arrangement increased, long, curving microtubules crossing each other within apical dendritic trunks was obvious, which is of great difference from control apical dendritic trunks showing parallel microtubules. Increased incidence of curving microtubules within taxol-treated apical dendritic trunks but far less in vehicle-treated dendrites suggests microtubule depolymerization is involved in dendritic trunk modification during compression. Quantitative analysis and effects of taxol on decompression-induced modification is undertaken to elucidate how microtubule remodeling affects dendritic plasticity.

O29

The regulation of cholinergic innervation and trophic factor on the remodeling of cortical dendritic spines

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Compression reduced the dendritic spines of the underlying cortical pyramidal neurons quickly and decompression failed to reverse this. Simultaneous NGF administration upon decompression resulted in the restoration of dendritic spines and thickening of the cholinergic axon bundle at the border of motor and cingulate cortices. This and the well-documented association of loss of cholinergic neurons with loss of dendritic spines on cortical neurons in dementia-related diseases and in addition the fact that in the adult brain NGF receptors are expressed in cholinergic neurons but not cortical pyramidal neurons led us to investigate whether cholinergic neurons regulate the densities of dendritic spines on cortical pyramidal neurons. Sensorimotor cortical cholinergic denervation, over 90% in 5 weeks, was achieved by injecting the immunotoxin, 192 IgG-saporin directly into the nucleus basalis magnocellularis (NBM) and substantia innominata (SI) unilaterally. Intracellular dye injection revealed that in the denervated cortex, dendritic spines on cortical pyramidal neurons were reduced by half in layers I-III and 25% in layer V, but unchanged in layer IV. Loss of dendritic spines was accompanied by specific decreases of the postsynaptic marker protein PSD-95 and the dendritic spine protein spinophilin suggesting that excitatory synapses, thus cortical functions were compromised. Our results suggest cholinergic inputs affect cortico-cortical synaptic transmission more than direct peripheral informational processing as thalamic inputs end predominantly in layer IV where dendritic spine densities were cholinergic-independent.

NGF applied intraventricularly to the cholinergic denervated cortex failed to increase the dendritic spines on its cortical pyramidal neurons in layers I-V suggesting that cholinergic innervation plays a crucial role in NGF’s promotion of dendritic spines on cortical pyramidal neurons.

O30

(計畫名稱：An Investigation of The Compression-induced Plasticity of Cortical Receiving Neurons and Thalamocortical Inputs)

Effects of primary somatosensory cortex compression on rat blood-brain barrier and thalamocortical connections

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Compression of cerebral cortex is usually induced 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, depending on the mass location and the damage it cause to surrounding brain tissue. We used a primary somatosensory cortex (S1) compression model to investigate whether cortical compression leads to functional deficit and/or blood-brain barrier (BBB) disruption. After S1 compression, male SD rats were grouped and allowed to survive for 1 day, 3 days, 1 week, 2 weeks, and 3 months. Contrary to the contralateral side, the touch sensitivity of whisker pads, forepaws, and hindpaws of animals was abruptly reduced on the lesion side at 1 day but gradually restored during 3 days to 3 months, evidenced by von Frey test. By measuring Evans Blue extravasation, BBB permeability began to increase in compressed S1 at 1 day, peaked at 3 days, but recovered at the remaining time points. Using immunohistochemical labeling, we found that the expressions of glutamate receptor subunits glutamate receptor 1 (GluR1) and *N*-methyl-D-aspartate receptor 1 (NMDAR1) 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 $GABA_A$ and $GABA_B$ receptor subunits were upregulated at 1 day and 3 days following cortical compression. These findings suggest that the physical compression of primary somatosensory cortex leads to impairment of somatic sensation in the early period but the sensory function recovers rapidly. The mechanism may involve the regulation of BBB and/or the thalamocortical connections.

O31
(計畫名稱：印尼紅溪河整治效應之科際整合研究)

Toward a Humanistic Relocation: The Case of Angke River in Jarkata and Its Meaning to Charity Aid
人文安置：印尼紅溪河的案例對慈善援助的意義
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The primary findings highlighted above have consistently illustrated the positive sides of the Angke River Project. The dramatic changes, especially the effects on education, religious understanding and ethnic relationships, have been so obvious as to be a good model for further projects. These progresses can be interpreted with some inspiring theories in social sciences as advocated by Foster, Vygotsky, Bourdieu, and Maslow. As to interethnic relationships, the perspectives set forth by Glazer and Moynihan, and De Vos and Hsu seemed to be appropriate. Yet this is by no means that the aiding program has been perfect. There are some complaints about strict regulations on residents' behaviors like gambling and alcohol drinking, and fewer chances to make a living as the new location is a little distant from the riverbank where they had well adapted for years. To make a compassion program successful, more factors need to be taken into account.
Key words: Angke River Project, Charity aid, Indonesia

O32

(計畫名稱：紅溪河整治方案之公共衛生影響評估)

印尼大愛一村肺結核防治健康促進計畫之成效評估

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依據世界衛生組織發表的報告指出，肺結核病為印尼主要死亡原因之一；肺結核盛行率在慈濟大愛村座落的西雅加達地區，更遠高於爪哇與全印尼肺結核病之盛行率。幾次焦點團體座談的結果也突顯出，肺結核病是大愛一村居民與醫護人員首要關心之健康問題。依據世界衛生組織發展出的健康促進原則與策略，我們設定了以對抗肺結核病之健康促進計畫工作目標。本計畫執行工作由印尼慈濟分會主導，召集學校老師、社區志工、慈濟醫院醫師與慈基基金會工作人員等組成的抗肺結核病行動團隊，並著手在大愛一村推動抗肺結核病健康促進介入工作。此行動工作團隊同時也成立六個工作小組，主要任務如下：小組一負責與政府部門建立夥伴與合作關係；小組二負責學校學生與社區居民教育計畫；小組三負責所有志工之訓練計畫；小組四負責尋覓肺結核病新病例；小組五負責肺結核病患之治療計畫；小組六負責評估治癒率之評估。

本研究目的在評估整體健康促進計畫，是否能提升居民與學生對肺結核病之認知，進而改善其態度與行為，做好肺結核防治的工作。在計畫執行前，我們分別於印尼慈濟教育體的小學完成了 170 份問卷、初中完成了 153 份問卷、高職完成了 107 份問卷；同區作為對照組的學校，完成了 188、140、95 份問卷。在居民部分，我們分別在大愛一、二村（二村為對照組）完成了 400 及 290 份問卷。前測的結果顯示，慈濟的學生與大愛一村居民對肺結核普遍的認識上，較優於對照組的學生與居民，並在多項問題的答對率上達統計顯著水準；這些可歸功於之前的篩檢活動。但許多關於肺結核防治與症狀等較深入的問題，實驗組與對照組答對率皆偏低，且無顯著差異；這表示肺結核防治的健康促進計畫仍有其必要性。後測將在計畫推行後 6 – 8 個月後實施，藉以評估建促計畫之成效。預計 2009 年 6 月可以完成後測，12 月前完成整個計畫成效之評估。

O33
(計畫名稱：宗教會遇經驗)

宗教合作的倫理關懷與實踐
Ethical Concerns and Practices in Religious Cooperation

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本研究以慈濟基金會在雅加達所興建的「大愛一村」、「大愛二村」居民，以及慈濟所濟助的奴魯雅伊曼習經院師生為研究對象，探討伊斯蘭教徒如何接納佛教慈濟團體的協助，宗教生活和信仰是否因和慈濟的接觸產生變化，如何看待慈濟的助人行為。計畫也有興趣於瞭解他們如何面對災難和貧窮，和慈濟志工比較，他們在何種信仰脈絡下助人。研究目的在探究國際賑災的宗教會遇經驗，藉此瞭解兩大宗教（佛教和伊斯蘭教）如何看待苦難，以及人如何超越苦難的考驗，並進一步討論不同宗教信仰者在面對苦難的互動中，開展何種宗教對話，和目前舉世所關注的宗教與全球倫理有何關聯。

我們在兩年內做了三次的田野訪談，受訪人數約四十人，除印尼的穆斯林外，亦包括華人的慈濟志工，我們亦參與觀察慈濟的志工活動。研究發現，慈濟對這些紅溪河畔遷來的災民而言，扮演和印尼政府合作，救災建屋的慈善角色，並未影響居民的宗教生活和信仰。雖然災民起初擔心他們會被迫信仰佛教，後來知道大愛村設有伊斯蘭禮拜堂，且慈濟附設的中小學未干涉女老師包頭巾，仍設有伊斯蘭教課程，才解除戒心。慈濟志工和村民的互動，雙方在宗教信仰方面保持禮敬的距離。慈濟志工不傳教，但傳播證嚴法師的「靜思語」，受訪的村民、宗教教師和伊曼習經院院長，都從道德教育的基礎上肯定「靜思語錄」之為實際的品行指導，且以此視慈濟的價值觀和伊斯蘭教理相同，如幫助別人，維持和平，尊重其他宗教。大愛村和伊曼習經院均有多人參與慈濟志工活動，這些穆斯林志工藉慈濟的助人行為，更了解並堅定自身的信仰，而慈濟也提供他們提升自我尊嚴，發揮良善潛能的機會。本研究反映宗教對話的行動合作模式，面對人類的苦難，雙方以各文化共同關懷的德行以及倫理實踐，產生宗教會通與交流。

O34
(計畫名稱：跨文化能力、學習投入與利他表現)

The Effects of Educational Aid to Learning Performance among Disadvantaged Students: The Case of Jakarta Tzu Chi School in Indonesia

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Education is a powerful way to eliminate poverty and resist inferiority, and also a radical way to make a country powerful. The educational report of the World Bank suggested the NPO to focus their educational aid on providing access to, creating equity, promoting educational quality, and increasing educational resources. The educational aid program enhanced by Indonesia Tzu Chi Foundation on the Angke River Relocate Project adequately responded this recommendation. In this report the authors intend to evaluate the effects of this educational aid program with a set of data based on the fieldwork we conducted between 2006 and 2008, the interviews and observations of students, teachers, parents and volunteers, plus the related documents. Accordingly we found that: the Jarkata Tzu Chi School does provide a better environment for students' learning and good quality of teaching so as to improve students' learning performance and provide a better quality of competence for future employment. Given that the effects of education investment is hard to come into view in a short time, the educational aid of Indonesia Tzu Chi Foundation indeed has satisfied to some extend the students' needs of "knowing and self-actualization" and the feeling of hope. By expanding more secure guarantee and educational opportunity, those disadvantaged students moving from the Angke River Bank to Great Love Village I for the first time have the chance to make a dream of their future. Indonesia Tzu Chi Foundation has played a key role as a well functioned NPO, not only preserving teenager's education rights, but providing a good opportunity to the disadvantaged students and in the time strengthening their educational competency.

Key Words: Indonesian Tzu Chi Foundation, educational aid, disadvantaged students, learning performance, Non-Profit Organizations

O35
(計畫名稱：族群關係與文化發展)

Interethnic Relations in Jarkata Great Love Village

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To explore how the relocation program moving from the Angke River to Great Love Village, we adopted both quantitative and qualitative methods to more than 100 residents from 2006 to 2008. It was found that (1) the villagers demonstrated rather positive stereotypes toward Chinese. The top ten highest frequencies given by the interviewees are (1) eager to progress, (2) hard working. (3) intelligent, (4) neat, (5) strong family bond, (6) honest, (7) polite, (8) compassion and love to give, (9) suspicious, (10) trustworthy, (11) good heart, (12) orderly, and (13) friendly. All traits but suspicious are positive terms attributing to Chinese. The comparisons of residents' evaluation toward selves and Chinese indicated significant differences, with self-evaluation better than that of Chinese, illustrating strong self-identity. Yet, the stereotype scores toward the Chinese reported by the residents are quite positive in average, which shows their good impression of Chinese. Regarding the interethnic interactions, about 30% residents have interacted with Chinese quite often or always, even though relatively fewer interactions were given to commercial and financial relations. These results testified to the fact that the interethnic relations between the residents and Chinese are not as intense as the interethnic history may have suggested. To enhance better relations, we recommend more interactions with the youngsters as they demonstrated more distance with the Chinese as they have to work off the village such that have fewer chance to interact with the Chinese.

Key words: interethnic relations, Angke River Project, charity aid and culture

O36

正向心理的發展與實踐：科際整合研究

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從 1990 年大量出現的正向心理學研究，固然是對目前學術界過度聚焦於人類非常態現象的反省，轉而以正向心理特質為主軸，但無論是正向或負向的行為及心理反應，都構成人類外顯及內隱行為反應的一部份，因此對人類行為與心理的完整瞭解，不應過度偏重於負面問題的探討，應該同時探索正向心理的本質、發展與影響。正向心理學強調智慧、勇氣、人道關懷、正義、修養、心靈超越等六大面向，其下並細分為 24 項正向的品格或美德，或稱「人類長處」。本研究從發展面、學習面、復原力及感恩心四向度瞭解正向特質建構的歷程。研究方法包含量化研究與質性研究;研究對象以大學生及成人為主。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、帶領師培生進行課程設計、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。

O37

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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正向心理學關注個人的優點，重視建立人生中美好的事項和修補不美好的事務，人們藉由發揮長處，重新思考可以達成的目標，並實踐關懷行動，可以開展生命正向的經驗。本研究以大學生為對象，進行正向心理的調查與介入課程研究。首先，完成「大學生生活態度量表」之編製，本量表共有 6 個分量表，總計 86 題：感恩分量表有 6 題，翻譯自 McCullough、Emmons 和 Tsang（2002）發展的感恩自陳量表；樂觀分量表有 15 題，由 Chang、D'Zurilla 與 Maydeu-Olivares（1997）所編製，結合使用正向預期及負向預期，探討個人悲觀與樂觀的特質；希望感分量表 12 題，採用王沂釗（2006）修改與增訂自 Snyder（1997）的「The Goals Scale」，分成目標導向決心(goal-directed determination)與實現目標的計畫(plan of ways to meet goals)；快樂分量表有 23 題，採用 Peterson 與 Seligman（2001）編製之「The Values in Action」真正的快樂量表；幸福感分量表 10 題，係陸洛（2003）所編製；憂鬱症狀分量表 20 題，翻譯自 Radloff（1997）所編製的「流行病學研究中心憂鬱感量表」，包括憂鬱情緒、正向情感、身體症狀、人際問題等向度。本研究以慈濟大學 242 名學生為預試對象進行內部一致性檢定，總量表的 Cronbach'α 為 .97，感恩、樂觀、希望感、快樂、幸福感、憂鬱分量表別為.81、.85、.83、.93、.86、.92，顯示量表之信度良好。在介入成效部分，共有 25 名大二至大四的學生選修「正向心理學」課程，其中男生 8 名，女生 17 名，選修學生於修課前後均接受「大學生生活態度量表」之施測。課程中除正向心理學相關知識的教授外，並將進行個人長處實踐方案，本次選修課程之學生在「美的欣賞」、「感恩」、「生活意義」、「寬恕」、與「愛」等長處較為突出，接下來的課程將就這些面向引導學生規畫實踐方案。另外，本課程也包括小組服務方案的實施，最後將彙整相關質性與量化資料，了解正向特質、正向情緒與正向行為間的關聯性與教學成效，以提供未來相關研究與教學之參考。

關鍵字：大學生、正向心理學、健康方案

O38
(計畫名稱：問題導向服務學習對師培生正向心理的影響)

問題導向服務學習對師培生正向心理的影響~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六項：一是瞭解偏遠地區原住民學生課業學習困難的各項因素。二是設計大學師培生適用的「問題導向服務學習方案」。三是探究「問題導向服務學習方案」實施中，師培生所遇到的各項問題以及解決問題的各種方法。四是分析「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所產生的影響與成效。五是省思「問題導向服務學習方案」中，師培生在正向心理上所產生的轉變及其轉變的因素。六是建構「問題導向服務學習方案」實施模式，並將這樣的服務學習經驗推廣到中小學學校教育中。本研究對象為慈濟大學師資培育的學生，約 20 名。使用的工具包括：正向心理量表、問題導向服務學習方案、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主要是交朋友與吃午餐，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，運用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、帶領師培生進行課程設計、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。

關鍵詞：問題導向服務學習、正向心理學

O39

(計畫名稱：正向情緒在認知、壓力後的生理復原及適應力所扮演的角色)

正向情緒在壓力事件之後的生理復原及適應所扮演的角色

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臨床心理學對心理功能或適應力的瞭解，多從失功能或心理問題為切入點，對個體的差異進行探索或提供解釋。然而這樣一種對於問題行為或適應失調的瞭解或觀點，未必能解釋何以某些人能適應良好或發揮優於平均的心理功能。本研究是立基於現有的正向心理學的理論基礎上，探討正向情緒對於認知功能的的影響。

許多研究均指出：快樂的人比起不快樂的人，有較好的功能、有較多的社會產出和社會活動、和較高的收入，而他們對事情的歸因型態較傾向於自我拉抬(self-enhancing)，也因此可能較容易產生正向的觀點來處理與自我有關的訊息 (Erez & Isen, 2002; Wadlinger & Isaacowitz, 2006)。研究也指出愉悅的情緒狀態或許可以促進較為正向的認知，而正向的認知又可以激發個體的愉悅情緒 (Huppert, 2005)。另外許多的觀察資料顯示： 對一相同的經驗，快樂的人會以較為正向的方式解釋，而快樂的人也比較少對負向的回饋作出回應 (Isen, Niedenthal, & Cantor, 1992)。然而這些研究的共通點是在其研究設計上皆以橫斷法來搜集資料，並作出結論。其缺點是很難以此結果證明正向情緒是影響認知的原因。

本研究是以正向情緒的擴展-建構模式為理論參考架構，以實驗室法探討正向情緒是否有助於個體較彈性的運用其認知資源，如較豐富的語言聯想力，以及能將刺激卡中的抽象圖案組織成較有意義的整體？受試者隨機分派至三個情緒情境 (愉悅、悲傷和中性情緒)，在觀看完引發情緒的影片後，三組的受試者都將進行二項與認知功能有關的任務，分別是 fluency task 以及羅夏克墨漬測驗；前者是評估受試者的語言聯想力，後者則是測量受試者知覺組織能力。在任務結束後受試者需要評定自己在影片結束時的情緒狀態、 進行任務時的情緒狀態、以及自己在進行任務時表現。本研究感興趣的問題是：是否個體在不同的情緒狀態，其認知功能和自我的評估會有不同的表現。

此一仍在進行中的研究，其結果對於發展階段的正向情緒的擴展-建構理論，可提供支持的證據或是修正的建議。另外在諮商實務工作的應用方面，本研究的結果可以對「適應」的機制，提供較周延的解釋。

O40

慈濟志工之正向心理研究

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本研究擬運用人類學的深度訪談及問卷調查法，探討為數眾多的慈濟環保志工，特別是曾經遭逢受苦經驗者，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）。本計畫預定研究期限三年，以台灣北中南東四區的慈濟志工為對象，在各區邀請 15 位（總計 60 位）環保或醫療志工參與研究，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。本計畫第一年度研究重點主要為：(1)已出版的慈濟環保志工的文獻資料，包括慈濟月刊及大愛台相關節目之文本資料，以及(2)對花蓮地區環保志工深度訪談之資料。本年度為第二年度計畫，報告重點為：目前已收集到的各地區環保志工訪談資料的初步分析結果。

O41

Inflammation and thrombosis in cardiovascular and hepatic diseases: an integrative study from cell biology, animal models, to clinical diseases

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The processes of inflammation and thrombosis interact at multiple points and there is abundant evidence to suggest that there are mechanisms common to both these processes. The possibility exists that anti-inflammatory agents could be utilized to manage thrombotic processes underlying disease. The current program project has set goal in elaborating the role as well as the links between the two system of thrombosis and inflammation in common cardiovascular diseases and hepatic diseases such as coronary artery diseases, cardiomyopathy, and hepatic failure induced by iron loading seen in patient of thalassemia. The 4 component projects were listed in the following, with Project 1: G-CSF induce inflammatory-dependent cardiac thrombosis in iron overload heart in mice. Project 2: *In vitro* and *in vivo* studies on the molecular mechanisms of adiponectin in the protection of liver from iron overload and its possible therapeutic implication. Project 3: The role of adiponectin in reactive oxidative stress related cardiomyopathy induced by adriamycin or iron overload. Project 4: Acute ischemic syndrome: Chest pain center concept with research on genomic, biomarkers, proteomic and cell markers. Although distinct project title as well as different methodologies, including in vitro cell culture studies, in vivo animal model studies, and clinical studies were used among our 4 component projects, close interaction and good cohesiveness can be easily found among the projects. For example: mouse model of iron loading were used in project 1, 2, and 3; therapeutic implication using AAV as vector and adiponectin and HO-1 as targets were used in project 2 and 3; expression assays such as RT-PCR, Q-PCR, IHC in project 1,2, and 3; transgenic and gene targeted mice and littermate in project 1, 2, and 3; proteomic analysis with 2D SDS gel electrophoresis, PF-2D, and MALDI-TOF analysis in project 4. The first 3 projects (1-3) were basic research in elucidating either molecular mechanism or establishing disease animal model, while project 4 may hopefully will be the extension of our conclusion resulted from *in vitro* and *in vivo* animal studies (projects 1-3) with further clinical application.

O42

(計畫名稱：G-CSF Induce Inflammatory-dependent Cardiac Thrombosis in Iron Overload Heart in Mice)

G-CSF induces inflammation-dependent cardiac thrombosis in mice can be attenuate by statin therapy: an in vivo disease model

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Objectives: Granulocyte colony-stimulating factor (G-CSF), a hematopoietic cytokine, was recently used to treat patient of acute myocardial infarction with intention to mobilize autologous stem cell thus replace infarct cardiac muscle cell. Although improved cardiac function was reported, controversy existed as some patients developed re-stenosis and worsen the condition post G-CSF delivery. However, the mechanism underlying this G-CSF related thrombosis is still not clear. We present here a new in vivo disease mouse model to study the G-CSF induced cardiac thrombosis. We hypothesize that iron loading could injure cardiac endothelium and further administration of G-CSF can induce cardiac thrombosis.

Methods and Results: We demonstrated that seven of ten iron and G-CSF treated mice (I+G) showed impaired cardiac diastolic function and thrombi formation in the left ventricular chamber, while no mice showed abnormality in other experimental groups. Endothelial fibrosis, increased macrophage infiltration and tissue factor expression were observed in I+G hearts. Supplement of simvastatin to I+G mice abrogated such thrombus formation by attenuating inflammatory profiles and systemic leukocytosis, which was likely due to activation of the pAKT signaling pathway.

Conclusion**:** Our in vivo disease model demonstrated that G-CSF induces cardiac thrombosis through inflammation-dependent pathway and can be attenuated via statin therapy. Present study provides a mechanism and potential therapy for G-CSF induce cardiac thrombosis. cardiomyocyte.

O43

In vitro and *in vivo* studies on the molecular mechanisms of adiponectin in the protection of liver from iron overload and its possible therapeutic implication

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Adiponectin, an adipocytokine first described as the most abundant protein produced by adipocytes, acts as an anti-inflammatory protein, suppresses cytokine production by activated macrophages, and displays anti-atherogenic property. The anti-inflammatory and anti-oxidative properties of adiponectin might decrease the iron-induced toxicity in liver. Adiponectin has been shown to activate a peroxisome proliferator-activated receptor-alpha (PPAR-α). Motif promoter analysis predicts a PPAR-α binding in the upstream of heme oxygen (HO)-1 promoter at the position of -888 to -871. HO-1 has been shown to involve in iron reutilization in liver that might also play synergistic effects on the protection. Therefore, we explore the potency of adiponectin as a HO-1 inducer and the molecular mechanisms of the protection and therapeutic implication of hepatic iron overload by adiponectin. We observed that adiponectin activated PPARa that consequently induced Cox-2 and HO-1 in hepatocytes. The induction of Cox-2/HO-1 elicited the anti-inflammatory and –oxidative effects against iron-mediated liver damage. The action of adiponectin via a PPARa-depedent mechanism was confirmed using a PPARa agonist, Wy-14643, which mimics the protective effects of APN in iron-mediated liver damage. Interestingly, the protective effects of APN from iron toxicity involved in Cox-2/HO-1 induction were partially reversed by using inhibitors of Cox-2 and HO-1, NS398 and SnPP, suggesting their crucial roles in this event. We also provide the evidence of the protective effect of adiponectin in vivo iron overload animal model of balb c57 and PPARa-knock out mice. Interestingly, adiponect not only decreased iron-induced apoptosis and inflammation, but also eliminate iron deposition both in vitro and in vivo, whereas the effects were reversed in PPARa knockout mice, suggesting the importance of PPARa in the protection of apn in iron-mediated liver damage. In this study, we first demonstrated the molecular mechanism and therapeutic effects of adiponectin in the protection of liver iron overload.

O44

(計畫名稱：The Role of Adiponectin in ROS-related Cardiomyopathy Induced by Doxorubicin or Iron Overloading)

Adiponectin blocks G-CSF plus iron-induced cardiomyopathy and iron deposition via PPARα-dependent hemoxygenase-1 activation

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Background- Chronic inflammatory and immunological reaction induced by GCSF/ iron that leads to a structural or functional deterioration of the heart been described previously. In addition decreasing level of adiponectin, a cytokine secreted from heart, adventitia and adipocytes, are associated with heart dysfunction induced by G-CSF/iron. We over-expressed adiponectin to the heart by AAV8-adiponectin (AAV8-APN) resulting in a dramatically lower thrombi formation (from approximately 80% to 15%) iron deposition and relative cardiac functions also are increased in overexpressed APN mice following G+I treatment. In addition, several inflammatory markers (number of infiltrating neutrophils, myeloperoxidase activity and induction of MCP-1, TNF-α, IL-6. ICAM) all were lower in WT mice infected with AAV8-APN. As for the mechanism of inhibition of G-CSF/iron induced cardiomyopathy, we found that APN increased hemeoxygenase-1(HO-1) expression via PPARα dependent pathway in vitro and reduced HO-1 expression is observed in PPARα knock out mice following infused with APN.

Conclusion: Together, these results demonstrate, APN may act as an anti-inflammation signal molecule inducer and induce HO-1 expression via PPARα dependent pathway in cardiomyocytes that attenuated thrombi formation and inflammation response in G+I induced heart cardiomyopathy.

O45

Acute coronary syndrome: Chest pain center concept with research on genomic, biomarkers, proteomic and cell markers

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Inflammation has been shown to play an important role in atherosclerotic cardiovascular disease. In recent 3 years, we have analyzed the association of inflammatory maker gene polymorphisms with serum level of C-reactive protein (CRP) and metabolic syndrome. A sample population of 617 individuals was enrolled for analysis. By genotype/haplotype analysis, using 5 *CRP* single nucleotide polymorphisms (SNPs), we have found significant association of *CRP* gene variants with CRP levels. Two haplotypes inferred from 5 SNPs (GCGCG and AAGCG) were associated with increased CRP level ($P = 0.017$ and $P < 0.0001$, respectively). Interaction analysis also revealed interaction of obesity with *CRP* genotypes/haplotypes with CRP level. These results suggested that *CRP* genotypes/haplotypes interact with obesity to set CRP level. (*Atherosclerosis*, 2009, in press). In addition, we also demonstrated significant association of soluble intercellular adhesion molecule-1 (sICAM1) with insulin resistance and metabolic syndrome in Taiwanese, which is independent to traditional and emerging cardiovascular risk factors. These data provide further evidence of the mechanisms of sICAM1 as a molecular marker for atherosclerosis (*Metabolism*, 2009, in press). For further elucidating the importance of Inflammatory gene polymorphisms on metabolic syndrome, six SNPs on the *ICAM1* gene were investigated, which showed significant association between SNP rs5491 and the sICAM1 level ($P<0.001$). We further combined *ICAM1* and *CRP* SNPs and found independent association between combined *ICAM1-CRP* genotypes and metabolic syndrome in multivariate analysis (submitted for publication). In conclusion, we have first elucidated that inflammatory maker gene polymorphisms, including *CRP* and *ICAM1* polymorphisms, play an important role on the occurrence of metabolic syndrome in Taiwanese. Further study will be necessary to understand the role of inflammatory maker gene polymorphisms on atherosclerosis heart disease.

O46

(計畫名稱：第一型與第二型糖尿病病程與併發症之生物醫學整合研究)

Biomedical Integrative Studies in the Progress and Complication of Type I and II Diabetes Mellitus in Rats

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Diabetes mellitus (DM) is one of the top ten death cause in Taiwan, and also related to cardiovascular, retinopathy, nephropathy, and distal organ and limbic amputation. Therefore, in this integrated study, we focused on the influence and clinic applications, such as inhalation anesthetics and hyperbaric oxygenation (HBO) in the type I and II DM animals. We used the frequency-domain analysis of telemetric systemic arterial pressures (SAP) and pulse-pulse intervals (PP) to quantify the parameters of blood pressure variability (BPV) and heart rate variability (HRV) for elucidating the effects of HBO and inhalation anesthetics on cardiovascular autonomic functions in diabetic rats. We found that recovery of vascular sympathetic modulation (BLF) was early in the diabetic rats with HBO treated when used sevoflurane than halothane and desflurane. We also found that type I DM rats showed that SAP, vascular sympathetic indicators were significantly decreased; PP, parasympathetic indicators were significantly increased by HBO. Cutaneous collateral circulation measured with laser Doppler flowmetry defined by the flux changes after epigastric flap elevation were significantly increased. Furthermore, we used type II DM rats to examine the expression levels of circadian clock genes in livers and found that changes of circadian clock genes expression are related to the functioning or daily pattern of metabolic processes in livers. In conclusion, these findings of the integrated study are valuable and may provide benefit at the clinical application in DM patients in the future.

O47

(計畫編號：Relationships between Rhythm-related Genes and Type I and II Diabetes Mellitus.)

Changes of Circadian Clock Genes Expression in Livers of Diabetic Rats

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Population of obesity and type 2 diabetes mellitus (T2DM) is increased with high rate on well-developed and developing countries. Except some related to heredity, most of obesity and T2DM are related to the energy imbalance. Recently some studies found that the circadian clock genes are important to maintain not only the biological clock but also the peripheral physiological functions in the energy balance. In this study, we tried to establish the animal models of T2DM using streptozotocin (STZ) and nicotinamide (NA) in male Spraque-Dawley rats to confer the role of circadian clock genes in pathophysiological progress of T2DM. For monitoring the pathophysiological responses after treatments, we measured the food intake, body weight, fasting plasma glucose level, and insulin resistance by the intraperitoneal glucose tolerance test. The treated groups displayed gradually increase of fasting glucose level, and exerted the trend of insulin resistance even DM after treatment. The expression levels of circadian clock genes, such as *period1 (Per1)*, *Per2*, *Clock*, *Bmal1*, *Cryptochrome 1 (Cry1)*, and *Cry2*, in the liver were examined by real time reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR). The gene expression except *Clock* showed the diurnal rhythm and treated groups showed the different trends in livers. In conclusion, changes of circadian clock genes expression in livers are related to the functioning or daily pattern of metabolic processes.

O48

(計畫編號：To Evaluate the Effects of Different Inhalation Anesthetics on Cardiovascular Neural Regulation of Autonomic Nervous System in the Streptozotocin Induced Type I and Type II Diabetic Rat)

Effects of Hyperbaric Oxygenation and Inhalation Anesthetics on Cardiovascular Autonomic Neural Regulation in Streptozotocin Induced Diabetic Rats

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Inhalation anesthetics increase heart rate (HR) in vivo both in animal and humans but decrease heart rate in isolated hearts. Clinical studies indicate that diabetes mellitus is associated with changes in autonomic nervous system control of cardiovascular function. This study is to elucidate the effects of hyperbaric oxygenation (HBO) and inhalation anesthetics on cardiovascular autonomic functions in diabetic rats. We measured daily blood pressure variability (BPV) and HR variability (HRV) of diabetic Sprague-Dawley rats with HBO treatment and exposure the different inhalation anesthetics (halothane, desflurane and sevoflurane) until recovery. Frequency-domain analysis of telemetric systemic arterial pressures (SAP) and pulse-pulse intervals (PP) were used as the parameters of BPV and HRV. High frequency power of HRV (HHF) and low frequency power of BPV (BLF) was referred to the cardiac vagal modulation and vascular sympathetic modulation, respectively. Normalized low-frequency power (LF %) of the RR spectrogram was regarded as the cardiac sympathetic modulation. Our results show that diabetic rat with HBO treated was associated with significant decrease of HR and a higher trend of HHF among the three inhalation anesthetics. Mean BP and LF% were not changed. BLF was significantly lower till at PA 90 in sevoflurane group but at PA 30 was significantly lower at both halothane and desflurane groups. In conclusion, recovery of vascular sympathetic modulation was early in the diabetic rats with HBO treated when used sevoflurane than halothane and desflurane. These results provide partial benefit at the clinical anesthesia at diabetes patients with HBO treated.

O49

(計畫編號：Effects of Hyperbaric Oxygen on the Dysfunctions of Cardiovascular Neural Regulation and Cutaneous Collateral Circulation in Type I and II Diabetic Rats)

Hyperbaric Oxygenation Improves the Cardiovascular Autonomic Neural Regulation and Cutaneous Collateral Circulation in Diabetic Rats

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Past studies demonstrate that programmed hyperbaric oxygen therapy is able to optimize the cutaneous oxygenation and attenuate the autonomic neural dysfunction in diabetic patients with foot complications. The specific aim in this study is to elucidate the effect of hyperbaric oxygenation (HBO) on cardiovascular autonomic functions and cutaneous circulation in diabetic rats. We measured blood pressure variability (BPV) and heart rate variability (HRV) in freely moving and conscious streptozotocin (STZ, 60 mg/kg, ip) induced diabetic Sprague-Dawley rats during the courses of HBO. Frequency-domain analysis of telemetric systemic arterial pressures (SAP) and pulse-pulse intervals (PP) were used to quantify BPV and HRV. Cutaneous microcirculation was measured with laser Doppler flowmetry before and after the elevation of epigastric flap. Our results in type 1 diabetes (T1D) showed that SAP, vascular sympathetic indicators were significantly decreased; PP, parasympathetic indicators were significantly increased by HBO. Cutaneous collateral circulation defined by the flux changes after epigastric flap elevation were significantly increased. T2D model was developed eventfully by applying more than 10 combinations of ages, STZ and nicotinamide doses, and surgical stress. Insulin resistance with an elevated blood sugar at 120 mins of glucose tolerance test played a strong indicating role for developing T2D. In conclusion, HBO improves the cardiovascular autonomic regulation in diabetic rats by increasing the cardiac vagal modulation and decreasing the vascular sympathetic activities. Cutaneous collateral circulation is also increased by HBO

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O50

台灣原住民幽門桿菌感染與胃癌發生之關係—整合分子流行病學、致病機轉與臨床研究

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胃癌是台灣常見且高死亡率的腸胃道癌症，佔十大癌症死因的第四位，其發生主要和環境中的危險因子，如飲食、幽門桿菌感染等有關。根據衛生署的統計，發現台灣原住民族群胃癌之發生與死亡率遠高於平地漢人，尤其是台灣的東北部和東部山地鄉鎮更是胃癌的高死亡率地區，原因除可歸咎於原住民的傳統飲食習慣外，我們初步的研究結果顯示原住民族群幽門桿菌感染之盛行率高於台灣其他族群，因此深入探究台灣原住民族群胃癌發生與幽門桿菌感染及致癌因子之關係，將有助於找到導致胃癌發生的真正「病因」，並提出診斷、治療及預防胃癌發生的方針。本研究現階段共整合了五個子計畫，分別為子計畫一「原住民易感基因與幽門桿菌感染在胃癌生成上的相互關係」、子計畫二「以系統性方法分析台灣東部原住民幽門桿菌之致病基因」、子計畫三「幽門桿菌大鼠感染模式之建立與應用」、子計畫四「幽門桿菌感染對台灣原住民胃部黏蛋白表現之影響」及子計畫五「建立幽門桿菌感染與胃癌生成之指標因子」。本研究是利用PCR-RFLP、實驗動物模式、基因體系統性方法探討台灣東部原住民免疫調控相關基因之單核甘酸多型性(SNPs)及幽門桿菌感染與胃部疾病嚴重度之關係，並藉由蛋白質體學(proteomics)尋找胃癌相關之特異指標因子，以期作為基因診斷與治療之依據。近兩年半(95 年 11 月 1 日～98 年 3 月 31 日)的研究成果顯現，原漢二族群間感染菌株毒性因子型別(*vacA* 及 *iceA*)有差異性存在，且宿主本身與天然免疫機制之遺傳因子 *ICAM-1* K469E 之基因型分佈亦有差異，另外 ICAM-1 K469E 的 K/K 型別與 NOD1 E266K 的 A/A 型別可能是幽門桿菌感染較易引發胃部疾病的潛在 SNPs (子計畫一)；以免疫組織化學染色法發現 MUC2 與 Ki-67 可作為評估胃癌發生早期的標誌因子(子計畫四)；在胃癌組織中則發現有許多致癌基因被誘發產生，且與正常胃黏膜組織蛋白質表現圖譜不同(子計畫五)；另外亦建構幽門桿菌之突變株與基因表現資料庫，以供篩選幽門桿菌與致癌直接相關之基因(子計畫二)；並且建立了幽門桿菌感染之大鼠模式的最佳條件，從組織病理切片上看到被感染的大鼠有胃炎的發生，COX-2 及 iNOS 蛋白的表達皆高於無感染的大鼠，顯示被幽門桿菌感染之大鼠的胃部亦會有發炎反應產生，並且以毒力較強之菌株感染大鼠後，產生胃炎的時程較毒力弱之菌株短，因此目前所建置之感染大鼠模式是可提供作為篩選幽門桿菌之毒力因子引發病變之能力的測試(子計畫三)。

O51

Relationship between susceptible genetic polymorphisms of the aborigine in Taiwan and *Helicobacter pylori* infection on gastric carcinogenesis

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Helicobacter pylori infection is closely associated with gastroduodenal diseases, such as chronic gastritis, gastric and duodenal ulcers, gastric carcinomas, as well as mucosa-associated lymphoid tissue (MALT) lymphoma. Both microbial and host factors influence the clinical course of *H. pylori* infection, and the disease outcome is determined by multiple factors, including the genetic composition of the infecting *H. pylori* strains and the genetic make-up of the host, and especially in the host immune responses. In Taiwan, there is definite geographic variation in gastric cancer incidence and death, with the higher rates seen in the aboriginal than non-aboriginal groups. It has been thought that there maybe different susceptible factors existing between the two populations. In the past 2 years, we have shown that (1) the prevalence of *H. pylori* was higher, with the age of the patients being younger, in aboriginal population than non-aboriginal groups; (2) the distribution of virulence factors, *vacA* and *iceA*, of *H. pylori* was significantly different between the two groups; (3) the single nucleotide polymorphisms of K469E of intercellular adhesion molecule 1 (ICAM-1), and E266K of nucleotide oligomerization domain 1 (NOD1), were both statistically different among ulcer patients, gastritis patients and healthy control subjects. In the third year, we used RT-PCR and immunohistochemistry to detect the expression of the mRNA and protein of β-defensins (hBD 1 and 2) in the gastric biopsy samples from *H. pylori*-infected and noninfected patients. The results show that hBD1 was expressed consistently in both groups, but hBD2 was significantly higher in *H. pylori*-infected patients than the noninfected ones.

O52

Isolation of pathogenic genes in *Helicobacter pylori* from east Taiwan Aboringines by systemic approach

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Helicobacter pylori, a spiral gram-negative microanaerobic bacillus colonizing the human stomach, is related to gastritis, peptic ulcer, and gastric malignancies, such as adenocarcinoma and mucosa associated lymphoid tissue (MALT) lymphoma. However, despite the high prevalence of this infection, only a small portion of infected patients incur significant clinical sequelae. Most patients remain asymptomatic throughout their lives. The difference in clinical outcomes may be ascribed to the host genetic background, environmental factors and virulence of bacterial strains. According to the prior study results, we know that the most common cancer sites of Taiwan Aborigines were stomach. Therefore, the aim of our study is to examine the special virulence factor of *H. pylori* from Taiwan Aborigines by systemic approach. At the beginning, thirty-six clinical isolates of *H. pylori* from Taiwan Aborigines patients with gastritis or ulcer were collected. Molecular typing of these clinical isolates was performed by PCR, and then the most virulent strain among these clinical strains was isolated by *Helicobacter pylori*-infected mouse model. The proportion of hummingbird cells in the AGS cell line rapidly increased following the inoculation of the most virulent *H. pylori* strain A699 but increased more slowly with reference strain 26695. In order to elucidate the *H. pylor*i-host cell interactions that underline the neoplastic process and identify which host cell factors are involved, we tried to characterize proteins differentially expressed in *H. pylori*-infected gastric epithelial AGS cells. Firstly, we examined broad patterns of gene expression induced by the A699 in the gastric cancer cell line AGS cells in culture using the DNA microarray. We identified approximately 50 genes that showed marked changes. To further investigate the difference of proteins expression between A699 and 26695, we combined two-dimensional gel electrophoresis and mass spectrometry to separate and characterize the total proteins of these bacteria. Recently, we got several possible proteins were upregulated in the virulent strain A699. These virulence associated genes will be further analyzed. We hope these informations will be helpful for preventing *H. pylori* infection and provide useful to drug development in the future.

O53

Development and application of *Helicobacter pylori*-infected rat model

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Helicobacter pylori is one of the most common pathogens which has been thought to be associated with chronic gastritis, gastric and duodenal ulcers, gastric carcinomas, and mucosa-associated lymphoid tissue (MALT) lymphoma, with the pathogenesis mechanism largely remained to be studied. It has been demonstrated that *H. pylori* produces many deleterious factors in gastric epithelial cells, such as vacuolating cytotoxin (VacA), cytotoxin-associated gene A (CagA), and urease that enhance the secretion of a range of inflammatory mediators. In addition, to study the bacterial pathogenesis, ideal animal model is very important. In this study, to optimize the infection conditions for *H. pylori* to colonize the gastrointestinal tract of rats, we choose 2 *H. pylori* strains, both were *vacA*m1 type but one was *cagA*-positive and the other *cagA*-negative, which were isolated from the gastric biopsy of a gastric cancer and a gastric ulcer patient, respectively. The results showed that (1) six to eight-week-old male Wistar rats were suitable for *H. pylori* infection, (2) the best conditions for infection were feeding two times (with an interval of 1 hr) a day for two days with the cells (1 x 10^9 CFU/ml) cultured for 24 hr, (3) active chronic gastritis was observed in rats 4 weeks after *cagA*-positive *H. pylori* inoculation, and 26 weeks after *cagA*-negative *H. pylori* infection, and (4) expression of cyclooxygenase-2 (COX-2) mRNA in gastric mucosa increased significantly in all *H. pylori*-infected rats.

O54

Effects of *Helicobacter pylori* infection on mucin expression in gastric tissues of aborigines in Taiwan

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Helicobacter pylori is the major etiological agent of chronic gastritis and peptic ulcer disease, and may lead to gastric cancer through intestinal metaplasia via alterations of the gastric mucosa. One of the possible mechanisms is that the bacteria have effects on the human antral and body mucosal intracellular mucin contents. The characteristics of the gastric mucous layer reflect its physiologic function and changes in its composition can be noted following various insults. Using monoclonal antibodies against various mucins has allowed evaluation of the changes in composition and distribution among diverse pathological samples. The goal of this study was to determine the distribution of mucins (MUC1, MUC2, MUC5AC, MUC6) and Ki-67, a proliferation-associated nuclear antigen, in the gastric biopsy specimens with relation to the *H. pylori* status by the immunohistochemical analysis. These specimens were enrolled from the patients undergoing upper gastrointestinal endoscopy at Buddhist Tzu Chi General Hospital. The results reveal that (1) the protein level of MUC2 was higher, but MUC5AC was lower in the samples from *H. pylori*-infected aboriginal patients than noninfected ones, (2) the protein expression index of Ki-67 was higher in *H. pylori*-infected nonaboriginal than aboriginal patients, (3) the secretion levels of gastric mucins were lower in *H. pylori*-infected aboriginal patients than nonaboriginal ones, and (4) no significant differences were found for the levels of MUC1 and MUC6. In addition, we found that the protein levels of MUC2 were significantly correlated with intestinal metaplasia, and with concomitant decrease in levels of MUC5AC in the gastric surface mucosa cells. In conclusion, the present study indicates that the MUC2 mucin and Ki-67 antigen expression patterns are likely reliable markers implying the tendency towards carcinogenesis in *H. pylori*-infected patients.

O55

The association between *Helicobacter pylori* and specific antigen express in gastric cancer

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The Gram negative bacterium *Helicobacter pylori* is one of the major causes of human gastric carcinoma, which is not only the second most frequent form of cancer worldwide but also represents the fourth leading cause of cancer death in Taiwan. However, the detailed mechanisms leading to gastric cancer formation still remain elusive. In order to gain better understanding of the mechanisms underlying the pathogenic development of this malignancy, a systematic, proteome based approach was chosen to detect candidate antigens of *H. pylori* for future uses in diagnosis, therapy and vaccine development. Using this approach, we were able to identify a series of proteins that expressed in normal gastric mucosa but were frequently down-regulated or not expressed in gastric cancer. The ones differentially expressed in gastric cancer were further verified by RT-PCR. Increases in manganese dismutase(MnSOD), biliverdin reductase and nonhistone chromosomal protein HMG-1 (HMG-1) were observed, while decreases in glutatione-S-transferase (GST) and foveolin precursor (gastrokine-1) (FOV), an 18-kDa stomach-specific protein with putative tumor suppressor activity, were detected.

O56

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 previous study showed that some species of *Momordica charantia* have some growth inhibitory effects on Hep G2 and the viral containing Hep G2.2.15 cells, some anti-viral effects on hepatitis B virus and anti-diabetic effects on 3T3/L1 adipocytes. Results of this study indicated that the effects of *Momordica charantia* are variant and the components extracted from the stem and leaves are more bioactive than others. Compounds purified from that parts of *Momordica charantia,* MC-9, MC-10, MF-1, MF-5, MS-1 and MS-2 didn't show significant responses related to the crude extracts. MC-1, which is a novel compound extracted from Hualien species of *Momordica charantia*, showed the anti-tumor activity on Hep G2 cells.

O57

(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 in this study. 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. Results of protein expression showed that the expression of hemoxygenase-1 (HO-1), an oxidative stress-induced protein, was raised obviously after treating with MC-1 for 24 h. However, p53 and the down-regulated p21 were decreased after treatments with the increased doses of MC-1.

PARP, caspase-3 and 8 in Hep G2 cells were significantly cleaved after treating with MC-1 for 24 h. The increased expression of HO-1 in Hep G2 cell treated with MC-1 was dose-dependent. It indicated that MC-1 might be one of the possible components in *Momordica charantia* resulted in cell apoptosis.

O58

A study on the antigluconeogenesis activity of *Momordica charantia*

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We found two crude extracts that could promote adipogenesis and enhanced 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 this 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 instead.

We screened phosphotases and kinases required for adipogenesis using a shRNA library. We found 173 genes could block and 12 genes could promote adipogenesis when knocked down. Many of the genes among the 173 genes were well known genes involved in adipogenesis. Some genes such as Nrp2 were specific to NIH/3T3 cells not required for 3T3-L1 adipogenesis.

We also developed a high sucrose liquid diet for mice. This animal model of obesity and insulin resistance is easy to induce and at low cost. It serves as a platform to screen for in vivo hypoglycemic activity.

O59

(計畫名稱：Isolation and Characterization of Terpenoid Synthases and Ribosome Inactivating Proteins from *Momordica charantia*.)

Construction of a yeast functional expression system for positive selection of triterpenoid synthase from *Momordica charantia*

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The biosynthetic pathways of fungi sterols and plant triterpenoid share the same upstream steps and diverge at the cyclization of 2,3-oxidosqualene, which is cyclized to ergosterol in fungi and to cycloartenol in plants.

Two *Saccharomyces cerevisiae* mutants LS811-dCan and LS811-dHg were constructed by homologous recombination to replace the erg7 gene that encodes the yeast lanosterol synthase responsible for the cyclization of 2,3-oxidosqualene. These heterozygotic mutants were transformed with a recombinant erg7 and sporulated to obtain haploid yeasts carrying the plasmid pRS416-GAL-erg7.

When the haploid yeasts were grown on media containing 5-fluoroorotic acid (5-FOA), the URA3 gene on the pRS416-GAL-erg7 converted the 5-FOA into the toxic compound 5-fluorouracil and the growth of the yeasts was arrested. These haploid yeasts, LS811-hCan and LS811-hHg , were used as a positive expression system for the selection of plant cDNAs capable of cyclization of 2,3-oxidosqualene.

cDNA libraries of *Momordica charantia* were subcloned in a gateway plasmid pYES-DES-811 and will be transformed into LS811-hCan and LS811-hHg for rapid isolation of novel triterpenoid synthase. This system could be generally applied as a rapid and simple screening tool for oxidosqualene cyclase from other plant cDNA libraries.

O60

Nicotinic acetylcholine receptor and neurovascular function

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The proposed study is to examine the role of the nicotinic acetylcholine receptor in mediating neurotransmission in the central and peripheral nervous system, and the glia, and its influence by beta-amyloid (Aβ) peptides, cholinesterase inhibitors, and statins in regulating the neurotransmission and vascular function. Significant progress and important findings for each proposed project have been made. The major findings for each project are:
1. The predominant functional nAChR subtype located on perivascular sympathetic nerves of the pigs in Taiwan is α3β2 subtype, but is not the α7 subtype like that found in the pigs from the US. We also have discovered that palmitic acid methyl ester (PAME) is released from the rat preganglionic neurons of the superior cervical ganglion. PAME is found to inhibit the postganlionic nicotinic receptor, suggesting that PAME may play an important role in regulating ganglionic transmission. In addition, PAME is a potent vasodilator. This latter finding is expected to open a new field of research on the physiological and pathophysiological role of this new vasodilator. (project # 95005-1). 2. Nitrergic-glutamatergic fibers and preganglionic nitrergic-cholinergic neurons are present in the dorsal facial area (DFA) for regulation of the CCA blood flow. Microinjection of nicotine orglutamate into the DFA of the medulla increases blood flow of the common carotid artery (CCA). The nicotinic action primarily via α7-nAChRs causes a release of glutamate to activate the glutamatergic receptor, while the glutamatergic action does not induce a release of cholinergic substance to activate the nicotinic receptor. These findings provide important information for developing therapeutic strategy for Alzheimer's disease (AD), hypertension and cerebral ischemia.(project #95005-2). 3. Acute administration of Aβ selectively increases NMDA receptor function in central sympathetic neurons, and kinase-dependent mechanisms are involved in modulating Aβ sensitivity of NMDA receptors. Furthermore, different fragments of Aβ are found to have differential effects on the NMDA receptor functions. Multiple mechanisms underlie the regulation of NMDA receptor functions by Aβin rat central sympathetic neurons.(project # 95005-3). 4. Nicotine-induced NO release in the astrocytes and its modulations by Aβ and statins are similar to those found in the cerebral neurovascular function, suggesting that decreased release of NO or dysfunction of astrocytes may be involved in the pathogenesis of AD. (project # 95005-4)

In summary, the results are consistent with our hypothesis that nAChR of different subtypes may play important roles in regulating neurovascular transmission and the function of the glia. In 28 months, the overall progress and accomplishments of this program project grant are very good based on the published 9 SCI papers and 1 submitted manuscript.

O61
(計畫名稱：Sympathetic nAChR and Cerebral Nitrergic Neurogenic Vasodilation)

Sympathetic α3β2-nAChRs mediate cerebral neurogenic nitrergic vasodilation

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The nicotinic acetylcholine receptor (nAChR) on the sympathetic nerves innervating cerebral arteries of the pig crossbreed between Landrace and Yorkshire (LY) in Illinois is α7-nAChR. Nicotine-induced cerebral neurogenic vasodilation in pigs crossbreed among Landrace, Yorkshire and Duroc (LYD) in Hualien (Taiwan), however, is not blocked by α-bungarotoxin (α-BTGX, a highly selective α7-nAChR antagonist). The cerebral perivascular sympathetic nAChR subtype of LYD pigs, therefore, was examined. The nicotine-induced dilatation of isolated basilar arteries was not affected by α-conotoxin IMI (an α7-nAChR antagonist) or α-conotoxin AuIB (an α3β4-nAChR antagonist). The vasodilation was inhibited by preferential α3-containing nAChR antagonists (tropinone and tropane) and α-conotoxin MII (a selective α3β2-nAChR antagonist). Using reverse transcription PCR, α3-, α7-, β2- and β4-subunits of nAChRs were expressed in fresh superior cervical ganglia. The mRNA levels of α3-, β2- and β4-subunits were significantly higher than that of α7-subunit. Furthermore, nicotineinduced inward currents in α3β2-nAChR-expresssing oocytes were blocked by α-conotoxin MII but not by α-BTGX or α-conotoxin AuIB. In conclusion, the predominant nAChR on cerebral perivascular sympathetic nerves mediating cerebral nitrergic neurogenic vasodilation in LYD pigs is α3β2-subtype (supported by TCU & NSC).

O62

(計畫名稱：Control of Common Carotid Arterial Blood Flow by Nicotinic, Glutamatergic, and Nitrergic Actions in The Medulla of Cats)

Nicotine-induced glutamate release in dorsal facial area of the medulla leads to an increase of blood flow in common carotid artery in cats

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Microinjection of nicotine or glutamate into the dorsal facial area (DFA) of the medulla increases blood flow of the common carotid artery (CCA). Whether these two actions are mutually dependent or independent is not known. Various agonists and antagonists for the nicotinic and glutamatergic receptors were microinjected through a four-barrel tubing into the DFA of anesthetized cats. Drug effects were assessed by changes in the CCA blood flow. Microinjections of 30 nmol nicotine (a non-selective nAChR agonist), 40 nmol choline (a selective α7- nAChR agonist) or 30 nmol glutamate induced a modest increase of the CCA blood flow. The nicotine- and choline-induced responses were dose-dependently reduced by prior administration with α7-nAChR antagonists (α-bungarotoxin or methyllycaconitine) as well as a non-competitive NMDA receptor antagonist (MK-801) or a competitive AMPA receptor antagonist (glutamate diethylester, GDEE). Nevertheless, the exogenous- or endogenous glutamate-induced response was not affected by the pretreatment with either α-bungarotoxin or methyllycaconitine. In conclusion, the nicotinic action primarily via α7-nAChRs causes a release of glutamate to activate the glutamatergic receptor, while the glutamatergic action does not induce a release of cholinergic substance to activate the nicotinic receptor. These findings may provide important information for developing therapeutic strategy for diseases such as Alzheimer's disease, hypertensive disease and cerebral ischemia.

O63

(計畫名稱：The Effects of Amyloid Beta-peptides on The Function of Nicotinic and Glutamatergic Receptors in Central Sympathetic Neurons of Rats)

Effects of soluble amyloid beta-peptides in N-methyl-D-aspartate (NMDA) receptor activation in rat central sympathetic neurons

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Soluble oligomers of amyloid beta-peptides (Aβ) have recently been demonstrated to adversely affect synaptic structure and plasticity and considered to participate in the pathogenesis of Alzheimer's disease (AD). The vasoactive effect of Aβ on cerebral and peripheral vessels has been reported *in vitro* and *in vivo*. It has been proposed that the mechanisms of AD pathogenesis may involve a combination of the vascular and neuronal toxicity of Aβ. Several *in vitro* studies reported recently that Aβ affected the function of NMDA receptors, a subtype of ionotropic glutamatergic receptors. The effect of Aβ on central control of cardiovascular function is poorly understood. The present study was undertaken to examine the effects of amyloid beta-peptides (Aβ) on the function of NMDA receptors, a subtype of ionotropic glutamate receptors, in rat central sympathetic neurons. Firstly, we examined the effects of different segments of Aβ on NMDA receptor-mediated responses in sympathetic ganglionic neurons (SPNs) of neonatal rat spinal cord slice preparation by whole cell recording *in vitro*. Consecutive applications of NMDA every 5 min induced reproducible membrane depolarizations in SPNs. Significant increases in NMDA-mediated depolarizations were found 10-20 min after superfusion of Aβ (1-40, 0.1 and 0.3 μM) for 5 min. Superfusion of Aβ (1-42, 0.3 μM) had no significant effects on NMDA-mediated depolarization. Interestingly, superfusion of Aβ (25-35, 0.3M) significantly inhibited NMDA-induced depolarizations. Western blot analysis showed that incubation of Aβ 0.3 μM but not Aβ 0.3 μM for 30 min onto spinal cord slices increased the levels of phosphoserine 896 on NMDA receptor NR1 subunit in lateral horn regions. Secondly, we examined the effects of Aβ on NMDA receptor function in rat rostral ventrolateral medulla (RVLM) neurons *in vivo*. Repeated microinjections of NMDA (0.14 nmol) into the RVLM every 30 min caused reproducible increases in mean arterial pressure in urethane-anesthetized rats weighting 300-350g. Microinjection of lower doses of Aβ (1-40, 20 pmol, 200 pmol) into RVLM 10 min pre-injection of NMDA significantly potentiated NMDA-induced pressor effects. Interestingly, injection of high dose of Aβ (1-40, 1 nmol) inhibited NMDA-induced pressor effects. These results suggest that different fragments of Aβ may have differential effects on the NMDA receptor function and a multiple mechanisms underlying the regulation of NMDA receptor function by Aβin rat central sympathetic neurons.

O64

(計畫名稱：Effects of nAChR, Aβ and Statins on Glia Cell Function)

Effects of Aβ and statins on nAChR-mediated NO release in glia cells

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Alzheimer's disease (AD) is a progressive dementing neurologic illness and the most common dementia disease in the elderly. Regional cerebral vasoconstriction, extracellular deposits of amyloid β (Aβ) fibrils and activation of microglial cells are suggested to play important roles during the pathogenetic development of AD. This study is focused on how one type of glia cells, astrocytes, are affected by Aβ and to explore the possibility of these cells in the pathogenesis of AD.

It has been demonstrated that activation by nicotinic agonists of α7-nicotinic acetylcholine receptor (α7-nAChR) located on cerebral perivascular sympathetic neurons results in nitric oxide (NO) release and vasodilation. α7-nAChR and three forms of nitric oxide synthase (NOS) are expressed in astrocytes. Epidemiological observations reveal that statins may improve clinical symptoms of AD. In this study, we examined if nicotinic agonists stimulate NO production from astrocytes as well as if this process is affected by Aβ and statins. The preliminary results demonstrated that α7-nAChR agonists, nicotine (0.2 mM) and choline (2 mM), induced NO production. The choline-induced NO release was inhibited by Aβ (1.5 μM), and this inhibition was prevented by concurrent treatment of lovastatin (20 μM). In addition, the nicotine-induced NO release was also inhibited by Aβ (3.0 μM), and this inhibition was prevented by concurrent treatment of lovastatin (40 μM) or metastatin (40 μM). These results indicate that nicotine-induced NO release in the astrocytes and its modulations by Aβ and statins are similar to those occurs in cerebral neurovascular function, suggesting that decreased release of NO or dysfunction in astrocytes may be involved in the pathogenesis of AD.

O65

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, including hypertension and stroke. Thus, this integrated program was performed to investigate MA-induced neuronal toxicity related to behavioral dysfunction, neuronal activity and cardiovascular alteration. In component project 1, we determined the cognitive dysfunction and social interaction deficits after the repeated administration of MA. Data indicated that mice exposed to a sensitizing regimen or neurotoxic regimen of MA exhibited deficits in neuropsychological behavioral tests. The cognitive deficits induced by long-term MA exposure were improved by the microglia inhibitors, pentoxifylline and clenbuterol. In component project 2, the effect of MA on the neuronal activity in medial prefrontal cortex (mPFC) and amygdala (AMY) and the locomotor behavior were investigated simultaneously. Data showed that the moving distances and velocities were significantly enhanced as rats were administrated with MA. Meanwhile, systemic MA administration altered the unit firing rate in mPFC and AMY. In component project 3, both restraint and MA were administrated to induce mild Fos-immunoreactive (ir) cells in nuclei of mesolimbic and nigrostriatal systems. Importantly, restraint prior to MA challenge robustly increased the numbers of MA-induced Fos-ir cells. Restraint also potentiated MA elicited neuronal activation in bed nucleus of stria terminalis and central nucleus of amygdala (CeA), and endocrine/autonomic output. In component project 4, the cardiovascular responses elicited by MA in conscious rats were recorded by radiotelemetry. Fos protein expression as a neuronal activation marker at ventrolateral medulla was identified in response to blood pressure regulated by MA. Results showed that MA increased blood pressure in a dose-dependent manner in both anesthetized and conscious rats. Consistently, a significant increase in Fos protein expression was found in the rostral ventrlolateral medulla (RVLM) and caudal ventrlolateral medulla (CVLM).

Taken together, our present studies demonstrate that MA may affect the neural circuitry in the forebrain in response to the behavioral hyperactivity. Fos protein expression enhanced by MA was consistent to an increase in BP. Based on these valuable animal models, our long-term goal is going to understand the mechanisms of MA-induced neuronal and cardiovascular toxicity and to develop the novel therapeutic agents for MA-elicited neuropsychological and cardiovascular dysfunctions.

O66

(計畫名稱：Therapeutic Effects of GDNF Inducing Agents on Methamphetamine-induced Neuropsychological Impairment)

Neuropsychological dysfunction induced by chronic use of methamphetamine in mice

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Methamphetamine (MA) is a stimulant commonly abused in Taiwan and all over the world. In addition to addiction, long-term exposure to MA results in psychosis, persistent cognitive deficits as well as a general loss of dopaminergic terminals and transmission. With the increasing abuse of methamphetamine, the number of patients with neuropsychological impairments escalates. In this study, we determined the cognitive dysfunction and social interaction deficits associated with the dopaminergic and serotonergic neuronal damages in central nervous system after the repeated administration of MA. Male ICR mice were either treated with MA (4×5 mg/kg, s.c., 2 hr apart) or MA (2.5 mg/kg, s.c.) every other day for eight applications. The neuropsychological functions including novel object recognition test (NORT), novel location recognition test (NLRT), water finding test and social interaction were examined. Our data showed that mice exposed to a sensitizing regimen or neurotoxic regimen of MA exhibited deficits in four behavioral tests. Furthermore, the microglia inhibitors, pentoxifylline and clenbuterol, treated for 7 days reversed the cognitive deficits (NORT, NLRT) induced by both MA treatment regimens. The current studies provide the animal model in MA-induced neuropsychological impairments. Based on the valuable animal model, our long-term goal is going to develop the novel therapeutic agents for MA-elicited neuropsychological dysfunctions.

O67

(計畫名稱：Effect of Methamphetamine on The Neuronal Activities of The Forebrain Nuclei)

The effect of MA on the neuronal activity of medial prefrontal cortex and amygdala in rats

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Methamphetamine (MA) is one of the abused drugs recently because the primary ingredients of MA can be acquired and converted into the final product easily. MA induces rewarding effects and has drug dependence. Long-term or high dose of MA have neurotoxicity on the neurons of central nervous system. The neurotoxic effect of MA is associated with cognitive impairment and even psychosis. In this study, the effect of MA on the neuronal activity of medial prefrontal cortex (mPFC) and amygdala (AMY) were investigated and the change of locomotor behavior was also monitored simultaneously. Two eight-microwire electrodes were implanted in the mPFC and AMY. The multiple single-unit activities were recorded in awake Long-Evans rat with a Recorder system. Many units were recorded in these two brain area. MA was applied to the rat by i.p. injection (2.5 mg/kg). The moved distances and movement velocities of rats were significantly enhanced 20-30 mins after the application of MA. Meanwhile, systemic MA administration induced both enhancement and reduction in unit firing rate. The inconsistent changes of firing rate were showed in mPFC and AMY. The cross-correlation between the neuron pair were decreased after MA administration. These findings suggest that the application of MA affect the neural circuitry in the forebrain which may be conducted to the behavioral hyperactivity.

O68

The mechanism of stress-promoted response to methamphetamine

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Stress raises the risk of induction and relapse of drug abuse. However, the significance of these correlative observations on vulnerable to addiction behaviors has not yet been systemically characterized. First, we clarified if stress modulates methamphetamine (MA)-induced neuronal activation, including cell groups of mesolimbic system related to addiction and central autonomic system integrating stress responses. Either restraint (30 min) or MA (2.5 mg/Kg BW, ip) induced mild Fos-immunoreactive (-ir) cells in nuclei of mesolimbic and nigrostriatal systems, including nucleus accumbens (NA), ventral tegmental area, medial prefrontal cortex (PFC), and dorsal striatum. Restraint prior to MA challenge robustly increased the numbers of MA-induced Fos-ir cells in these areas. MA induced neuronal activation in both affective nuclei, bed nucleus of stria terminalis and central nucleus of amygdala (CeA), and endocrine/autonomic output, the paraventricular nucleus. Restraint potentiated the activative effects of MA in these areas. FosB involves in neuronal plasticity and is accepted to play a role on development of addiction. Single administration of MA did not significantly increase the numbers of FosB-ir cells in NA, PFC and CeA, whereas restraint increased FosB expression in these cell groups, except CeA. Restraint and MA mutually increased the number of FosB-ir cells in CeA. Next, we will clarify if the integrator of stress response, corticotrophin releasing factor (CRF), involves in the synergistic effect of stress on MA-induced neuronal activation by using CRF receptor antagonist and injection of tracer for searching CRF origins. Moreover, we will measure the dopamine release before and during restraint by voltammetry.

O69

The central mechanisms of cardiovascular toxicity induced by methamphetamine

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The cardiovascular effects of acute methamphetamine (MA) use include tachycardia, myocardial ischemia and hypertension. MA produces increase in blood pressure and the response of heart rate mainly via sympathetic nervous system. In the present study, radiotelemetry was use to record the cardiovascular responses elicited by intraperitoneal (i.p.) injection in conscious rats. Blood pressure changes by intracerebroventricular (i.c.v.) administration of MA were used to assess the central effects of the drug in anesthetized rats. Fos protein expression was used as a neuronal activation marker at ventrolateral medulla to identify the imaginable central target of MA in blood pressure regulation. Our results showed that i.c.v. (50, 150 and 500 nmole) or i.p. (2 and 10 mg/kg) administration of MA increased blood pressure in a dose-dependent manner in anesthetized and conscious rats, respectively. A significant increase in the level of Fos protein was found in the rostral ventrlolateral medulla (RVLM) and caudal ventrlolateral medulla (CVLM) after central (by i.c.v.) or peripheral (by i.p.) administration of MA. MA (2 and 10 mg/kg; i.p.) induced a significant increase in the expression of the phosphoserine 896 protein (regulated by PKC) on NR1 subunit in the RVLM. The available data suggests that both the CVLM and RVLM may play important roles in the central regulated cardiovascular toxicity of MA. Phosphoserine 896 on NR1 subunit by PKC signaling pathway in the RVLM may be involved in the MA-induced pressor responses.

O70

間歇性低氧引發生理病理變化之機轉探討

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本整合型研究計畫以間歇性低氧之動物模式，探討睡眠呼吸中止與心臟血管疾病之相互關係。一般而言，心臟血管系統受到各種因素之調控，包括心臟自我功能、自主神經功能、血管收縮與舒張之恆定、血液因子、中樞調控等共同參與。本整合型計畫以自發性高血壓大鼠與配合相同週數之正常血壓對照鼠為實驗動物，每天於動物光亮期中暴露於間歇性低氧環境，間歇性低氧之周期為 1.25 分鐘/次(灌流 30 秒的氮氣後，再予灌流 45 秒的空氣)，每天持續六小時。實驗結果顯示，經過二天間歇性低氧處理後的大鼠，血中紅血球、血紅素、血容比及紅血球生成素即有顯著的上升，並隨著處理天數的增加其情況更顯著，此可能導致血液黏稠度逐步增加。第八天時，間歇性低氧更可活化自發性高血壓大鼠升壓中樞(RVLM)麩胺酸 NMDA 受體，導致交感活性輸出增加；但是正常血壓大鼠則無此變化。事實上，根據每天血壓連續之監測發現自發性高血壓大鼠約於暴露間歇性低氧第九天開始，即可觀察到具有增強交感神經活性及平均動脈血壓之現象，且在同時化學反射反應亦同步增加，然而空氣組動物卻只有隨著時間的增加呈現些微提高之反應。並且第十天可監測到自發性高血壓大鼠的左心室細胞呈現壞死現象，並隨著間歇性低氧時間增加其細胞壞死的比率亦隨之增加。而當事先給予超氧陰離子基驅除物，可以抑制間歇性低氧所引發化學反射活性、交感神經活性興奮與血壓上升之反應；並且亦可有效地消除間歇性低氧所造成自發性高血壓大鼠心肌細胞壞死之現象。再者，每天預先處理誘發性一氧化氮合成酶抑制劑、神經性一氧化氮合成酶抑制劑或 L-半胱氨酸(清除超氧陰離子基與一氧化氮結合生成之亞硝酸根)，亦具有明顯抑制間歇性低氧所引發之心肺反應與心肌壞死之現象；另也發現間歇性低氧可些許的增加動脈中誘發性一氧化氮合成酶含量。但是，間歇性低氧之處理並不會增加血漿中纖維蛋白原(fibrinogen)與凝血活性，同時亦無法明顯地提高 IL-1、IL-2、IL-6、IL-10 及 TNF 等發炎性介質的含量。根據上述之實驗結果可推測間歇性低氧可加速清醒自發性高血壓大鼠之血壓惡化程度，而其中可能與增加血比容、興奮延腦升壓中樞、過度增強的化學反射反應引發交感神經活性興奮、心室細胞逐漸壞死有關；並且可能因活性含氧物及活性含氮物之產生，而參與間歇性低氧引發各項心肺反應之過程。

O71

自由基對於間歇性低氧引發正常血壓大鼠與自發性高血壓大鼠之化學反射、自主神經功能及血壓變化之影響

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長期暴露於間歇性低氧之環境如同睡眠呼吸中止病患於睡眠期間處於反覆性呼吸中止現象，可能進而導致高血壓。反觀之，高血壓患者亦有較高比率罹患睡眠呼吸中止症。此外，間歇性低氧狀況如同發生缺氧後再給予氧氣供應，可改變各種自由基之釋放量，且此自由基含量之變化，亦可能參與於長期間歇性低氧所造成各種生理病理反應。研究報告指出，輕微高血壓患者或自發性高血壓大鼠，其周邊化學接受器之敏感性較高，並且其體內亦可產生較強的氧化壓力。根據上述之理由可推測，高血壓患者或動物當處於間歇性低氧情況下，可能因增強的化學反射反應與惡化的氧化壓力作用，導致心臟血管系統更嚴重之調控失常。本實驗採用八至九週大之自發性高血壓大鼠每天於動物光亮期中暴露於間歇性低氧環境，間歇性低氧之周期為 1.25 分鐘/次(灌流 30 秒的氮氣後，再予灌流 45 秒的空氣)，每天持續六小時，連續觀察三十天。實驗過程中，使用非侵入性無線遙測儀偵測每天動脈血壓變化，並將此動脈血壓訊號再經由頻譜分析，用以評估自主神經功能之活性。此外，利用小動物體積描記系統，每天將動物暴露於空氣及急性低氧(12% O_2，5 分鐘)下，測量清醒動物呼吸型態之變化，藉以評估大鼠化學反射之敏感性。本實驗結果顯示，暴露於間歇性低氧約於第八至九天開始即可明顯地增強心率變異性低頻比值(其為交感神經活性指標)及平均動脈血壓，且在同時化學反射反應亦同步增加，然而空氣組動物卻只有隨著時間的增加呈現些微提高之反應。而事先以腹腔注射之方式給予超氧陰離子基驅除物、誘發性一氧化氮合成酶抑制劑或神經性一氧化氮合成酶抑制劑，皆可以抑制間歇性低氧所引發化學反射活性、交感神經活性興奮與血壓上升之反應。再者，每天預先處理 L-半胱氨酸(L-cysteine)，藉以清除超氧陰離子基與一氧化氮結合生成之亞硝酸根(peroxynitrite)，亦具有明顯抑制間歇性低氧所引發之心血管及呼吸反應。相反地，無論是間歇性低氧、空氣暴露或事先處理各種藥物，皆無法有效地改變心率變異性中高頻成份（其為心臟迷走神經活性指標）及心跳之快慢。根據上述之實驗結果可推測間歇性低氧可加速清醒自發性高血壓大鼠之血壓惡化程度，其中可能與過度增強的化學反射反應引發交感神經活性興奮有關；並且可能因活性含氧物及活性含氮物之產生，而參與間歇性低氧引發上述各項心肺反應之過程。

O72

(計畫名稱：間歇性低氧引發之高血壓：大鼠前腹外側延腦中麩胺酸神經傳導與活性氧種之角色)

慢性間歇性低氧對清醒之自發性高血壓與正常血壓大鼠心血管作用評估

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慢性間歇性低氧(intermittent hypoxia，IH)導致許多嚴重的病理生理狀態，包括心肌壞死、認知功能障礙以及系統性高血壓。研究報告指出，自發性高血壓大鼠(SHR)與對照之正常血壓大鼠(WKY)對於重複性壓力可產生不同的心血管反應。本研究中，我們使用遙測電極記錄清醒的 SHR 及 WKY 在 IH 處理過程中血壓的反應變化。藉由控時電磁閥，實驗動物被給予每天持續 6 小時之 IH 處理(交替灌入 30 秒氣體壓縮鋼瓶 100%的氮氣與 45 秒鼓風式抽氣馬達所抽取的空氣)，連續七天，並於第八天犧牲動物，取其 RVLM 核區，分析麩胺酸離子通道型受體 NMDA 之受體蛋白量變化，作為受體活化之指標。同時另處理一組動物，經 IH 處理三天後，於第四天犧牲，亦量測 RVLM 核區 NMDA 之受體蛋白量變化，作為對照比較。我們的結果顯示：SHR 的收縮壓與舒張壓會於 IH 處理之六小時內有逐漸下降之趨勢；但此下降趨勢會隨著 IH 天數之增加而逐漸減緩。不同於 SHR，WKY 大鼠於 IH 處理之七天中，其六小時低氧過程中血壓數值仍具顯著下降之情形。然無論是 SHR 或 WKY，七天之 IH 處理皆不足以改變其基礎血壓值。NMDA 受體蛋白表達量之結果則顯示：經七天 IH 處理後，SHR 之 RVLM 區上，NMDA 受體次單位蛋白 NR1 與 NR2B 較其正常通氣之 SHR 控制組，有顯著並具統計意義之增加；但對 WKY 大鼠而言，IH 組與正常通氣組，其 RVLM 上 NR1 與 NR2B 表現量並無統計上之差異。而僅經三天 IH 處理之動物，無論 SHR 或 WKY 其 RVLM 上 NR1、NR2A 或 NR2B 之表達皆與正常通氣組無異。據此研究結果推估：SHR 於六小時 IH 處理過程中，血壓減低之趨勢，隨著天數逐漸減緩，可能部分來自活化 RVLM 麩胺酸 NMDA 受體，導致交感活性輸出增加所致。而 SHR 與 WKY 大鼠對於 IH 的挑戰，顯現不同的反應，也許可用來解釋此二品系動物對低氧環境展現不同的調適與防禦能力。

O73

間歇性低氧對於凝血功能以及動脈血管的影響

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在中風、心肌梗塞及深層靜脈血栓的患者中有高比率的人有阻塞性睡眠呼吸中止(obstructive sleep apnea)症，顯示阻塞性睡眠呼吸中止是心血管疾病的重要危險因子。為釐清阻塞性睡眠呼吸中止對於凝血功能以及動脈血管的影響，我們給予 WKY 大鼠每天 6 小時的間歇低氧或是空氣處理，經過 2 天、5 天及 20 天後，分析比較他們的血液組成、凝血活性、血漿中細胞激素的含量以及動脈血管的變化。

和空氣處理組比較，每天暴露在間歇低氧 6 小時的 WKY 大鼠的血液中的紅血球數量、血紅素含量以及血容比均顯著上升，而且隨著間歇低氧處理的天數越長，紅血球數量、血紅素含量及血容比的上升越明顯。血漿中紅血球生成素的量在間歇低氧處理後也顯著上升，顯示間歇低氧引發大鼠紅血球及血紅素上升的原因應該是低氧導致腎臟釋放紅血球生成素進而刺激紅血球及血紅素生合成。白血球及血小板在經過 20 天的間歇低氧後並無明顯變化。間歇低氧處理組的老鼠的血漿凝血時間(aPTT 及 PT)與空氣處理組並沒有顯著差異，意味著每天 6 小時的間歇低氧環境(20 天內)並不會促進凝血活性。另外，間歇低氧並不會增加血漿中纖維蛋白原 (fibrinogen)、IL-1、IL-2、IL-6、IL-10 及 TNF 的含量，但是動脈中 iNOS 的含量有些許的增加。我們還觀察到大鼠暴露在間歇低氧的初期(2 天)有體重減輕的現象，隨後體重開始上升，在 20 天後體重就有了大弧度的增加。

綜合上述的資料，我們認為每天 6 小時的間歇性低氧環境會促使 WKY 大鼠的腎臟增加紅血球生成素的釋放量，刺激血紅素及紅血球的生合成，進而增加血液的帶氧能力。這樣的因應雖然讓老鼠再度暴露在間歇低氧的環境時體內缺氧的情況不會太嚴重，幾天後體重可以上升，但是明顯增加的血紅素卻也導致在正常氧氣量的環境下(18 小時)血液會有過多的氧氣，增加了氧自由基，促使動脈血管產生變化，像是增加 iNOS 的表達。雖然大鼠體內的凝血活性並沒有因為間歇低氧(20 天內)而增加，但是血管病變到一定程度還是可能會有血栓的問題。所以間歇性低氧環境似乎是先導致血管變化，間接促進血栓的形成。

O74

(計畫名稱：間歇性低氧造成心肌細胞死亡機制之探討)

長期間歇性低氧引起正常血壓及高血壓大鼠心臟細胞死亡機制之探討

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間歇性低氧是一種在許多病生理情況中可以發現的症狀，例如阻塞性睡眠呼吸中止症(obstructive sleep apnea)。在臨床的研究發現阻塞性睡眠呼吸中止症的患者常併發許多心血管相關的疾病，包括高血壓、心室肥厚及左心室功能不良。先前的研究指出間歇性低氧造成的細胞死亡機轉類似於缺血再灌流損傷，此種損傷可能與缺氧到回復氧氣的過程中產生大量的自由基而造成細胞的死亡有關，但確切機制目前尚待釐清。為了解間歇性低氧造成心肌細胞死亡的機制，並比較高血壓合併間歇性低氧對心肌細胞造成的傷害，因此我們利用正常血壓和自發性高血壓大鼠給予每天六小時(上午 10 時至下午四時)反覆性的間歇性低氧(30 秒 5 %與 45 秒之 21 % 氧氣)或正常氧分壓處理，連續 10 天、20 天及 30 天。間歇性低氧處理後，犧牲動物取出心臟並測定心肌組織脂質過氧化程度和超氧化物歧化酶活性，以確定間歇性低氧是否造成心臟組織氧化壓力的增加或累積。首先證明間歇性低氧是否造成心肌細胞壞死或凋亡，我們利用 propidium iodide 染色來分辨細胞膜的完整性，以區分正常細胞與壞死細胞；細胞凋亡則是以 TUNEL assay 與 DNA ladder 作為偵測。為釐清引發心肌細胞凋亡的機制，我們利用西方點墨法來偵測細胞內蛋白質的變化，包括 HIF-1、PARP 及活化態的 caspase-3，並進一步游離粒線體來分析 cytochrome C、AIF 是否釋放到細胞質。目前的研究顯示，經過連續 10 天、20 天及 30 天的間歇性低氧處理之後，正常血壓及高血壓大鼠心臟組織中 HIF-1 的表現量並無顯著的變化，但會造成自由基的增加並造成心肌細胞死亡的現象。在正常血壓的大鼠，間歇性低氧造成心肌細胞同時產生壞死及凋亡的現象，並且其引發細胞凋亡的機制與粒線體中 cytochrome C 釋放和 caspase-3 活化有關，而與 AIF 的釋放無關；而在高血壓的大鼠，間歇性低氧會造成氧化壓力的增加並造成心臟細胞的壞死但並無觀察到有凋亡的現象，且也無 cytochrome C 的釋放及 caspase-3 的活化，若在連續處理間歇性低氧前先給予腹腔注射 superoxide 清除劑 MnTMPyP 及 peroxynitrite 清除劑 L-cysteine，則可以抑制細胞壞死的現象。由以上實驗結果證明，連續間歇性低氧處理會引發大鼠心臟細胞內活性氧化物及活性氮化物的增加，導致心肌細胞的死亡。

O75

（計畫名稱：當代佛教音樂記憶－慈濟歌曲的民族音樂學研究）

佛法人間化—慈濟音樂在體驗佛教現代性的角色

Civil Buddhism – Tzu-Chi Buddhist Musicology and its Modernized Embodiment

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慈濟音樂的流傳與運用，是「慈濟人」社會生活的一部份，也是特定場景表達活動概念與情感象徵力量上極有力量的一環。慈濟基金會於 1966 年由證嚴法師創辦後，可稱為是目前台灣最大的非營利宗教組織，是一個活動能量與宗教傳播跨越國界的現代佛教團體，主要著力在「慈善、醫療、教育、人文」四大志業。本文首先描繪了慈濟音樂廣泛使用在這些非宗教、甚至是世俗的生活領域，進而透過音樂現象的分析，以檢驗「慈濟人」有別於傳統佛教團體的主要現代化與創新特質。

由於慈濟除了經營傳統寺院做為出家眾或在家居士修持法門的道場之外，另立龐大的居士團體，實踐「佛法人間化」的理想。出家與在家界線的模糊，讓廣大的在家居士甚至是專業工作者負擔了實踐佛教志業的責任，而非僅是單向的從寺院聽取佛教義理。證諸慈濟歌曲在 1983-2008 年的發展過程中，可以發現在家眾傳唱的現代曲調逐年普遍流通，題材也貼近城鄉市民生活，簡潔曲調也易朗朗上口，並充分運用在慈濟的內、外部活動中，凝聚了一種風格獨特的美學情感建構。

本研究中關於慈濟歌曲與手語劇的分析雖然是民族音樂學下的形式分析，但問題意識探討慈濟音樂如何結合傳統中國佛教音樂元素，而在現代化創新的佛教人文及慈善實踐中持續發揮其一貫影響力。

關鍵詞：音樂現象、慈濟歌曲、手語劇、佛教現代化、民族音樂學

O76

Separation and analysis of biological particles：Rapid Identification of Pathogens in Urinary Tract Infections by Capillary Electrophoresis and MALDI-TOF Mass Spectrometry

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Capillary electrophoresis (CE) has become one of the most significant techniques in biological research, due to its simple method development, high separation efficiency, low sample consumption and short analysis time. These advantages may also be realized for the analysis of microorganisms. In this study, we have cleaned up the clinical urine samples by simple filtration and centrifugation, than the pellets were redispersed in buffer solution. We used the CE to separate and identify pathogens in urinary tract infections (UTI). This method is rapid and needs only 20 minutes to identify bacteria in urine specimens; it was much faster than the conventional biological methods (about 2–3 days). In some special cases, we collected the microbial pathogens from CE, made a careful check by MALDI-TOF mass spectrometry; it was extremely simple and fast to identify pathogens through protein fingerprinting. We successfully separated and identified five species including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. However, the work presented with the advent of powerful CE and MALDI-TOF methods, for an accelerated discovery and diagnosing of diseases in human.

O77

(計畫名稱：地景記憶再創造－藏彝走廊離散人群的環境永續策略)

傳統生態知識中的地景觀點—地理資訊民族誌在區域研究中的應用

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地理資訊系統透過彙整大量的地景資料與傳統生態知識，能夠充分呈現部落文化主位觀點下的自然地景，發掘其中濃厚的社會與象徵意涵。對於無文字而經常透過地景儲存歷史記憶與集體情感的民族而言，地理資訊系統更是一種能夠幫助研究者探索其文化概念與社會關係的重要輔助工具。在揭示其學術研究價值的同時，本文主張地理資訊民族誌亦可在部落教育與區域政策等實務方面發揮積極的效用。藉由對於部落人才素質的提升，凝聚社群共識以及提供文化主位觀點的諮詢，地理資訊系統的實質應用，將可望成為當代少數民族反思傳統價值與生態現代化過程的新感知工具。

關鍵字：傳統生態知識，地景，地理資訊系統，民族誌

O78

「精確」與「解構」的對話－李立揚英詩中的文化對立.迷惘和整合

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本計畫旨在承襲筆者近五、六年的研究脈絡，將以德希達的解構理論為研究方法，探討美國華裔詩人李立揚詩中的主體定位問題。李立揚是二代移民，父親當過毛澤東的私人醫生，外祖父是袁世凱，特殊和顯赫的家族背景使他或許比大多數移民後代背負著更多歷史記憶和傳承文化的責任。然而，一個相當程度主導當今世界的強勢（美國）文化卻一波接著一波的不斷向他襲來，幾乎要把他吞沒。處在這種文化衝突當中的李立揚將如何適應和回應，及是本論文探討的核心。本研究共討論李立揚已出版之四本詩集中的十四首詩- 其中，我們將以較多的篇幅探討「柿子」，因為它與德希達的解構之間展開了一次極其精彩而有效的對話。

A Dialogue between Precision and Deconstruction Cultural Confrontation, Disorientation, and Reconciliation in Li-Young Lee's Poetry

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A continuation in methodology and subject of my research in recent years, the present study employs the Derridean deconstruction to address the problem of identity that informs the poetry of Li-Young Lee, a Chinese American poet of the second- generation immigrant. Lee's father was a personal physician to Mao Zedong while in China, and his grandfather is Yuan Shi-kai, the first President of the Republic of China. Because of this distinctive legacy of the family, Lee may be said to be freighted with more memory of and more obligation to Chinese culture than most of other immigrant descendants. On the other hand, however, he is overwhelmed by an ongoing impact of the dominant Euro-American culture. What the poet's mindset is in response to such cultural confrontation is hence the pivotal concern of this essay. In this study fourteen poems taken from all of Lee's four published collections are used to explicate Lee's responses to the stalemate of the cultural conflict. Of these poems we devote much greater space to the interpretation of "Persimmons" in that it holds a most valid dialogue with Derrida's deconstruction, a strategy that may lead Lee a little farther away from his sense of displacement.

O79

The Learner's Response: an investigation into the suitability of a non-EFL textbook for integrated English skills courses for English majors in Taiwan

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This investigation focuses on the student reception of a textbook designed for use by mainly Native Speaker students in American colleges (*The Writer's Response*, McDonald and Salomone, 2004) in order to determine to what extent may be appropriate for serious consideration as the core text for Integrated English Skills courses for students majoring in English at a university in Taiwan. The study draws upon research which examines the following: the place of culture in EFL materials, cross-cultural and intercultural factors in SLA, and the psychology of SLA. A preliminary 'impressionistic' evaluation instrument was designed and applied. Data was collected from two groups of students over a two-year period by means of structured and semi-structured instruments. While the study reveals a broadly favourable student response to the textbook, it finds that carefully selected ancillary curriculum for listening and speaking is required. The study also finds that students believe the book has several important strengths, but that their realization in the classroom depends significantly on teaching approach and methodology.

O80

(計畫名稱：戰後日本文學記憶的「殖民地台灣」－以坂口ネ零子「蕃地」作品為始)

戰後日本文學記憶的殖民地－探討霧社事件相關作品

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1930 年 10 月 27 日在霧社小學運動會當天發生了大規模的台灣原住民反抗事件，亦即霧社事件。事件發生至今已歷經 70 年以上，但仍被透過各種型態傳述著。若將事件視為記號（Code），那記號的指示內容隨著時代變遷而呈現出多樣型態。大家對於霧社事件的印象，並未隨著事件或殖民地時期的結束而畫下句號，甚至現在還被各種媒體所敘述著。

歷史事件意義是從敘述過程中所產生，而敘述內容會反映出各時代背景，所以絕非一成不變。本計畫研究主要目的是透過分析從戰後到 70 年代為止的文學作品對於霧社事件這個發生在 30 年代的台灣歷史集體記憶的詮釋方式，來探討殖民地記憶的連續與斷層。具體而言，會透過分析比較曾經歷過殖民地生活的坂口禎子與未曾經歷過殖民地生活的稻垣真美這兩人的霧社事件相關作品，藉此提出閱讀霧社事件相關作品的新觀點。

選擇坂口與稻垣作品的理由如下：這兩人雖然世代、經驗、立場皆不同，但皆選擇霧社事件為寫作題材。這些作品的共通點在於，透過複雜的人際關係，也就是無法單純地被還原於支配／被支配的二元對立構造，來呈現殖民地「和解」的可能性。「和解」意味著從殖民地政策衍生出來的歷史悲劇與期待這悲劇今後的走向。

O81

Pharmacological enhanced ^{18}F-FDG PET imaging for evaluation of Parkinson's disease in rats

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After methamphetamine enhanced, ^{18}Flurodeoxyglucose (^{18}F-FDG) PET (positron emission tomagophy) imaging was able to applied for the evalution of Parkinson's disease. Six rats were subjected to imaging analysis including three with 6-OHDA unilateral lesion and other as control group. The pharmarcological challege induced significant difference in the metabolism activity of striatum between normal rats and lesioned ones. Drug enhanced rotation behavior and immuno-histo-chemistry staining for tyrosine hydroxylase (TH) confirmed the depletion of dopamine cells behind the striatum area in the ipslateral side in the lesioned animals. Being an advantaged PET tracer in the convenient and lower cost, ^{18}F-FDG could also successfully apply for the grading of Parkingson's disease. This new approching method could help scientists or clinicians to better understand the progression of PD and may potentially lead to realize the activitive cerebral area in PD patients.

O82

Pathophysiology of Urothelial Dysfunction in Patients with Interstitial Cystitis/Painful Bladder Pain Syndrome – Increased Apoptosis and Decreased Junctional Protein Expression of Urothelium due to Suburothelial Inflammation

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Interstitial cystitis/painful bladder syndrome (IC/PBS) is a heterogeneous syndrome characterized by the symptoms of bladder pain and associated with frequency and nocturia. Recent findings have proposed several pathophysiological mechanisms including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity and occult infection. The human bladder urothelium and urothelial cells play an important role in the normal defense mechanism. One of the most common findings in IC/PBS patients is denudation or thinning of the bladder epithelium, suggesting an altered regulation of urothelial homeostasis. IC/PBS was found to associate with increased urinary adenosine triphosphate (ATP) and increased stretch-activated ATP release by bladder urothelial cells, suggesting augmented purinergic signaling in the bladder. Urine from patients with IC/PBS has been shown to inhibit urothelial proliferation through antiproliferative factor (APF). In urine samples of IC/PBS patients, significantly increase of APF, decrease of heparin-binding epidermal growth factor (HB-EGF) and increased levels of EGF were discovered. APF expressed by the urothelial cells induces increased permeability in cell culture, regulates expression of other cytokines, including upregulating HB-EGF and down-regulating EGF. These cytokine abnormalities were also related to increases in purinergic signaling, which mediates increased bladder sensation. Abnormal uroplakin, chondroitin sulfate and tight junctional protein zonula occludens-1 (ZO-1) expressions strongly suggest abnormal differentiation in the IC/PBS bladders whereas elevated E-cadherin expression may represent an adaptation to increased bladder permeability. The epithelial damage may precede the other histopathologic findings in the bladder wall. Local inflammatory process might be induced through the afferent and efferent nerves in the suburothelial interstitial cellular network which integrate the transmission of signals from the urothelium to the detrusor muscles in the bladder wall. Investigation of the relationship between chronic inflammation and the urethelial dysfunction such as urothelial apoptosis, expression of junctional protein and inflammatory reactions in suburothelium might demonstrate this hypothesis. Through investigating the changes of urothelial dysfunction and suburothelial inflammation at baseline and after BoNT-A injections could provide evidence for the existing pathophysiology of IC/PBS. Key words: IC/PBS, apoptosis, inflammation, urothelial dysfunction

O83

In vitro study of human and guinea-pig gallbladder smooth muscle function

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Several peptides can cause either contraction or relaxation of guinea-pig and human gallbladder through interacting with their receptors on gallbladder. Two gastrointestinal peptides were investigated by measurements of contraction and relaxation of gallbladder muscle strips. In the first experiment, the effect of natriuretic peptides in gallbladder was investigated. We measured relaxation of isolated human and guinea-pig gallbladder strips caused by natriuretic peptides. Results in the human gallbladder were similar to those in the guinea-pig gallbladder. The relative potencies for natriuretic peptides to cause relaxation were CNP>>BNP≧ANP. These indicate the existence of the natriuretic peptide receptor-B (NPR-B) mediating the relaxation. These results demonstrate that natriuretic peptides cause relaxation of human and guinea-pig gallbladder muscle through interaction with the natriuretic peptide receptor-B.

In the second experiment, proteinase-activated receptor-1 (PAR_1) and PAR_2 mediate contraction in the guinea-pig gallbladder were studied using the same method. We measured contractions of isolated human and guinea-pig gallbladder strips caused by PAR agonists. The PAR_1 agonists, thrombin, TFLLR-NH_2 and SFLLRN-NH_2, as well as the PAR_2 agonists, trypsin, SLIGKV-NH_2 and SLIGRL-NH_2, caused contraction in both human and guinea-pig gallbladders. These indicate the existence of PAR_1 and PAR_2 mediating gallbladder contraction. Three PAR_4 agonists, GYPGKF-NH_2, GYPGQV-NH_2 and AYPGKFNH_2, did not cause any contraction or relaxation. These results demonstrate that both PAR_1 and PAR_2 but not PAR_4 mediate muscle contraction in the human and guinea-pig gallbladder.

Bile acids are synthesized from cholesterol in the liver, stored in the gallbladder and secreted after meals to increase absorption of fat in the intestine. Recently, bile acids were found to be ligands for the bild acid receptor, a G-protein-coupled receptor. Bile salts have been found to inhibit the cholecystokinin induced guinea pig gallbladder contraction. There is no information available on the effects of bile acids in the human and guinea pig gallbladder. So, in the third experiment, the effect of bile acids in the gallbladder was investigated. The results will be reported.

O84

Analysis of Retinal Nerve Fiber Layer and Macular Thickness Measurements in Taiwanese Individuals

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Purpose: To investigate the reproducibility of peripapillary retinal nerve fiber layer (RNFL) and macular thickness measurements in healthy Taiwanese subjects using optical coherence tomography (Stratus OCT).

Methods: Fifty-two eyes of 52 healthy Taiwanese subjects (32 females and 20 males) were enrolled in the cross-sectional study. A randomly chosen single eye from each healthy subject underwent thickness measurements using OCT, before and after apillary dilation, by three trained and experienced operators. Average measurements of peripapillary RNFL and macular thickness were calculated. Comparisons of thickness measurements before and after apillary dilation, and among the three operators were performed.

Results: The RNFL thickness measurements were higher in the inferior peripapillary area by RNFL scan (average: 107.4 ± 17.8 μm) and the macular thickness measurements showed a ring-shaped hump in the 3 mm perifoveal area by macular scan (average: 252.8 ± 8.9 μm). Comparing the peripapillary RNFL and macular thickness before and after apillary dilation, there was no significant difference (p>0.05) in average, superior, inferior, temporal or nasal peripapillary areas, in total macular volume and foveal thickness, nor in 1 mm, 3 mm and 6 mm perifoveal areas, whether before or after apillary dilation and irrespective of which operator performed the measurements.

Conclusions: The inferior RNFL area and the 3 mm perifoveal area showed higher thickness measurements in the peripapillary region and macular region, respectively. The thickness measurements performed using OCT showed no significant differences before and after apillary dilation, and showed good reproducibility.

Key Words: retinal nerve fiber layer thickness, macular thickness, optical coherence tomography, Taiwanese

O85

AAV Mediated Immune Regulatory Gene Therapies for Malignant Tumors

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Malignant tumors are currently lacking effective treatment modalities. Gene therapy represents a promising approach for these diseases. Gene therapy with immune regulatory gene can elicit the specific remembered immune responses to seek and attack uninfected tumor cells.

IL-15 is a four α-helix bundle cytokine displaying IL-2 like functions, such as the induction of T and NK cell proliferation. Moreover, IL-15 can provide a costimulus to induce B cell proliferation and Ig secretion. Therefore, IL-15 has been proposed as a possible candidate for the development of cancer immunotherapy or gene therapy approaches.

First, the isolated IL-15 genes were constructed into the same plasmids. Second, because the AAV gene delivery system is considered as the safest viral delivery system, the AAV viral vector was utilized to express IL-15 protein. The AAV viral vector that encodes IL-15 proteins was used to produce recombinant therapeutic AAV in cGMP laboratory at Tzu-Chi Hospital. We further investigated the tumor suppressive effect of this method in different kinds of malignant tumor in different mice models, including JC breast cancer in BALB/c mice, DBTRG malignant human brain tumor, Hela human cervical cancer cell lines in BALB/c nude mice.

The tumor suppressive effects were noted by the use of IL-15 in these tumor mice models pretreated with single time intramuscular injection of AAV vectors carrying IL-15 4-5 weeks before tumor implantation. The survival time of tumor bearing mice could also be prolonged by this method. We also found the tumor suppressive effect of AAV-IL-15 gene therapy passed the apoptotic pathway.

O86

Sympathetic α3β2-nAChRs mediate cerebral neurogenic nitrergic vasodilation

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The nicotinic acetylcholine receptor (nAChR) on the sympathetic nerves innervating cerebral arteries of the pig crossbreed between Landrace and Yorkshire (LY) in Illinois is α7-nAChR. Nicotine-induced cerebral neurogenic vasodilation in pigs crossbreed among Landrace, Yorkshire and Duroc (LYD) in Hualien (Taiwan), however, is not blocked by α-bungarotoxin (α-BTGX, a highly selective α7-nAChR antagonist). The cerebral perivascular sympathetic nAChR subtype of LYD pigs, therefore, was examined. The nicotine-induced dilatation of isolated basilar arteries was not affected by α-conotoxin IMI (an α7-nAChR antagonist) or α-conotoxin AuIB (an α3β4-nAChR antagonist). The vasodilation was inhibited by preferential α3-containing nAChR antagonists (tropinone and tropane) and α-conotoxin MII (a selective α3β2-nAChR antagonist). Using reverse transcription PCR, α3-, α7-, β2- and β4-subunits of nAChRs were expressed in fresh superior cervical ganglia. The mRNA levels of α3-, β2- and β4-subunits were significantly higher than that of α7-subunit. Furthermore, nicotine-induced inward currents in α3β2-nAChR-expresssing oocytes were blocked by α-conotoxin MII but not by α-BTGX or α-conotoxin AuIB. In conclusion, the predominant nAChR on cerebral perivascular sympathetic nerves mediating cerebral nitrergic neurogenic vasodilation in LYD pigs is α3β2-subtype (supported by TCU & NSC).

O87

The influence of antipsychotic agnent to heart eart rate variability: evaluation in schizophrenic patients switched from typical antipsychotic agents to amisuliride and olanzapine:

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Background: Schizophrenia is a severe mental disorder and need nearly whole life medication, the information on the cardiovascular safety and tolerance of antipsychotics is significantly clinical importance. Atypical antipsychotics was used in schizophrenia patients since 1990s, more and more patients switched from typical antipsychotics, but the cardiovascular safety remain without a accessible evaluation tool. In the study, we try to use the computer-assisted measurement of 5-minutes resting heart rate variability (HRV) from schizophrenia patients, who switched to atypical antipsychotic agents (amisulpiride and olanzapine)

Methods: Fifteen patients switched to amisulpiride and eighteen patients switched to olanzapine, HRV was evaluated before medication switched and followed every month after switched. Frequency-domain analysis of short-term and stationary RR intervals was performed to evaluate low-frequency power (LF; 0.04-0.15 Hz), high-frequency power (HF; 0.15-0.40 Hz), the ratio of LF to HF (LF/HF), and LF in normalized units (LF%).

Results: Our results showed significant increases in mean, variance and HF of RR interval in the amisulpirde group, no obvious change in LF, LF/HF, LF%, but without any obvious change in olanzapine group€.

Conclusions: The results indicate that amisulpride have a more vagotonic effect, suggesting a more cardiovascular safety, as compared with olanzapine when subjects switched from typical antipsychotic agents. Further studies with less confounding factors may be interesting in the future.

O88

Methyl palmitate is a retinal relaxing factor

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Retinal circulation has no autonomic innervation and is therefore thought to be regulated by autoregulatory mechanisms and local factors released from neighboring cells. “Retinal relaxing factor (RRF)” was accidentally discovered, but the identity was still unknown. Here we demonstrate that PAME is released from retina tissue and a RRF.

Materials and Methods: The retina and thoracic aorta were separated from Sprague-Dawley rats. A modified superfusion bioassay cascade system and Gas chromatography-mass spectrometry (GC-MS) were used.

Results: palmitic acid methyl ester (PAME) is released from retina and cause vasodilation.

Conclusion: PAME is a RRF.

O89

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.

O90

Caffeine induces reinforcing behaviour via inhibition of adenosine A_{2A} receptor associated with phosphorylation of DARPP-32 in mice

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Caffeine, an antagonist of adenosine A_1 and A_{2A} receptor, is the most widely used psychoactive substance in the world. Evidence indicates that caffeine interacts with the neuronal systems involved in drug addiction. Although adenosine A_1 and/or A_{2A} receptor have been found to play important roles in the locomotor stimulation and probably reinforcing effect of caffeine, the relative contribution of the A_1 or A_{2A} receptors to the motor activation and reinforcing effects of caffeine has not been completely resolved. For studying the role of adenosine A_1 and/or A_{2A} receptor in the motor activation and reinforcing effects of caffeine, we choose a selective A_1 antagonist (DPCPX), a selective A_{2A} receptor antagonist (SCH58261), and caffeine to exam their locomotor stimulation and behavioral sensitization in C57BL/6 male mice following acute and chronic administration. Conditioned place preference (CPP) was used to evaluate the drug-seeking potential of these compounds. Furthermore, striatal membrane from behaviorally sensitized mice was prepared for the Western blot analysis of the expression of phospho-Thr75-DARPP32 (dopamine- and cAMP-regulated phosphoprotein of molecular weight 32 kDa). Our study showed that caffeine and SCH58261 but not DPCPX induced locomotor sensitization and CPP. The behavioral sensitization of locomotor activity after chronic treatment with caffeine and SCH 58261 was associated with increased DARPP-32 phosphorylation at Thr75 in the striatum. These results indicate that caffeine-induced reward and behavioral sensitization is mediated by antagonism at adenosine A_{2A} receptor. These effects are associated with phosphorylation of DARPP-32 at Thr75 in the striatum.

O91

Exercise training protects against cardiomyocytes death in irreversible ischemia-reperfusion injury

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Coronary artery disease is the major cause of death in most countries of the world. The primary pathological expression of coronary artery disease is myocardial injury resulting from an ischemia-reperfusion (IR) insult. Numerous animal studies demonstrate that consecutive bouts of endurance exercise provide cardioprotection against IR injury, but the exact mechanisms responsible for this protection remain elusive. Therefore, the purpose of present study is to investigate the mechanism of that exercise-induced cardioprotection attenuate cardiomyocytes death induced by intracellular ion disturbance in cytoplasm, mitochondria, and endoplasmic reticulum resulted from IR-induced oxidative stress. Primary culture adult cardiomyocytes were isolated by Langendorff technique. The change of intracellular free radical, ion concentration, and ion channel on membrane and mitochondria opened or closed during IR were measured by microspectrofluorometry and confocal microscopy in a single cardiomyocyte; besides, the methods such as western blot, RT-PCR, cytochemistry staining, immunochemistry staining, TUNEL assay…etc. are used to detect the cardioprotection against IR-induced injury. Thus, if this study is finished as expected, it will be understood how the mechanism from the system to cell molecule of cardioprotection attenuate the cardiomyocytes death resulted from IR-induced oxidative stress. These results will be a great benefit to popularize regular exercise preventing the heart disease and to invent a new medicine for protecting IR injury in the future.

O92

Promising role of a plant extract (TChi-2) in the post-treatment of LPS-induced acute lung injury in the rat

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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 urethane anesthetized male Sprague-Dawley rats. 24 hrs after its application, LPS (10 mg/kg, iv) significantly decreased white blood cells, elevated plasma tumor necrosis factor-α, and thickened interalveolar septa in lung tissues. These changes were prevented by TChi-2 (15 mg/kg, iv or 30 mg/kg, ip), administered one and six hr after LPS-challenge. These treatments also caused significant attenuation of LPS-induced increase in plasma NO, and inhibition of LPS-induced iNOS expression, phosphorylation of IκB (an inhibitor of NF-κB), and activation of NF-κB in lung tissues. These results suggest a promising role of TChi-2 in treating LPS-induced acute lung injury.

O93

The molecular mechanisms for Tanshinone IIA to inhibit the proliferation of human lung cancer H292 cells

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OBJECTIVE: To investigate the inhibition of Tanshinone II A in human lung cancer H292 cells and its mechanism.

METHODS: MTT was used to measure the levels of the proliferation of H292 cultured with various concentrations of Tanshinone II A. The effects of Tanshinone II A on cell cycle of H292 were detected by FACS. After treated with Tanshinone II A(6μg/ml) for 6, 12 and 24hrs, the proteins expressions of P53, p21, bax, bcl-2 and cyto-c in H292 was tested by western-blot method.

RESULT: The proliferation of H292 was obviously inhibited by Tanshinone II A in a dose and time dependent manner. The percentages of viable cell relative to control were 70.77±0.75%, 67.2±0.61%, 63.81±0.5%, 55.96±0.47%.50.34±0.19% respectively, when cultured with various concentrations of Tanshinone II A(control, 1 , 2, 3, 4 and 5 μg/ml) for 24 hrs. The percentages of viable cell relative to control were 59.45±0.36%, 50.41±0.38%, 42.61±0.44%, 38.94±0.24%.34.35±0.33% respectively, when cultured with various concentrations of Tanshinone II A(control, 1 , 2, 3, 4 and 5 μg/ml) for 48 hrs.The percentages of viable cell relative to control were 59.29±1.32%, 45.81±1.6%, 39.89±0.65%, 34.85±0.98%.30.68±0.12% respectively, when cultured with various concentrations of Tanshinone II A(control, 1 , 2, 3, 4 and 5 μg/ml) for 72 hrs. The results of FACS showed the percentages of sub-G1 phase were increased respectively, when colo 205 cells were cultured with various concentrations of Tanshinone II A(control, 3 , 6 and 12 μg/ml) for 48 hrs.The outcome of western blot showed that the protein expressions of p53, p21 , bax and cyto-c were increased , but proto-onco gene bcl-2 was notably decrease, after cultured with Tanshinone II A(6 μg/ml) for 6, 12 and 24 hrs.

CONCLUSION: Tanshinone IIA can inhibit the proliferation of H292 and induce the apoptosis of the cell through up-regulated the expressions of p53, p21, bax, cyto-c and down-regulated the expression of bcl-2. Developing Tanshinone IIA as a lung cancer preventive or therapeutic agent is possible in future.

O94

Involvement of Sympathetic Function in the Sleep-related Change of Gastric Myoelectrical Activity in Rats

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The gastric myoelectrical activity fluctuates across sleep-wake states as a result of modulation by the brain-gut axis. The role of the autonomic nervous system in this phenomenon, however, was not fully elucidated. Through simultaneous recording and subsequent continuous power spectral analysis of electroencephalogram, electromyogram, electrocardiogram, and electrogastromyogram in 16 freely moving Wistar rats, sleep-wake states of the animals were defined and indices of cardiac autonomic regulation and gastric myoelectrical activity were calculated. We found that both cardiac autonomic regulation and gastric myoelectrical activity fluctuated through sleep-wake cycles. Correlation analysis further revealed significant correlations between EGMG power and each of the R-R interval, the high-frequency power, the low-frequency power, the low-frequency power to high-frequency power ratio, and the normalized low-frequency power of heart rate variability with respect to their trend of change across different sleep-wake states. These results suggested that the sleep-wake-related change of gastric myoelectrical activity was related with sympathovagal balance. Sympathetic nerve may play a more important role in the central modulation of gastric myoelectrical activity than previously perceived.

O95

Immediate Impacts of Electromagnetic Treatments on Cardiac Autonomic Function in Schizophrenia Patients

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Background: Electromagnetic Treatments, electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (rTMS), were effective treatment models in Psychiatry. However, the influence of ECT or rTMS to autonomic function is unclear till now.

Methods: Thirteen female schizophrenic were enrolled and 5-min ECG recording for frequency–domain heart rate variability (HRV) analysis was used to assess the function of ANS at baseline and post-ECT or post-anesthesia. On the other hand, 9 schizophrenic patients with active AH received a single blind trial of rTMS (9 days of active stimulation at 90% of motor threshold and 2 days of sham stimulation), and 5-min ECG recording for HRV analysis was also measured before and after each stimulation.

Results: HRV data after ECT showed: First, the high-frequency (HF) power component of HRV was suppressed by the electric shock, but not by the anesthetic. Second, the anesthetic seemed have eliminated the escalating effect of the sympathetic modulation brought by the electric shock. HRV data after rTMS showed: Low-frequency power (LF%) and ratio of low-frequency power to high-frequency power (LF/HF), significantly increased after active stimulations.

Conclusions: ECT and rTMS were both conducted by passing electricity through the brain directly or indirectly, but their impacts to human cardiac autonomic function were not the same. Even though TMS resembles a modality of ECT without anesthesia, rTMS does not suppress vagal control as what electric shock does in ECT. Sympathetic escalation seems to be induced gradually in rTMS trials, but this phenomenon did not found after ECT.

Reference: Lai IC, Yang CC, Kuo TB, Shieh KR, Wang YC (2008) Immediate impact of electroconvulsive therapy on cardiac autonomic function in schizophrenia: A preliminary study. Schizophr Res 100(1-3): 353-5.

O96

The impacts of prospective payment system implementation on health insurance in Taiwan — The influences and relative factors analysis on Diagnosis related groups

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Background: Taiwan diagnosis-related groups (TW-DRG) have been introduced as a reimbursement system for in-patient care. The aim of this study is to evaluate the impact of TW- DRG implementation on hospital adoptive strategies in Taiwan.

Data Sources: This is a cross-sectional study to assess strategy differences in performance, cost shift, quality control and medical documentation change between hospitals in Taiwan. The primary data are longitudinal, administrative claims for databases sampled registry of National Health Insurance provided by National Health Research Institutes. Outcome measures are questionnaire data from hospital survey on 100 hospitals and 1,000stuffs with experiences of DRG system in Taiwan.

Study Design: A quantitative analysis including administrative data from National Health Research Institutes over a time period of 8 years (2002–09) . Characteristics and trends of case-mix index, number of cases, average age and length of stay (LOS) will be studied in detail. The contents of questionnaire include four dimensions to evaluate the impacts of performance, cost shift, quality control and medical documentation change between hospital s in Taiwan. Statistical Analysis includes descriptive and inference methods, responses to questions of interest will be assessed for the differences in socio-demographic variables with student t test and in the values of CMI(case-mixed index) in various groups were assessed using the ANOVA test. Multivariable logistic regression with stepwise model-building methods will be used to determine variables likely to significantly predict the adoptive strategies . Variables included in the model will be those that had a *P* value of 0.05. All data analyses were performed using SAS 9.0 (SAS Institute, Inc., Cary, NC).

Conclusions: National Health Research Institutes over a time period of 4 years (2002–05) appear the first impact of DRG system implementation is the case-mix index values increased after the introduction period 1n 2002. At the same time, characteristics and trends of case-mix index is significant associated with average cost, the ratio of complication/co-morbidity, numbers of procedure , mortality rate, 14-day readmission rate and average age over 2002-2005. The R-square of multiple regression model researches over 0.7. The another adoptive strategies of hospitals include up-coding, patient dumping and quality change will approach continuously in the future.

Limitations: The implementation of the TW-DRG system will begins at July 2009 in Taiwan, the change information of impact in hospital in-patient setting will be collection after 2009.As reported in the other countries where per case payment has been used in payment system and the most countries revise the DRG version for the factor of socio-demographic, however, in contrast, the DRG classification will some differences around the world.

Keywords: DRG, payment system, impact, length of stay

O97

Validation of three different nutrition screening tools in hospitalized patients for primary nutrition assessment

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Background: Malnutrition is the common problem in hospitalized patients. The nutritional status is often neglected in spite of the fact that poor nutritional status may adversely affect the disease recovery, length of stay, complications, as well as mortality rate. There are many tools exist; however, none has been universally accepted by nurses. A sensitive but simply, quick and easy-to-use applied nutritional screening tool is needed for practice nurses for early detection of malnutrition.

Aim of study: The aim of this proposal is to test the reliability and validity of three potential tools, the malnutrition universal screening tool (MUST), the admission nutrition screening tool (ANST) and the Subjective Global Assessment (SGA), in order to identify patients who are malnourished on admission to hospital or at risk of becoming malnourished during their hospital stay.

Method: A sample of 150 patients was selected from medical, surgical and oncology wards. The reliability of the screening tools will be tested by physicians, nurses, and dietitians with completing the screening tool on the same patient. These results of inter-observer reliability will be compared to determine whether the screening tools are reproducible with different observers. The validity will be assessed by the results of the sensitivity and specificity of the tools.

Result: The results will determine which tool will detect malnutrition status of patients efficiently.

Key words: malnutrition, nutrition screening tool

O98

Identification of genes that play potential roles in megakaryocytic differentiation

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The process of cell differentiation is regulated by the coordination of cell type-specific gene activation. Our previous studies indicated that anthrax lethal toxin (LT) could suppress TPA (12-O-tetrdecanoylphorbol-13-acetate) induced megakaryocytic differentiation in human erythroleukemia (HEL) cell line. Based on microarray data, hundreds of genes were up-regulated after TPA treatments and down-regulated upon LT-pretreatments. Those genes may play important roles in normal and LT-suppressed megakaryocytic differentiation. Genes encoded transcription factors known to involve in development or differentiation processes were first considered as candidate genes. Flow cytometry was used to study whether the differentiation abilities (specific surface marker expression and DNA polyploidy) of HEL cells were blocked by shRNA knock down of candidate genes. In this study, we have identified four genes (NF1/X, ZFP36L1, ZNF541 and FOSB) that play potential roles in megakaryocytic differentiation.

O99

An Animal Model of Adolescent Toluene Exposure

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Abuse of toluene-containing volatile solvents by adolescents is a significant public health problem. The present study was to establish an animal model for adolescent toluene exposure. Male NMRI mice received injections of either toluene (750 mg/kg) or oil during postnatal day (PN) 35-48. The blood levels of toluene were determined with the head-space gas chromatography at 0, 0.5, 1 and 3 h after toluene exposure. The biological half time of toluene in the blood was 1.6h. The blood concentrations of toluene were 103.8±3.3, 63.1±2.6 and 24.8±4.0ug/ml at 0.5, 1 and 3h respectively, and the levels were in the range of that in toluene abusers. In adolescent exposure regime, male mice received one injection per day of either toluene (600 mg/kg) or oil during PN35-37 and (750 mg/kg) during PN38-39 and PN42-46. A variety of psychiatric disorder-relevant behavioral tests were examined at PN56-84. The toluene-exposed mice were significantly deficient in the social interaction test, nesting behavior, social dominance tube test, and novel objective recognition test. However, toluene exposure did not affect locomotor activity and behavioral profiles in the forced swimming test, tail suspension test, emergence test and elevated plus maze. These results provide evidence to suggest that toluene exposure during adolescence is associated with long-lasting schizophrenic negative symptom-like behaviors and cognitive impairment, but not depression-like or anxiety-like behaviors at adulthood in mice, which correlate with schizophreniform psychosis observed in toluene abusers. This animal model is suitable for research into neurobiological abnormalities found in chronic toluene abusers and development of the therapeutic strategies.

O100

Intravenous immunoglobulin ameliorates thrombocytopenia through modulating the selectin pathways

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Intravenous immunoglobulin (IVIg) has been widely used in various diseases, for example, autoantibody-mediated thrombocytopenia and ischemia-induced inflammatory responses. However, the action mechanism of IVIg is yet unclear. It was suggested that IVIg might exert its anti-inflammatory effect through selectin pathways, because IVIg could inhibit P-selectin-dependent rolling and β2 –integrin-dependent adhesion of leukocytes *in vitro*. In this study, we performed antibody-induced thrombocytopenia in selectin knockout mice (*P-sel*$^{-/-}$, *PSGL1*$^{-/-}$, *L-sel*$^{-/-}$ and *E-sel*$^{-/-}$), and we found that P-selectin, compared to PSGL1, L-selectin and E-selectin, is more significantly involved in IVIg mediated amelioration. It had been reported that IVIg mediated amelioration was linked to the regulation of FcγRⅡB and FcγRⅢ receptors on effecter cells in spleen of animal model using FcγRⅡB or FcγRⅢ knockout mice. Here we found that experimental ITP levels perfectly associated with in vitro platelet-macrophage engagements. For example, FcγRIII KO mice were showed to have higher resistant against ITP and we found that FcγRIII null splenocyte-platelet engagements were indeed lower than the WT splenocyte-platelet engagements. Using this approach, different mononuclear cell-platelet combination among different genotype (WT, *P-sel*$^{-/-}$, *PSGL1*$^{-/-}$, *L-sel*$^{-/-}$ and *E-sel*$^{-/-}$) of mice were analyzed. It revealed that, when compared with *P-sel*$^{-/-}$ mice, platelets isolated from IVIg-treated WT mice had lower splenocyte-platelet engagements. Since IVIg priming *in vitro* can't reduce the responsiveness of platelets, our data might suggest that platelets were regulated by leukocytes indirectly *in vivo*. To investigate the hypothesis, we adaptively transferred *in vitro* IVIg-treated splenocytes to WT ITP mice. These IVIg-primed leukocytes had ameliorative effect on the recipient mice. In order to investigate the involvements of platelets and platelet P-selectin in this ameliorative effect, we isolated platelets from these recipient mice and subjected to our phagocytosis assay system. It revealed that platelets from recipient WT, but not *P-sel*$^{-/-}$ mice, would have a lower responsiveness to phagocytic leukocytes. It seems that these IVIg-treated splenocytes could modulate P-selectin$^{+/+}$ platelets in vivo and lead to a low responsiveness of platelets in response to splenocytes. It might suggest that there is a P-selectin-mediated leukocyte-platelet crosstalk involved in IVIg-induced amelioration on ITP. The sub-population of splenocytes to modulate platelets will be further investigated.

O101

Expression and function of FGF7 during liver regeneration

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Previous studies show that fibroblast growth factors (FGFs) are involved in the process of liver injury repair. Liver regeneration is impaired in transgenic mice expressing dominant-negative FGFR2b in hepatocytes. Among FGFR2b ligands, FGF7 is expressed by activated hepatic stellate cells (HSCs) in fibrotic liver. However, the function of FGF7 after partial hepatectomy (PH) has not been addressed. The results showed that FGF7 protein was detected in quiescent liver and increased in regenerating liver. FGF7 expression was examined in HSCs, which was transiently activated after PH. The expression level of FGF7 increased in accordance with the activation of HSCs after PH. High expression level of FGF7 was also detected in culture-activated HSCs. To study the function of FGF7, it was overexpressed specifically in the liver using hydrodynamics. Results showed advanced and greater number of BrdU-positive cells in FGF7-overexpressed livers after PH. Activation of mediators downstream of FGF signaling was also analyzed. Phosphorylation levels of Erk1/2 and p38, but not Akt, were higher in FGF7-expressing liver after PH. In conclusion, HSCs expressed FGF7 and this expression was increased as HSCs were activated during liver regeneration. Overexpression of FGF7 accelerated hepatocyte proliferation after PH, which suggested a possible role for FGF7 in promoting liver regeneration.

O102

Changes in Egr family proteins in mice hippocampus after chronic treating with methamphetamine

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Methamphetamine is a well-known psychostimulant that can induce psychosis, and methamphetamine-treated rodents are used as model psychosis in human. Early growth response (Egr) genes encode transcription factors that are induced by stimuli that cause synaptic plasticity. Recent study showed that gene expression of Egr family is down-regulated in dorsofrontal cortex of patients with schizophrenia as compared with control, suggesting that Egr members may involve the pathophysiology of schizophrenia. In addition, several lines of evidence show that Egr2 associates with attention, while Egr3 plays an essential role in learning and memory.

In the present study we used western blot to measure Egr family proteins expression in frontal cortex and hippocampus of mice after four weeks' treatment with methamphetamine. We detected increased expression of Egr1 and 3 proteins in hippocampus of mice after chronic methamphetamine treatment as compared with control animals. In addition, we found that Egr2 protein was up-regulated in human lymphoblastoid cells of schizophrenia compared with controls.

These data showed that chronic treatment with methamphetamine modulate the expression of Egr proteins that might be involved in the etiology and the pathophysiology of schizophrenia. In the future, exploration of trans/cis mechanisms and the downstream signaling cascade of Egr family genes will contribute to our understanding of the molecular basis of schizophrenia.

O103

CXCR2(IL-8Rβ) Was Upregulated by hSecurin Through Direct Transactivation

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hSecurin has been found to be over-expressed in most metastatic adeno-carcinomas. It also has transcription activation activity. Thus, one of the possible mechanisms that hSecurin's metastatic promoting ability may be through the regulation of its target genes that are involved in metastatic pathway. To explore the relations between hsecurin and tumor metastasis, we carried out microarray screening of HCT116 cells under different hSecurin status and identified CXCR-2(IL-8 receptorβ) as one of the genes whose expression was significantly elevated by ectopically overexpressed hSecurin in hSecurin null HCT116 cells (18-2). . IL-8 is a chemokine that activates multiple intracellular signaling pathways through two GPCR (CXCR-1 and CXCR-2). Increased expression of CXCR-2 has been characterized in cancer cells, suggesting that CXCR-2 may function as a significant regulator within the tumor microenvironment. Based on our microarray profile, we hypothesized that one of the mechanisms how hSecurin promotes cancer cell metastasis is through upregulating CXCR-2. Both RT-PCR and western analysis supported our hypothesis that expression level of CXCR-2 correlated with the expression level of hSecurin. . In order to figure out how CXCR-2 was upregulated by hSecurin, we isolated CXCR-2 regulatory sequence and serially deleted the regulatory sequence of CXCR-2 gene, and then analyzed their functions in breast cancer cells, MDA-MB-231 and MCF-7. The functional assay showed that the deletion of the region containing putative hSecurin binding site caused the greatest reduction of CXCR-2 promoter activity in both MDA-MB-231 and MCF-7 breast cancer cells, suggesting that CXCR-2 is a direct target of hSecurin. CHIP analysis showed that hsecurin interacted directly with this promoter region of CXCR-2. hSecurin specific shRNA knocked down 231 cells caused reduced expression of CXCR-2 These findings suggested that increased expression of hSecurin could directly activate CXCR-2 and may play important role in cancer metastasis.

O104

The role of Min system in *Helicobacter pylori*

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Cell division accuracy is important for rod-form bacterial proliferation. In *Escherichia coli*, the Min system consists of three proteins, MinC, MinD and MinE, which prevent the cell division at the poles. In previous studies, Min system is important for the midcell plane position and morphological maintenance. *Helicobacter pylori* is an etiologic agent of human gastritis and peptic ulceration. In addition, *H. pylori* can convert its morphology from rod-shape to coccoid form, with intermediate U form. The coccoid form is viable but not culturable in vitro. The importance of nonculturable coccoid form of *H. pylori* is thought to be in disease transmission and insensitive to antibiotic treatment. To clarify the relationship between Min system and morphological change in *H. pylori*, we mutated the *minC, minD and minE* gene in *H. pylori* ATCC43504, respectively. The *min* mutants formed the filamentous cells and the cell length is longer than wild-type strain. Complementation of *minC*, *minD* and *minE* gene in each mutant strain, approximately 91%, 53% and 41% bacterial cell length was restored, respectively. In reverse-transcription PCR showed *H. pylori minD* mutants have polar effects on expression of *minE* gene. However, complementation of *minDE* gene in *minD* mutant strain resulted in cells recovered of approximately 79%. These results show the Min system alters the morphological phenotype in *H. pylori*.

O105

Active Tuberculosis due to Double Strains in Eastern Taiwan

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To evaluate the extent of patients infected with double strains of *Mycobacterium tuberculosis* in eastern Taiwan - a high tuberculosis prevalence area, we collected 185 pulmonary tuberculosis patients notified by Tzu Chi General Hospital from October 2007 to September 2008. A modified multiplex polymerase chain reaction was developed using primers described by Warren et al to detect the Beijing and non-Beijing family genotypes in sputum cultural isolates. Of the 185 patients, 46.5% (86/185) were infected with Beijing family genotype strains, 42.2% (78/185) with non-Beijing family genotype strains, and 11.3% (21/185) with double strains. Among the three groups, there were no significant differences associated with the sexual ratio, the age groups, race, concurrent diseases, the treatment history of tuberculosis, the results of sputum acid-fast stain, and cavitation on chest radiograph. The Beijing family genotype strains had significantly higher multidrug resistant rate (17.5%) than those of the non-Beijing family genotype strains (3.8%) and double strains (4.8%) ($p = 0.035$). This study demonstrates that double strains of tuberculosis infection are present in patients with active tuberculosis in a high prevalence setting. Most importantly, the initial infection is unable to prevent from the subsequent infection.

O106

Studies of blood-brain barrier permeability change during rabies infection and therapeutic achievement after passive immunity delivering into CNS

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Rabies infection is known to be lethal in human. Treatment with passive immunity for the rabid patients is effective only when the patients have not shown neural sign. The blood-brain barrier (BBB) is a complex functional barrier and triggers the therapeutic development in neurological diseases. The goal of this study is to determine the integrity of BBB and to assess the possibility of enhancing BBB permeability combination with passive immunity in late stage of rabies virus (RV) infection. We measured the integrity of BBB permeability by quantitative ELISA for either total IgG or albumin levels in the cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI). The BBB permeability was changed after day 6 post-infection and significantly enhanced at day 8-9 post-infection, which the clinical neurological signs were observed. We also assessed the development of immunity; RV specific antibody was not induced high enough in neutralization test. To investigate whether the BBB permeability disruption with or without passive immunity is associated the lethal outcome of RV infection, we setup the osmotic breakdown of BBB model in rats and generated RV neutralizing monoclonal antibodies. The RV-infected rats administrated with osmotic arabinose (1.6M) to open BBB alone had similar lethal outcome to the control. However, the intravenous injected with monoclonal antibody 8-10E with or without osmotic arabinose treatment lengthened the survival. Forty to 50 percent Lew/SsN rats of rabies virus-infected could survive to day 40 post-infection accompanied discordant movements. The results suggest that the immune effectors, especially specific neutralizing antibody, delivering into central nerves system may have therapeutic values in rabies infection.

O107

Immunization of vaccine with complement (C3dg) and CpG-B ODN induce SLE-like syndromes in Balb/c mice

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Autoantibody produced by autoreactive B cells in SLE patients is a clinical characteristic during the disease development. Previous studies found that antibodies to human cytomegalovirus pp65 antigen (anti-HCMV pp65) were elevated in SLE patients but not in other connective tissue disease patients and healthy people. Furthermore, immunization of HCMV pp65 not only exacerbated SLE-like autoimmunity in autoimmune-prone mice, but also induced autoimmunity in Balb/c mice. To further investigate the pathogenic potential of HCMV pp65, we evaluate a new design of vaccine with complement (C3dg) and CpG-B ODN as adjuvant. The complexes of C3dg opsonized antigen have been demonstrated to activate anergic B-cells and exacerbate autoimmunity. To investigate anti-HCMV pp65 induced tolerance break and autoantibody secretion, Balb/c mice were challenged with HCMV pp65 peptides ($AA_{1\text{-}167}$ and $AA_{336\text{-}439}$) or plasmid ($AA_{1\text{-}167}$ and $AA_{336\text{-}439}$). Following the initial challenge, mice were boosted three times with $AA_{1\text{-}167}$ or $AA_{336\text{-}439}$ conjugated with C3dg/CpG-B ODN. The results showed that anti-dsDNA antibodies were detected in all mice following four weeks of immunization. The titers to anti-dsDNA antibodies were elevated in mice immunized with plasmid encoding $pp65_{336\text{-}439}$. Elevated autoantibodies to Hela antigens were also detected in mice received immunization of HCMV pp65 $AA_{336\text{-}439}$. Immune-complexes mediated glomerulonephritis is an important clinical feature to both human SLE patients and model animals. We found that $AA_{336\text{-}439}$ immunized animals often associated with higher frequencies of lesion and antibody deposition on their glomeruli. In conclusion, our data showed that HCMV $AA_{336\text{-}439}$/C3d complex is capable of initiating autoimmunity and induce SLE-like pathological anti-dsDNA antibody and kidney lesion in normal strain of BalB/c mice.

O108

Characterization of the *Helicobacter pylori* bacteriophage, ϕHP1

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Helicobacter pylori is a gram-negative pathogen which naturally specifically colonizes the human stomach and causes gastro-intestinal disease. Eradication of *H. pylori* by antibiotics to cure patients with chronic gastritis has improved life quality of human being. Since antibiotics are frequently used to treat bacterial infection, drug-resistant *H. pylori* were increasing daily. To develop the alternative therapy, bacteriophages were applied to many cases of multiple-drug-resistant bacterial infection. Bacteriophage which infects *H. pylori* was only reported once in 1993, but no further character was defined after that. In this study, we isolated a novel temperate bacteriophage of *H. pylori*, ϕHP1, which carrying linear double-stranded DNA. One-step growth data showed inefficient infection character. The genome size of ϕHP1 is ca 27 kb, and the predicted coding regions possess homologous amino acid sequence to *H. acinonychis* prophage II. In consideration of applied to phage therapy, this phage was tested to be capable of infecting over half of clinically isolated *H. pylori*. These results indicated that ϕHP1 could be the candidate to apply to phage therapy and genome sequence data would reveal evolutional history between different Helicobacter species.

P01

Effects of PGE_2 and VEGF on sperm functions: possible involvement in endometriosis-associated infertility

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Background – Endometriosis, characterized by the presence and growth of endometrial cells outside the uterus, affects 10% of reproductive women. Pelvic pain, dysmenorrhea, and infertility are typical and often devastating symptoms. Endometriosis is the primary cause of infertility in women, with the prevalence rate ranging from 25% to 50%. A lot of cytokines, growth factors, and angiogenic factors, such as interleukin-1, prostaglandin E_2 (PGE_2), and vascular endothelial growth factor (VEGF), are released from immune cells in implanted endometriotic lesions of affected women, changing the composition of the peritoneal fluid. Past studies have proved that overproduction of cytokines in endometriotic patients have negative effect on sperm functions or embryo development. Obviously higher level of PGE_2 and VEGF in peritoneal fluid of endometriotic patients was shown in previous studies; however, no direct impact of PGE_2 or VEGF on sperm has been described. Therefore, we focused on the effect of these cytokines on sperm functions in this study.

Methods – Fresh semen samples were obtained from healthy volunteers after a minimum of 3 days of sexual abstinence. Following swim-up, washed sperm were divided into several aliquots and incubated with varying concentrations of PGE_2/VEGF. Sperm motility was evaluated by computer-assisted sperm analysis. Acrosome reaction was evaluated by *Pisum sativum* agglutinin-FITC staining.

Results – PGE_2/VEGF resulted in a dose-dependent decrease in the percentage of acrosome reaction induced by the Ca^{2+} ionophore A23187. Statistically significant reduction was observed at 2, 20, 200 ng/mL and 10, 100 ng/mL of PGE_2 and VEGF, respectively. Besides, after exposure to 100 ng/mL of VEGF for over 2 h, a significant decrease in sperm motility and progressivity was observed (vs. control, $p = 0.007$ and 0.018, respectively). On the other hand, PGE_2 did not have significant effect on sperm motility.

Conclusion – The present data indicate that high concentration of PGE_2/VEGF in endometriotic patients may inhibit sperm acrosome reaction and lead to infertility.

P02

Calcium Oscillation and Osteogenic Differentiation in Human Bone Marrow derived-Mesenchymal Stem Cells

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Bone marrow-derived mesenchymal stem cells are multipotent and differentiate into specific cell lineages such as osteoblasts and adipocytes. Intercellular interaction, microenvironment and intracellular signal transduction are important during tissue developement. The mechanism by which bone marrow mesenchymal stem cells differentiate into bone is currently unclear. The changes in Ca^{2+}concentration are important during intercellular interaction and intracellular signal transduction. Since hMSCs were found to have spontaneous Ca^{2+} oscillations we hypothesized that Ca^{2+}concentration may change in the osteogenic differentiation of BMSCs. In this study, we isolated human BMSCs in vitro and characterized them with CD markers: CD34 (-), CD45 (-), CD90 (+), CD117 (-), HLA-ABC (+) and HLA-DR, P, Q (-), We measured the spontaneous $[Ca^{2+}]_i$ oscillations with loaded Fluo-3 AM and studied the differentiation of BMSCS. We induced hBMSCs to differentiate into osteogenic cell lineages by culture them in *differentiation medium*. At 2, 7, 14 and 21 days we loaded Fluo-3 AM and 10μM IP_3 receptor agonist (ATP) in perfusion culture medium to measure Ca^{2+} oscillation in differentiating hMSCs. We measured the fluorescence *intensity fluctuations in* hBMSCs cultured with osteogenic differentiated medium and compared them with control group which contained hBMSCs cutured without osteogenic differentiated medium. We found that hBMSCs cultured with osteogenic differentiated medium had less intensity of fluorescence of $[Ca^{2+}]_i$ oscillations at day 2 in comparison with control group. At 10 days the osteogenic differentiation of hBMSCs were confirmed by positive alkaline phosphatase staining. In conclusion, intracellular $[Ca^{2+}]_i$ oscillations decreased in hBMSC after osteogenic differentiated medium was added. In this study we showed that $[Ca^{2+}]_i$ oscillation changed early and before osteogentic differentiation developed. Ca^{2+} signal transduction may play an important role in the process of differentiation.

P03

An investigation of the therapeutic effect of spinal cord stimulation on cerebral infarction

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Spinal cord stimulation (SCS) has been proposed as an alternative treatment in the management of ischemic limb pain and angina. A few clinical reports have demonstrated the beneficial effect of SCS in the treatment of vegetative patients caused by cerebral focal ischemic stroke as some of patients appeared to have improved. In animal model, studies as early as 1976 proposed that SCS improves the vascular tone of peripheral circulation while recent study showed that SCS helps to reduce the infarct volume after ischemic stroke, but how does it affect ischemia-affected cortical neurons remains enigmatic. In light of this, we use a rat model of permanent middle cerebral artery occlusion (MCAO) by ligating the MCA at where it emerged from the lateral fissure to explore where SCS is neuroprotective. Neurological functions of the animals following MCAO alone and MCAO followed by SCS were evaluated, area of infarct was identified with 2,3,7-Triphenyltetrazolium chloride (TTC) staining and the morphology including dendritic spines of cortical pyramidal neurons analyzed following Golgi-Cox impregnation. Preliminary showed that MCAO resulted in localized cerebral infarct in 67% of the SD rats studied while 60% of the rats subjected to SCS still showed an identifiable but smaller infarct. MCAO resulted in obvious loss of staining and in addition, deterioration of the morphology of stained cortical pyramidal neurons within 4-6 hours of surgery. We are currently analyzing the neuronal morphology of the Golgi-Cox-impregnated cerebral cortex of these animals and the results will be presented at the poster on site.

P04

Oxidative Stress Caused by Intermittent Hypoxia Induces Cell Death in Rat Cerebellar Granule Cells.

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Episodic cessation of airflow during sleep in patients with sleep apnea (SA) syndrome results in intermittent hypoxia. The condition with cycles of hypoxia and reoxygenation resembles ischemia-reperfusion injury. Increased oxidative stress caused by reoxygenation in SA patients has known to be associated with increased risks of cardiovascular diseases and with neurocognitive deficits.

To investigate whether intermittent hypoxia would induce cell death and to find the mechanism, we used Sprague-Dawley neonatal rats for primary culture of cerebellar granule cells. Cells were subjected to either room air (RA, 20% O_2) or intermittent hypoxia (IH, 20% O_2 and 5% O_2, 30 minutes alternatively) for one to four days. Quantitative assessment of cell death, including apoptosis and necrosis, showed that IH significantly induced more cell death than RA ($p< 0.05$). Reactive oxygen species ($O_2^{\bullet -}$ and OH^-) increased significantly in IH group than RA group ($p< 0.05$). Using various blockers for different sites of cell death pathway, including OH^- generation and PARP, cell death in IH group decreased significantly ($p< 0.05$). Caspase activation was not noted, but apoptosis inducing factor (AIF) translocation to nucleus was found significantly more in IH group ($p< 0.05$). These findings indicate that oxidative stress caused by IH induces cell death in rat cerebellar granule cells, including apoptosis and necrosis. PARP activation results in ATP depletion and AIF translocation to nucleus, and contributes to apoptosis and necrosis.

P05

Differential regulation of NMDA receptor-mediated depolarization by different fragments of β-amyloid peptides in rat sympathetic preganglionic neurons

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Many studies have reported that the serum β-amyloid peptide (Aβ) levels are elevated in several mutations linked to familial Alzheimer's disease (AD). Additionally, hypertensive individuals had increased amount of senile plagues and neurofibrillary in their brain, and a higher chance for developing AD. It has been proposed that the mechanisms of AD pathogenesis may involve a combination of the vascular and neuronal toxicity of Aβ. Studies have reported the vasoactive effects of Aβ on cerebral and peripheral vessels *in vitro* and *in vivo*. The effect of Aβ on central control of cardiovascular function is poorly understood. Several *in vitro* studies reported recently that Aβ affected the function of NMDA receptors, a subtype of ionotropic glutamatergic receptors. This study investigated the effect of Aβ on NMDA receptor function in sympathetic preganglionic neurons (SPNs) located in the lateral horn region of spinal cord and involved in the regulation of cardiovascular function and sympathetic outflows. Consecutive applications of NMDA every 5 min induced reproducible changes in membrane potential in SPNs of neonatal rat (7~14 days) spinal cord slice preparation using whole-cell patch recording. Superfusion of $A\beta_{1-40}$ (0.3 μM) for 5 min caused no change of membrane potentials but significantly potentiated NMDA-induced depolarizations. In contrast, perfusion of $A\beta_{1-42}$ (0.3 μM) didn't influence NMDA-induced depolarizations. Interestingly, perfusion of $A\beta_{25-35}$ (0.3 μM) significantly inhibited NMDA-induced depolarizations. Western blot analysis showed that incubation of $A\beta_{1-40}$ but not $A\beta_{1-42}$ for 30 min onto spinal cord slices increased the levels of phosphoserine 896 on NMDA receptor NR1 subunit in lateral horn regions. These results suggest that different fragments of Aβ may regulate differentially the NMDA receptor function in rat sympathetic preganglionic neurons.

P06

Role of RpoS in stress response and catalase expression of *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus is a food-borne pathogen that inhabits in blackish seawater throughout the world. For microorganism, to survive in a variety of diverse and harsh stresses during life cycle, it's important to regulate gene expression responding to the continuous change of surrounding environment. Replacement of σ factor, a subunit of RNA polymerase, is a common way for microorganisms to switch the gene expression pattern. It is believed that RpoS (σ^E) participates in general stress responses in many bacteria. To understand the stress response in *V. parahaemolyticus*, we constructed *rpoS* mutant and analyzed its viability under different stresses. Although there was no significant difference between wild type and mutant when growing at 37 ^oC, *rpoS* mutant showed decreased survival rate in cold (4 ^oC), acid (pH 4.5), oxidative (1 mM H_2O_2) and starvation (artificial seawater, ASW) conditions. It indicated *rpoS* plays an important role for *V. parahaemolyticus* adaptation to stressful environments. We further used zymogram to assess the catalase activity during normal growth condition. The catalase activity of group C was faint in the mutant strain. Those data imply that *rpoS* regulates several stress responsive genes in *V. parahaemolyticus*. In order to find *rpoS*-dependent genes, proteomic analysis of cellular response to stresses will be performed.

P07

Establishment of a method for random mutagenesis of *Thermus* spp. and *galE* gene characterization

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Most bacteria are capable of forming surface-associated, architecturally complex communities of cells, called biofilm, which consist of extracellular matrix that holds the cells together and play an important role in helping bacteria to defend the environmental stresses. *Thermus* spp. are Gram-negative bacteria and which also have the ability to form biofilms. Previous study performed that differential protein expression profiles between planktonic cells and sessile cells by two dimensional gel electrophoresis analysis, one of significant differences was UDP-glucose 4`-epimerase which was encoded by *galE* gene in *Thermus thermophilus* HB8. To understand the role of *galE* gene during biofilm formation in thermophilic bacteria, we constructed a GalE overexpressed strain and generated a GalE mutant by homologous recombination with thermostable bleomycin-resistant gene as selective marker. In the future, the biofilm forming ability comparison and other different phenotypes between all of them will characterize the role of GalE. On the other hand, transposon mutagenesis is most applicable method for mutant library construction. However, there is no useful tools for random mutagenesis in *Thermus* spp. We tried to construct a Tn*10* transposon which containing *ori* of ColE1 and a thermostable bleomycin-resistant gene. In the future, more than 3,000 mutants of thermophilic bacteria will be screened for the phenotypes such as biofilm formation, motility, and survivability in high temperature. Here we will focus on the biofilm-forming ability between different mutants by two strategies: the *galE* single gene study and random tranposon mutation to identify and characterize the essential genes that involved in biofilm formation of thermophilic bacteria.

P08

Roles of MIP-1α in primary murine Listeriosis

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MIP-1α is an important chemokine, which has been shown to be chemotactic to macrophage, neutrophils, NK cell, and activated T cell. Nonetheless, its role in innate defense of primary Listeria monocytogenes (LM) infection has never been investigated. Here, we showed that administration with 1.25 x 10^4 CFU of LM, revealed a differential survival rates for MIP-1α-deficient and wild-type mice (84.6% and 30.4% for wild-type and MIP-1α-deficient mice respectively; Mann-Whitney Test; $p = 0.001$). Enumeration of splenic LM number 24, 48, and 72 h post-infection (p.i.) showed that MIP-1α-deficient mice had more bacteria than had wild-type mice ($p = 0.01$, 0.0007, and 0.0007 for 24, 48, and 72 h p.i. respectively). Analysis of macrophage revealed that its recruitment after infection occurred in two phases, one at 24 h p.i. and the other at 72 h p.i.. The recruitment of macrophages in MIP-1α-deficient mice was relatively ($p = 0.0001$ and 0.001 for 24 h and 72 h p.i. respectively). On the contrary, the early recruitment of neutrophils was not affected by the absent of MIP-1α; however, MIP-1α-deficient mice were unable to sustain high percentage of neutrophils by 72 h p.i. ($p < 0.0001$). Interestingly, TNF-α expression on macrophages and neutrophils also showed this two-phase pattern. The percentages of macrophages expressing TNF-α in MIP-1α-deficient mice were significantly less than those of wild-type mice at 24, 48 and 72 h p.i. Surprisingly, we consistently found that there were more neutrophils expressing TNF-α in MIP-1α-deficient mice at 24 h p.i. This suggests that MIP-1α has an opposite effect on the expression of TNF-α on macrophage and neutrophils. We also examined the expression of CD69 and MAC3 on macrophages. The expression of these two markers on macrophages in wild-type mice reached peak at 48 p.i., and slightly turned down at 72 h p.i. On the contrary, MIP-1α-deficient macrophages had a sharp decline in the expression of CD69 and MAC3 at 72 h p.i. ($p = 0.0004$ and 0.039 for CD69 and MAC3 respectively). In contrast to CD69 and MAC3, expression of CD200R, a receptor implicated in deactivation of macrophage, showed an opposite pattern in MIP-1α-deficient mice, and failed to turn down at 72 h p.i. Our result suggested MIP-1α have complicated action during murine listeriosis.

P09

Analysis of a bifunctional catalase-peroxidase KatG in *Vibrio parahaemolyticus*

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In order to cope with oxidative stress, bacteria need to produce antioxidant enzyme (e.g. catalase, peroxidase, superoxide dismutase) to remove reactive oxidative species. In our previous study, two copies of *katG* (VPA0453 and VPA0768) were found in the genome of *Vibrio parahaemolyticus*. Both *katGs* encode bifunctional catalase/peroxidase with a molecular weight of approximately 80 kDa. Both deduced amino acid sequences showed 78% identity and 88% similarity. To investigate the functions of VPA0453 and VPA0768, mutants are generated by allelic exchange via suicide vector approach. The VPA0768 mutant has been constructed successfully, but the VPA0453 is keeping on generating. Compared to the wild-type strain, the catalase/peroxidase activity of VPA0768 lysates was decreased obviously. Furthermore, zymogram was also applied to differentiate catalase activity on native PAGE. The result showed catalase patterns of VPA0768 mutant were clearly different from that of wild-type strain. In the future, we will compare the specific catalase activity, zymogram profile, survival under hydrogen peroxide treatment between VPA0768, VPA0453 mutant and wild-type strain to explore the role of bifuncitonal catalase-peroxidase KatG in *V. parahaemolyticus*.

P10

Characterization of the relationship between chaperonin and biofilm formation in *Thermus* spp.

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Thermus aquaticus YT-1 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. *Thermus aquaticus* YT-1 were analyzed by proteomic analysis in order to identify some of the proteins involved in biofilm formation. Two-dimensional gel electrophoresis revealed distinct and reproducible different protein expression pattern between sessile and planktonic cells. Then the extractive proteins were analyzed by ESI-QUAD-TOF or MALDI-TOF. In addition, compared data with Matrixscience database. Result shows those proteins was involved in different kind of protein functions. According to previous results, I have selected chaperonin (GroE) for further study. The GroE can be divided into GroES and GroEL; it was ubiquitous and essential protein folding machines. In genetic approach, *groEL* mutants were generated by disrupting the gene via the double crossing over from *Thermus thermophilus* HB8 and *Thermus aquaticus* NTU103. The mutant without the targeted fragment in the chromosome of *Thermus thermophilus* HB8 and *Thermus aquaticus* NTU103 was confirmed by PCR using primers *groEL*-F and *groEL*-R. Result shows *groEL* was not be amplified. In biochemistry approach, *groES* was cloned to pET-30a plasmid then purified by nicol-chelate affinity chromatography and ready for antibody preparation. Western-blot analysis showed that sessile cell GroES expression more than planktonic cell in difference time points. In addition, the function of GroES protein folding was tested by protein refolding assay and enzyme protection assay. Result shows GroES did not have refolding and protection functions suggest only GroES was not insufficient. The relationship of GroELS and biofilm formation will be analyzed in the future.

P11

Preparation of capillary immunoaffinity column for chiral separation of phenylalanine and its analogs with high performance liquid chromatography

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Chiral separation of drugs is an important issue in pharmaceutical industry. Many prescription drugs have enantiomers. Sometimes, only one of them is curative, and the other is ineffective and toxic.

Recently, in our laboratory, chiral quantitation of methamphetamine and its analogs (MDMA, PMMA) in urine had been achieved by high performance liquid chromatography with a self-made capillary immunoaffiniy column (250 μ m-ID).

By using the same methodology, D- phenylalanine specific polyclonal antibody will be obtained by immunized rabbit with BSA conjugated D- phenylalanine and purified the antiserum with D- phenylalanine stationary phase. The antibody obtained will be used to prepare a capillary immunoaffinity column for chiral separation of phenylalanine and its analogs in this study.

P12

Analysis of Conserved Motifs Among the RNA Dependnt RNA Polymerase (RDRP) Encoded by RNA Virus

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RNA-dependent RNA polymerases (RdRps) function as the catalytic subunit of the viral replicase required for the replication of the majority of RNA viruses. In previous studies, six consensus motifs among the RdRp sequences encoded by plus-, minus- and double-strand RNA virus were identified based on the sequence and structure analysis. As the RdRp collection methods applied in previous studies may miss the sequence dissimilar RdRps, in my study, the “keyword” search approach against the UniRef50 database is used to collect the RdRp sequences from the plus-, minus- and double-strand RNA virus separately. We then further analyse the features of the RdRp sequences in each type by multiple sequence alignment and grouping methods. The preliminary result shows some unreported conserved regions in each of the RdRp group in the plus-strand RNA viruses. Next, I will use protein secondary structure prediction and clustering approaches to analyze the grouping features among the collected RdRps.

P13

Screening of effective Chinese herbs against Aβ caused neurotoxicity

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Alzheimer's disease (AD) is a progressive neurodegenerative disease and the common cause of dementia among older people. AD is characterized pathologically by the deposition of beta-amyloid (Aβ) involving extracellular senile plaques and intracellular neurofibrillary tangles. The aggregation of Aβ (1-42) has been shown to induce lipid peroxidation, protein oxidation, neurotoxicity, and reactive oxygen species generation in neurons. The constitution of *Fructus Aurantii Immaturus* (Zhi Shi) contains eliminate phlegm, gastroptosis, prolapse of rectum. It is function has been demonstrated to be effective for hypertension and improving blood supply of the heart and brain. In the present study, we investigated the effect of *Fructus Aurantii Immaturus* (Zhi Shi)) on neurotoxicity induced by Aβ (1-42) in cultured PC-12 cells. Results indicate that the extract of *Fructus Aurantii Immaturus* (Zhi Shi) may protect PC-12 cell against Aβ induced neurotoxicity.

P14

The Effect of Androgen Receptor on D1 Cell Osteogenic Differentiation

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Androgen receptor (AR) plays an important role in the function of several organs including primary and accessory sexual organs, skeletal muscle, and bone, making it a desirable therapeutic target. AR acts as a ligand-regulated, DNA-binding transcription factor to regulate gene expression by binding the androgen response element (ARE) within target genes. AR was shown to affect osteogenic differentiation. However, the detailed molecular mechanisms of AR in regulating bone cell differentiation remain elusive. In this present study, we determined the role and molecular mechanism of AR in D1 cell (multipotent mouse mesenchymal stem cells) osteogenic differentiation. First, we examined the expression levels of AR during the osteogenic differentiation of D1 cells by semiquantitative RT-PCR. Dual-luciferase reporter assay was also performed to determine the transcriptional activity of AR during the osteogenic differentiation of D1 cells. The transcription factor Runx2/Cbfa1 and osteocalcin are known to be essential for multipotent mesenchymal cells to differentiate into osteoblasts. Therefore, we investigated further on the effects of AR on the expression of Cbfa-1 and osteocalcin by semi-quantitative RT-PCR. The transcriptional regulation mechanisms, by which AR controls their expression, were determined by chromatin immunoprecipitations (CHIP) and dual-luciferase reporter assays. Our study will elucidate the action of AR in affecting the osteogenic differentiation of D1 cells by regulating the expression of Cbfa-1 and osteocalcin.

P15

Effect of androgen receptor on K562 cell erythroid differentiation

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Androgen receptor (AR) belongs to the nuclear receptor family that are ligand-dependent transcription factors to regulate the expression of genes critical for cell growth and differentiation. Androgen receptor regulates gene expression by binding to a specific DNA sequence called androgen responsive element. Androgens 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 remain poorly understood. In the present study, we examined the role of AR and its molecular mechanism on erythroid cell differentiation using K562 cells an erythroleukemia cell line. We examined the change of the of AR expression level during K562 cell erythroid differentiation by Western blot assay. The effect of androgen-AR signaling on K562 cell erythroid differentiation was examined by benzidine staining and flowcytometry for the increase in their hemoglobin level and glycophorin expression. Because GATA-1, Myc or other transcription factors that are involved in erythroid differentiation contain androgen response elements in their promoter regions, we further investigated whether their expression level were indeed affected by androgen-AR signaling. Chromatin immunoprecipitation was preformed to demonstrate the binding sites of AR in their promoter region. Reporter gene assay was used to demonstrate the transcriptional regulation of AR on the potential binding sites in promoter regions of GATA-1 and Myc genes. This study will provide new insights into the roles of androgen receptor in erythropoiesis and its molecular mechanism on erythroid cell differentiation.

P16

Searching for cellular factor(s) facilitating HCV replication

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Hepatitis C Virus (HCV) infects 170 million individuals in around the world. Infection with HCV frequently causes chronic hepatitis and progresses to liver cirrhosis and hepatocellular carcinoma (HCC). HCV belongs to the genus Hepacivirus, within the family Flaviviridae. HCV is an enveloped virus, its genome is a single-stranded positive-sense (9.6 kb) RNA. Viral isolates are further classified into six genotypes (1–6) and over 100 subtypes. Currently, no safe and effective vaccine is available to prevent HCV infection. Conventional treatment, using the combination of pegylated interferon-α (PEG-IFN-α) and ribavirin is only effective in about 50% of the patients and is associated with important side-effects.

Cyclosporin A (CsA) , a widely used immunosuppressive drug, has been reported to be effective against HCV infection. The antiviral action of CsA is mediated by blockade of actions of cellular CsA-binding proteins, the cyclophilins. Therefore, cellular cofactors affecting hepatitis C virus replication could be the potential anti-viral targets.

In this study, we are going to search for the cellular factors that can facilitate HCV replication. Microarray analysis was used to screen the genes with differential expression level between the HCV replicon cell line and mutant cell line (without HCV replicon). Genes over-expressed in HCV replicon were knocked-down by shRNA technology individually to determine their effect on the HCV replication. The replication of HCV was monitored by the expression of HCV NS5A protein.

The cellular factors PLCG2、ELOVL4 and PLA1A were identified through this screening system and all of these genes are involved lipid metabolic pathways. Therefore, other genes involved in the metabolic pathways were also analyzed. Knock-down of these selected genes involved in the metabolic pathways could also repress HCV replication. Moreover, PLCG2 inhibitor U73122 could also inhibit HCV replication. We are going to confirm these results in the system using JFH-1 infectious clone and the ex vivo system using liver biopsy sample.

P17

Involvement of Integrin-mediated Signaling for TPA Induced Migration of HepG2 cell.

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Hepatocellular carcinoma (HCC) is one of the major malignancies in the world. The prognosis of HCC is poor due to frequent intrahepatic metastasis and tumor recurrence. Target therapy blocking the signal pathway leading to intrahepatic metastasis is promising for improving prognosis of HCC.

We have been studying the signal pathway mediating tumor progression of hepatoma cell line HepG2 triggered by the phorbol ester tumor promoter TPA. Previously, we found protein kinase C (PKC), the cellular receptor of TPA may cross react with reactive oxygen species (ROS) to sustain mitogen activated protein kinase (MAPK) activation required for TPA-induced cell migration. Subsequently, we found integrin-mediated signal pathway were involved. In this study, we found TPA may induced phosphorylation of integrin related signal components including paxillin (p-Tyr 31, p-Ser 178) FAK (pTyr-397) and Src (p-Tyr416). These TPA-induced activation event may be prevented by PKC inhibitor BIS (Bisindolylmaleimides) and ROS scavenger (DPI(diphenyleneiodonium chloride)、DTT(Dithiothreitol)、CAT（H_2O_2:H_2O_2 oxidoreductase）、SOD (Superoxide oxidoreductase)). Furthermore, IP/Western for PKC indicated TPA may trigger interaction of PKC and integrin β. On the other hand, TPA-induced generation of ROS and PKC activation were also dependent on integrin related signal as have been found by student in our Lab. Taken together, it is possible that TPA may induce cross talk of PKC and integrin mediated signal cascade for sustained ERK activation and cell migration of HepG2. More experiment must be performed to address this issue.

P18

Simultaneous determination of pseudoephedrine and ephedrine and other cold medicines in urine sample by liquid chromatography-tandem mass spectrometry (LC-MS-MS)

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L-(+)-Pseudoephedrine(PSE) is the (S,S) diastereoisomer of ephedrine, It is a naturally occurring alkaloid. Pseudoephedrine and ephedrine are widely used in combination with other drugs for the clinical treatment of common cold, respiratory allergies .Pseudoephedrine and ephedrine , like other stimulants has been abused , are used as key ingredient for the production of the illicit drug (D)-methamphetamine.

The objective of this study was to develop a liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for the analysis of 13 active ingredients commonly found in cold medicine, including pseudoephedrine and ephedrine in urine samples. The study is used to screen urine for the presence pseudoephedrine and ephedrine at a cutoff concentration of 500 ng / mL. The urine samples were extracted by liquid phase extraction (LLE) after adjustment of its pH . The extracts were analyzed directly in to ESI-MS system .The mobile phase was 50% water containing 0.1% formic acid of and 50% methanol containing 0.1% formic acid .The analysis was performed at a flow rate of 0.2 mL / min and monitored with multiple reaction monitoring (MRM) at an selected collision energy of 11 to 24 e V . The method was applied to determine whether pseudoephedrine and ephedrine were co-administered with other ingredient in cold formulations. The cold medicine contains other ingredient such as analgesic, antihistamine, decongestant and antitussive.

P19

Analysis of Colistin-resistant Associated Genes in *Acinetobacter baumannii*

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Acinetobacter baumannii is a gram-negative bacterium which causes serious nosocomial infection. There are many new strains of *A. baumannii* recently isolated and found to be resistant to commonly used antibiotics and named multiple-drug resistant *A. baumannii* (MDRAB). At present, colistin can effectively treat *A. baumannii*, however, there is sporadically colistin resistant strains found clinically. Therefore, the aim of our study is to investigate the genes associated with colistin resistance. According to some review papers, it had been mentioned that the development of resistance to colistin may be due to the change in binding affinity between colistin and lipopolysaccharide. At the beginning, we want to examine whether lipid phosphoethanolamine transferase gene (A1S_2752) and its upstream genes (A1S_2750 and A1S_2751) are associated with colistin resistance. The expression levels of above-mentioned genes were analyzed and found the mRNA expression levels of these genes are significant higher in artificial induced colistin resistant strains than wild-type. In order to investigate more genes related to colistin resistance, we constructed an expression library from a colistin resistant clinical strain. Recently, we got another there genes (A1S_0008, A1S_3219 and A1S_3220) relative to colistin resistance from the expression library. These colistin resistant associated genes will be further analyzed. We hope this research could be the basis of removing the drug resistance strains, and provide further development of new antimicrobial agents in the future.

P20

Searching for cellular factors up-regulated after influenza A virus infection

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The flu is a contagious respiratory illness mainly caused by influenza A virus. Flu outbreaks due to emerging influenza A virus infection could cause millions death, e.g. 1918 Spanish flu (H1N1), 1957 Asian flu (H2N2) and 1968 Hong Kong flu (H3N2).

To search for cellular factors modulated by influenza A virus infection, microarray analysis was performed to detect the cellular genes modulated after influenza A virus WSN33 infection in A549 cells at different time periods (2 hrs, 4 hrs, 21 hrs). As expected, cellular genes related to immune responses were modulated after virus infection. Unexpectedly, various histone genes were up-regulated after virus infection. To determine the effect of histone gene up-regulation on influenza A virus replication, siRNA technology to knock-down histone gene expression in A549 cells will be used. If reduction of histone expression will inhibit virus replication (analyzed by plaque assay), the mechanism(s) regarding histone affects virus replication will be addressed.

P21

Reactive Oxygen Species generation mediate TPA induced singal transduction in Hep G2 cells

TPA 誘導肝癌細胞株 Hep G2 中活性氧生成的訊號傳遞

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Reactive oxygen species (ROS) were involved in tumor metastasis especially at the migration and invasion stage. ROS generation may be induced by growth factors (such as TGFβ and HGF), or integrin engagement, which are capable of triggering tumor progression. Previous study in our lab indicated that ROS may be generated during migration of hepatoma cell HepG2 induced by tumor promoter TPA in a protein kinase C (PKC) dependent manner. In this study, we further investigated whether integrin-related signal transduction is involved in TPA-induced ROS generation. The flow cytometry was used to analyze ROS generation using DCFDA as a ROS probe. In the time course study, ROS were found to be generated in HepG2 after treatment of TPA for 0.5 to 6 hr with maximal induction at 1 h. The TPA-induced ROS generation was not observed in cell suspension of HepG2 indicating that integrin triggered signal was requied. Furthermore, we found the PKC inhibitor Bis (bisindolylmaleimides), and inhibitor for the integrin-related signal cascade such as RGD peptide, Src kinase inhibitor, Rac inhibitor (NSC23766) may prevent TPA-induced ROS generation at 1 h. On the other hand, we also found that ROS generation was also required for TPA-induced (ERK) MAPK phosphorylation in attached HepG2 but not HepG2 in cell suspension. Taken together, we suggest that TPA may induce ROS generation via the integrin pathway, which is required for TPA-induced sustained ERK activation and cell migration of HepG2. More detailed mechanisms are still needed to be explored.

P22

Application of Capillary Electrophoresis and MALDI-TOF Mass Spectrometry to Rapid Identification of Anaerobes for Medical Diagnosis

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Capillary electrophoresis and mass spectrometry has developed to a well accepted analytical approach complementary and/or competitive to classical MS-hyphenated separation techniques, due to its simple method development, high separation efficiency, low sample consumption and short analysis time. Recently, CE has been used widely as a diagnostic tool in separation and identification of microbial mixtures. The rapid identification of bacterial pathogens is significant for the patient supervision and initiation of appropriate antibiotic therapy in the early stages of infection. Furthermore, anaerobic bacteria are the major component in all kinds of normal flora. It causes the infection of the tissues and organisms in human body (anaerobic infection). In the present study; we used CE and MALDI-TOF mass spectrometry for rapid identification of anaerobes. In some special cases, we collected the microbial infectious pathogens from CE, and analyzed by MALDI-TOF MS; it was simple and fast to identify pathogens through protein fingerprinting approach. This method was rapid and needed only one hour (CE and MALDI-TOF) with before culture time (2-4 days) to definitively identify bacteria in specimens; it was much faster than the conventional biological methods (about one week or more). However, the work presented with the advent of powerful CE and MALDI-TOF methods, for an accelerated discovery and specific diseases in clinical diagnostics.

P23

Dynamics of destruction on *Staphylococcus aureus* membrane during Gramicidin A treatment

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Gramicidin A has been recognized as an antibiotic peptide. It was generally believed that Gramicidin A performed its bactericidal ability by forming ion channels on bacterial surfaces. However, the detail mechanism for the bactericidal effect induced by gramicidin A is still not very clear. In this study, we attempted to apply biophysical techniques Fourier transform infrared spectroscopy, Atomic force microscopy and Flow cytometry to investigate the detail mechanism of the Gramicidin A induced bactericidal effect on *Staphylococcus aureus* surface.

According to Fourier transform infrared spectroscopy (FTIR) on Gramicidin A treated bacteria, several peaks corresponding to chemical bonds, such as peptide bonds and phospholipid bilayer polar and apolar group, disappeared during the treatment indicating the disruption membrane proteins and membrane lipids on membrane surface. The atomic force microscope (AFM) images of Gramicidin A treated bacteria indicated that the destruction of the bacteria by the treatment initiated by forming considerably large pores on bacterial surface. Materials in the bacteria then leaked through these large pores and subsequently made the bacteria flat. In final stage, the disrupted bacteria were found to be in dissolved like morphology possibly duo to the total destruction of the bacterial membranes as indicated by the FTIR spectroscopy. As hydroxyl radicals were found to be generated during the treatment according to flow cytometry, the destruction of the bacterial membrane lipids and proteins might be caused by these hydroxyl radicals.

This study reveal that during the Gramicidin A treatment on *Staphylococcus aureus*, apart form the normally believed ion channel formation, the bacteria are more likely be killed also by more complex ways such as large pore formation and reactive oxygen species (ROS) production during Gramicidin A treatment.

P24

Structural and functional analysis on engineered Sushi peptides

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Bacterial resistance to antibiotics has become one of the major problems in clinical treatments. As a result, there is an urgent need to develop or search for new antibacterial drugs to keep on the pace of the antibiotic resistance development by bacteria. Antimicrobial peptides were one of the new choices for this purpose.

Sushi 1 (S1) and Sushi 3 (S3) belong to a family of antibacterial peptides derived from the Sushi domain of Factor C, a lipopolysaccharide (LPS)-sensitive serine protease important in the horseshoe crab coagulation cascade. Although S1 and S3 have been shown previously to be affective against Gram-negative bacteria and the hypothesis of the bactericidal mechanism has been proposed, the details of the mechanism regarding to the structural point of view is still less certain. The research intended to apply structural biological and biophysical methods such as Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM) and flow cytometry to gather information regarding to the bactericidal mechanism during the peptide treatments. Bioinformatics, amino acid substitutions as well as structural biological methods were then used to search and study important amino acid positions for antibacterial activity in these two peptides. Subsequently, more effective antibacterial drugs can be designed and engineered based on S1 and S3.

P25

A Novel Seizure Detection Method Based on EEG zero-crossing of Time-histogram

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Introduction - Epilepsy affects 4~5% population in the world and the death rate of sudden unexpected death in epilepsy (SUDEP) is the top of death rate of this population. In order to detect epilepsy occurrences and/or prevention or prediction of SUDEP, it is necessary to develop an accurate, realizable, personalized automatic seizure detection method which is valuable for understanding epilepsy. Previously, İnan used fuzzy C-means (FCM) clustering algorithm to detect epileptic spikes. Xu tried to solve the same problem by improved morphological filter and compare to traditional filter.

Methodology - Our experiment data came from FSPEEG database with authorization with 21 subjects with epilepsy (Table 1). The EEG was segmented into time intervals vector by successful zero-crossing. An important advantage of this zero-crossing approach is that the time intervals are robust to noise components of the signal amplitude. After reconstructing by a window every 10 second, the lengths of data for processing are reduced. In addition, histogram is widely used in image and signal processing. If the seizure happened, the raw data's energy increased. It also means that the number of zero-crossing rate may increase. According to the hypothesis, the time zero-crossing intervals have been mapped to a histogram for presenting the number of the crossing rate. Instead of Fourier transform, short-term Fourier transform (STFT), eq.1, is applied on the histogram without overlap, which is so-called time-histogram transform. After the time-histogram transform, the energy and the low frequency are the features of seizure detection.

For seizure onset decision, summation of the histogram and energy of time-histogram transform are used to set the threshold. Because of baseline differences among persons, the threshold is set on average energy from window 1 to window 10. The summation of 1~300 points of energy is our first feature to identify seizure; the second feature is the total points of histogram.

Results – The method is fast and robust. It took only 6 seconds to process 1 hour EEG seizure data. The details of accuracy, sensitivity and specificity for each person are shown at Table2. The data marked as red is seizure onset duration wthin reference window. The total accuracy and specificity are 93.68% and 95.39%.

Conclusions and Discussions – Generally, our method offer a new way for seizure detection. This paper introduced an effective way to find a significant feature by using STFT. After reconstruction and building histogram, the dimension of data is decreased, but the system performance increased. Over all accuracy reaches 93.68%, including 2 data seizure onset in reference window.

The sensitivity still has the room to improve and the decision of threshold is critical. The problem hopefully could be solved by statistics or AI algorithms. Hence, finding more meaningful and significant features probably is a way to success.

P26

Identification of Major Depression Disorder (MDD) Based on HRV Analysis with comparison of relaxation and focus states

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Introduction - Depression is a significant public health concerned worldwide. WHO (2000) reported that depression becomes as one of the most important issue in 21st century. Its life-prevalence rate is up to 25%. There are about 3% of the populations who suffer for depression for each year. That is, in year 1993, there are 120 million people suffer for depression annually in the world. Major Depression Disorder (MDD) is a mental disorder with symptoms, such as low self-esteem, and loss of interest or pleasure in normally enjoyable activities. Therefore, it is an important society topic indeed. The aim of this research is to understand the physiological mechanism of depression and to develop the evaluation criteria for predicting the physiology stages the depression in advance.

Method – There are 31 MDD subjects (39.35±11.65 years old, including 14 males and 17 females) and 28 health controls (38.36±10.56 years old, including 13 males and 15 females) who were investigated. Their ECG signals have been recorded for 2 minutes at rest stages and 22 minutes at attention stages during TOVA test. All data collected in morning between 9:00am~12:00pm as control factors. Then, the RR tarchogram was applied the fast Fourier transform for heart rate variability (HRV) frequency analysis, including total power, VLF, LF, HF, and LF/HF. After frequency analysis, those factors from MDD and health groups in rest and attention stages were computed the significance by using t-test. The independent samples t-test was applied on different sample groups with same factors. In addition, the paired samples t-test was applied on the same sample group and same variables with different mental stages.

Results –

In the independent samples t-test: The LF/HF (rest: MDD(1.17±.73), controls (0.94±.34); attention: MDD(1.21±.52), controls (.88±.21)) at the attention stage shows significance at t-test. That is, it is an important factor for identification of MDD and normal controls.

In the paired samples t-test: HRV factors (total power, LF, HF, and LF/HF) on MDD subjects are highly correlated with relaxation and attention stages. However, the condition is not happen in health controls.

The VLF (rest(4.21±4.20 ms^2), attention (3.11±2.30 ms^2)) is significantly to identify the state at the MDD patients.

Discussions & Conclusions - The HRV do show the significant difference between the MDD subjects and the health controls. Specially, the LF/HF, the ratio of sympathetic and parasympathetic system, is the most difference between two groups. According our study, the MDD subjects have lower regulation on sympathetic or parasympathetic activities.

P27

The influence of fentanyl on PR interval variability (PRV)

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Introduction: Autonomic nervous system (ANS) is part of human's nervous system which divided into the sympathetic and the parasympathetic nervous systems. Sympathetic and parasympathetic innervate heart distictly; sympathetic nerve innervates predominately on ventricle; whereas parasympathetic nerve predominately on sinoatrial (SA) and atrioventricular (AV) node. In general, heart rate variability (RR interval variability) was most widely used for autonomic quantification.

In addition, atrioventricular conduction delay, which contributes to the length of PR interval in electrocardiogram (ECG), is also controlled by ANS. However, there is only little known on the mechanism of atrioventricular conduction delay. Since total cycle (RR interval) variability was known to be influenced by ANS, we hypothesized that PR intervals of heart cycle can demonstate different control power of sympathetic and parasympathetic nerve. Hence, this research investigates PRV under the influence of fentanyl and how PRV is influenced by ANS regulation.

Method: Fentanyl, an opioid frequently used for general anesthesia, was known to alleviate surgical stress by means of autonomic blockade. We recorded ECGs from 15 patients undergoing general anesthesia for different surgical procedure. At least 300 consecutive heart beats was recorded before and 3min after fentanyl 2 μg/kg administration. Both time domain and frequency domain methods were used for PR interval and RR interval variation analysis for comparison.

Results: Significant reduction in very low frequency (VLF), low frequency (LF), and high frequency (HF) was observed in HRV. Standard deviation but not mean length of RR interval was also significantly decreased after fentanyl given. No statistic change in PR interval variation was found by means of time domain and frequency domain analysis.

Conclusions: We concluded that AV node delay variability was not significant influenced by fentanyl. The result corresponds to the theory that fentanyl modulates heart rate primarily by sympatholysis, instead of modulation of vagal activity.

P28

發展以系統架構為基礎之隨身型生理訊號監測裝置

Development of the based on system structure of physical signal monitoring device

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研究動機 - 隨著國人健康意識型態的提升，工作之餘的休閒運動也漸受重視(如:騎自行車、慢跑、散步...)，衛生署日前公布 95 年國人十大死因，心血管疾病仍高居前 3 名，慢性病患除了維持正常作息及飲食習慣外亦需常常量測其生理情況，因此隨身型生理訊號監測裝置已漸受到重視。目前市面上到處都可以看到隨身型生理訊號監測裝置，如血糖機、電子血壓計、耳溫槍....等，隨身型生理訊號監測裝置確實有其發展潛力，若能研發出隨身型生理訊號監測裝置並結合目前民生用品(如手錶、手機、i-Pod..)使用將更能提高其實用性。本研究是探討如何將使用者心電圖訊號及狀態用以系統架構為基礎之隨身型生理訊號監測裝置來進行訊號偵測收集與傳輸。

研究方法 - 本研究試做一個同手機大小具智慧型之隨身型生理監測裝置，以 MSP430 單晶片為基礎核心，硬體用模組式開發設計方式，分為心電圖、三軸加速計、藍芽無線、Micro SD 儲存、面板顯示及操作等模組，利用 IAR 軟體開發系統功能來整合模組之間的運用。在軟體流程方面為了與 MSP430 單晶片相互溝通，在主程式一開始將單晶片的時脈、I/O、面板操控按鈕與搖桿、系統時間、3 軸加速計、LCD 面板、Micro SD 卡及面板上的選單初始化，再顯示選單並等候選擇後執行，主程式流程圖，在選單部份分別有 RealTime Mode、Sport Mode、Transmission Mode、Set Mode 及 Sleep Mode。本研究心電圖訊號是選在 RealTime Mode，在此模式選項可以偵測及時心電圖 QRS 波及 3 軸加速計訊號並用藍芽將二者訊號以無線方式傳出。以 RealTime Mode 程式流程來說，首先初始化數值後接著啟動藍芽功能，面板燈熄滅進入省電模式，接著執行心電圖 R 波的偵測及計算心跳數，再來進行 3 軸加速計的計算，最後送出二者訊號到 PC 端，若按下面板鈕就會退出此選單的執行指令，結束 RealTime Mode 此時藍芽功能也會停止。在收取心電圖及三軸加速計訊號部份是在硬體部份取得使用者之心電圖訊號及運動狀態訊號經 R,C 電路元件做初步濾波後，再將原始類比訊號送至 MSP430 單晶片，經取樣後轉換成數位訊號，並利用高通、低通數位濾波方式來濾除不要的雜訊，取得使用者心率、運動情況後以數字顯示心跳數於生理監測裝置面板上，同時 Micro SD 記憶卡可儲存二種生理訊號，並可透過此裝置選單將藍芽功能啟動，利用藍芽無線模組以 115200Hz boudrate 將智慧型之隨身型生理監測裝置上的資料上傳到 PC 端。

結果與討論 - 本次心電圖波形採用三導程電極方式貼上一般醫療用電極貼片，使用隨身型生理訊號監測裝置取得心電圖第二導程波形(Lead II)，利用此裝置藍芽無線傳輸功能將波形傳輸至具有藍芽接收功能的 PC 上並安裝 iHeart nono PC-Suite 軟體後，設定好 COM Port 後啟動接收功能即可收到心電圖波形及 3 軸加速計狀態。現階段試行只做到將心電圖及 3 軸加速計訊號以藍牙無線模組傳輸至個人端 PC，但在心電圖資料分析或者在姿態分析上還有待進一步研究。

P29

Functional Capacity Evaluations (FCE) on Persons with Low Back Pain and Healthy Controls by using Surface Electromyography (EMG)

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Introduction - Functional Capacity Evaluations (FCE) is widely applied for workers' compensation, insurance issues and case management decisions. In the United States, low back pain (LBP) workers are up to 27% of total populations. Even though FCE performance is an important factor of return to work, but "Pain" statuses are very hard to quantify. In recent years, many EMG-related studies on the paraspinal muscles reflect the importance of evaluation of muscular dysfunction in chronic low back pain. Hence, there are needs to find the relationship between pain states and EMG by using FCE.

Materials and methods - EMG data from ten volunteer subjects was collected from Tzu Chi university students and hospital staffs. The including criteria is based on the diagnosis of physician. The excluded criteria include unclear conscious, injured upper extremities, acute back pain or post surgery. Eight of ten subjects were identified as chronic and past history. The other two subjects are identified as normal.

Subjects were asked to execute FCE with randomly selected the lifting weights. The started weight is defined as 50% of maximum grasp power. Surface electrodes were placed bilaterally over the greatest convexity of the erector spine muscles at the L2/L3 and L4/L5 level with a fix distance at 5 cm. Biopac MP35 is used for EMG data acquisition with sampling rate at 1000 Hz. Both root-mean-square (RMS) voltage and spectra of short-term Fourier transformation (STFT) were investigated for the whole test period by the MATLAB software.

Results - By observing the spectrum of STFT, there are no distinct biomarkers that may classify LBP and normal control. It is promising to find that the slope of RMS can classify two groups with 75% sensitivity, 100% specificity, and 80% accuracy. Moreover, when the times of lifting are increased, the sensitivity is 100%, the specificity is 100%, and the accuracy is 100%.

Discussions and conclusions - For further analysis and method improvement, more data samples are necessary. Previous researches indicated RMS which is associated with subjects' motivation or cooperation to exert power, so more biomarkers are needed. In the future, fatigue index may apply as another factor for study.

P30

The Adventitia-derived Vasodilating Factor

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The adventitia (Adv) of the rat aorta has been shown to release yet to be determined substance(s) that can relax the medial smooth muscle cells. The aim of this study was to determine the nature of the adventitia-derived vasodilating factor (ADRF). The control (+Adv) aortic rings and those without adv (-Adv) of male Wistar Kyoto (WKY) rats were examined using both in vitro tissue bath and superfusion bioassay cascade techniques. Contractile responses to phenylephrine (Phe, 10^{-9} to 10^{-5} mol/L) and norepinephrine (NE, 10^{-9} to 10^{-5} mol/L) were significantly greater in aortic rings-Adv compared to aortic rings+Adv. These results are consistent with the reported findings by others. KCl (10-100 mmol/L)-induced aortic constrictions, however, were not different between aortic rings with or without Adv. The Krebs' solutions superfusing aortic rings resulting in aortic dilation were analyzed by GC-MS. The preliminary results indicated the presence of palmitic acid methyl ester (PAME) in the perfusates. The concentrations of PAME decreased drastically in aortic rings-Adv. Exogenous PAME in a concentration-dependent manner induced aortic relaxation. These results suggest that PAME is an ADRF.

P31

Effects of furosemide and water consumption on metandienone metabolites in human urine analyzed by GC/MS

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Metandienone is a synthetic anabolic androgenic steroid. There are at last five metandienone metabolites which we can analyze in human urine. Furosemide is a potent loop diuretic. Some athletes use diuretics to speed up the excretion of prohibited substances, so they may pass drug tests. The purpose of this study is to investigate the impacts of furosemide and/or large amount of water on the metabolism of metandienone.

Five healthy males were recruited to participate in four separate experiments. These experiments were: 1. the volunteers taking metandienone only; 2. the volunteers taking both metandienone and furosemide; 3. the volunteers taking metandienone and a large amount of water; and 4. the volunteers taking metandienone, furosemide, and a large amount of water. Urine was collected and analyzed with GC-MS. The analysis was focused on the concentration and the excreted amount of the three urine metabolites (AM3M2, AM3M4b and AM3M6) of metandienone at various time following its administration.

The results were that: 1. AM3M2 was found in the urine samples two hrs after taking 10 mg metandienone, 2. taking 10 mg metandienone and 40 mg furosemide effectively reduced the concentrations of the three metabolites in the early urine samples; however, the concentrations increased greatly at the 16th hr, especially for AM3M2, 3. taking 10 mg metandienone and 400 mL/hr water for 12 hrs could more effectively reduce concentrations in the early samples; however, concentrations increased 12 hrs after the intake, and 4. taking 10 mg metandienone, 40 mg furosemide, and 400 mL/hr water also effectively reduced the concentrations in the urine. Moreover, this combination can decrease the concentrations at the 16th hr.

In this experimentation, we found that taking furosemide could reduce the concentrations of the three metabolites in the urine and taking large amount of water appeared to be more effective in reducing the urine metabolite concentrations of metandienone.

P32

Neuroprotective Effects of Magnolol on Rotenone-induced Animal Model of Parkinson's Disease

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Magnolol, an active component extracted from *Magnolia officinalis*, has various pharmacological effects, including antioxidation, anti-inflammation, and antagonism of excitotoxicity induced by excitatory amino acids. In this study, we examined whether magnolol has the neuroprotective action against neuronal toxicity caused by rotenone, which acts as an inhibitor of mitochondrial complex I and has been used to induce an animal model of Parkinson's disease. To examine the effectiveness of magnolol in reversing the motor deficiency in mice, various doses of magnolol (1, 5 and 10 mg/kg) were administrated intraperitionially once before 30 minutes injection of rotenone, which was administrated into the dorsal third ventricle (D3V) to produce neuronal injury. Results showed that rotenone induced motor dysfunction, such as a strong increase in catalepsy, a decrease in locomotor activity and motor incoordination. Magnolol (5 and 10 mg/kg) prevented the mortality death induced by rotenone at higher dose (4.5μg). Magnolol (10 mg/kg) also improved the behavioral dysfunction induced by rotenone at 3.2μg. Furthermore, the reduction in methamphetamine (2.5 mg/kg) induced hyperlocomotor activity after rotenone exposure was reversed by pre-treatment of magnolol (10 mg/kg). Taken together our results indicate that magnolol is suggested to be a novel neuroprotective agent for PD treatment.

P33

The Interaction between SARS-Cov nucleocapsid and cellular pyruvate kinase Proteins

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Severe acute respiratory syndrome (SARS) is a respiratory illness caused by the infection of a novel coronavirus called SARS-CoV. SARS-CoV infects many organs, including lungs, liver, and immune cells. Therefore, infection with SARS-CoV could result in severe symptoms, including anemia. In our previous study, SARS-CoV nucleocapsid (NC) protein was found to interact with cellular pyruvate kinase (PK) protein Interaction between SARS-CoV NC protein and cellular PK protein was also demonstrated by IP-Western and confocal microscopy analyses. Furthermore, the PK activity was repressed by SARS-CoV NC protein in vitro. The effect of SARS-CoV NC protein on the cellular PK activity will be determined in the Vero cells. Our results suggest that SARS-CoV NC protein could interact with cellular PK protein and inhibit its activity. Reduction of cellular PK activity could cause anemia. Thus, our results suggest that anemia induced by SARS-CoV infection could be through NC protein.

P34

LMP1 Up-regulates Hypoxia Inducible Factor 1α (HIF1α) Gene Transcription through JAK/STATs Signaling Pathway

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Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric basic helix-loop-helix transcription factor composed of HIF-1α and HIF-1ß that is the central regulator of responses to hypoxia. The specific binding of HIF-1 to the hypoxia-responsive element (HRE) induces the transcription of genes that respond to hypoxic conditions, including vascular endothelial growth factor (VEGF). Our previous data reveal an elevated amount of HIF-1α transcript by LMP1 through C-terminal activation region 3 (CTAR3) in nasopharyngeal carcinoma (NPC) cell. As LMP1 activates JAK/STAT signaling pathway via CTAR3, HIF-1α expression was clearly suppressed using JAK3 specific inhibitor. In addition, we also showed a prolonged RNA stability of HIF-1α in NPC cells under LMP1 overexpression. Therefore, our preliminary results display a positive regulation of HIF-1α gene transcription by viral oncoprotein, LMP1.

P35

The role of Tenc1 during adipogenesis.

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Adipocyte function is crucial for the control of whole body energy homeostasis. Through used RNAi to screen mouse whole genomic kinase and phosphatase, we found the differentiation of NIH/3T3 cells were blocked when tensin-like C1 domain-containing phosphatase (Tenc1) was silenced. And the mRNA level of Tenc1 increase during adipogenesis. So we considered that Tenc1 was required for adipocyte differentiation in NIH/3T3 cells. Through previous researches, we knew that Tenc1 was a negative regulator of the Akt signal transduction pathway. So we used Tenc1 shRNA to knockdown NIH/3T3 cells that Akt gene is overexperssion. We found the inhibition of adipocyte differentiation is stronger. Hence Tenc1 could regulate adipogenesis via Akt activity.

P36

Interactions between HCV NS3 and cellular dNT-1 proteins

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Clinical evidence suggests that hepatitis C virus (HCV) is etiologically involved in liver cirrhosis, hepatic cancer, and B-cell lymphomas. In our previous study, HCV NS3 protease domain but not helicase domain was found to interact with dNT-1, cytosolic 5'(3')-deoxyribonucleotidase, in yeast two hybrid system. dNTs are present in most mammalian cells and have been involved in the regulation of intracellular dNTP pools by substrate cycles. Substrate cycles are relying on the interplay between a deoxynucleoside kinase and a nucleotidase, participating in the regulation of dNTP pools. Adenosine deaminase and phosphorylase, which could degrade deoxyribonucleoside, attain special importance in cells of the lymphoid system that are low in deoxyribonucleotidase activity, and, in their absence, dATP and dGTP specifically accumulate in B and T cells and cause diseases. In our previous study, interaction between exogenous HCV NS3 and dNT-1 proteins was also demonstrated by IP-Western and confocal microscopy analyses. When HCV subgenomic replicon cells were treated with IFN to get rid of HCV, the expression of dNT-1 was un-altered, but the activity of dNT-1 was enhanced. These results suggest that HCV would inhibit dNT-1 activity possibly through NS3 protein.To address this issue, cellular dNT-1 activity was determined after HCV NS3 gene was transiently transfected in the HuH7 cells. Indeed, cellular dNT-1 activity was repressed by HCV NS3 protein in this system. Our results suggest that the development of B cell lymphoma after HCV infection possible through inhibition of dNT-1 activity.

P37

Structural studies of the HCV sub-genomic replicons in lipid raft

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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 its structure is affected by the replicon-related genes are not clear. In this study, atomic force microscopy (AFM) and transmission electron microscopy (TEM) are used to characterize the HCV sub-genomic replicon structures. The membrane flotation assay and western blotting were used to separate membrane fraction and to identify fractions containing the HCV sub-genomic replicons, which were then visualized using AFM and TEM. Disk-like structures were observed by AFM from fraction 2 samples derived from HCV sub-genomic replicons but not those from parental Huh-7 cells. The same sample was also examined with TEM. In addition, lipid-raft related genes were knocked down in HCV replicons to see if the morphology is altered.

P38

Anti-proliferative and Co-cytotoxic Effects of *n*-Butylenephthalide on Danshen for Treatment of Hepatocellular Carcinoma Cells

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Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, and is the third highest cause of cancer-related mortality. Chinese herbal medicine is prevalently used to treat liver cancer, particularly in Taiwan. However, herbal therapy for cancer usually carry out controversial results. In some cases of treatment, it results in significant improvement, however, in other cases gives non-significant to severer condition. The reason for it remains unclear, though important. Ethanolic *extracts of* Danshen (*Salvia miltiorrhiza*) have been proved its anti-cancer role in many literatures, however, we found that treatment with low doses of *extracts* on hepatocarcinoma cell line HepG2 resulted in enhancing cellular proliferation, while cytotoxic effect was presence only up to higher doses. We suggested that the effect of enhancing cellular proliferation may cause higher growth rate of tumor cells in patient and thus limit dinical utilization of herbal medicine. In this study, the natural compound n-butylidenephthalide (BP), a component of derived from acetone extracts of Angelica sinensis, was used to evaluate their anti-proliferative and co-cytotoxic effects on Danshen (*Salvia miltiorrhiza*) against growth of HepG2 cells. Data showed that enhancement of cell proliferation caused by Danshen (*Salvia miltiorrhiza*) was neutralized by Bp, and co-cytotoxic effect was observed. Other strategies of decreasing proliferation and enhancing cytotoxic effects on herbal treatment against tumor cells are in progress. Our results supported that combination use of herbal medicine should give more efficacy and safety in cancer therapy.

P39

Association of p38/MAPK pathway on *Bacillus anthracis* lethal toxin suppressed erythrocytic differentiation

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Anthrax, a disease caused by *Bacillus anthracis* infection, usually coincides with hypoxic tissue damages, anemia and lethality; the mechanism associated with its high mortality is not yet clear. Anthrax lethal toxin (LT) is the major virulence factor produced by *Bacillus anthracis* that composed of two proteins: protective antigen (PA) and lethal factor (LF). PA binds to toxin receptors on the surfaces of target cells and allows entry of LF into the cytosol. LF is a zinc-dependent metalloprotease that cleaves the N-terminus of mitogen-activated protein kinase kinase (MKKs/MEKs), thus disrupt the mitogen-activated protein kinase (MAPK) pathways: the ERK (extracellular signal-regulated kinase), p38 and JNK (c-Jun N-terminal kinase). Since anemia and hypoxic tissue damages are involved in anthrax-mediated mortality, these observations prompt us to analyze whether LT could block erythroid differentiation. Base on our data, we found that LT pretreatments could block GTP induced erythrocytic differentiation in K562 cells. In this study, we characterized the signaling pathway of LT suppressed erythrocytic differentiation by Western blotting. Our data suggest that LT might suppress erythrocytic differentiation through blocking p38 pathway.

P40

Chromosomal Analysis of Patients with Autism Spectrum Disorders

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Autism spectrum disorders (ASDs) are general name of neurodevelopmental disorders with impairments in social interaction and communication, as well as abnormal behavior, includes autism, Rett's disorder, Childhood disintegrative disorder, Asperger's disorder and pervasive developmental disorder not otherwise specified(PDD-NOS). The prevalence of ASDs is estimated to be 6.0-6.5 in 1000, but the etiology is not clear yet. The heritability of ASDs are more than 64%, so genetic factor plays a major role in the etiology of this disease. ASDs are complex genetic diseases, and previous studies suggest ASDs displays a high degree of genetic heterogeneity. This makes ASDs research relative's and comparatively difficult. At present, linkage analysis and association study are useful methods to find out the candidate region involved and compare genetic difference between case and control. But both methods are limited to sample size to arrive significant result. Cytogenetic methods are the other choice to find candidate region on the chromosome of ASDs patients. In this study, we have performed chromosomal analysis by using the G-banding technique on 73 patients with ASDs. All analyze 20 cells in each case, and we identified 4 ASDs cases having chromosome structural abnormalities. Among them 3 cases only find an unusual cell in 20 cells, the chromosome structure of 19 other cells is all normal. Another one is more noteworthy: 11 out 20 cells were found to have deletion of 15q12. Whether the deletion is associated with ASDs needs further study. Our data provide a clear direction to find out the candidate gene of ASDs.

P41

Molecular genetic studies of sleep disorders in Taiwan

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Narcolepsy is a chronic neurological disorder in part of the brain that regulates sleep-wake cycle. It is a sleep disorder that is characterized by excessive daytime sleepiness, sudden loss of muscle control (cataplexy), hypnagogic hallucinations, and quick entrance into rapid eye movement (REM) sleep. Narcolepsy is a polygenic disease accompanied by environmental factors and the prevalence is approximately 0.02%-0.16% in adults. Many studies showed that narcolepsy is associated with HLA-DRB1*1501 and HLA-DQB1*0602, suggesting narcolepsy is an immunological disease as well. Tumor necrosis factor (TNF) is a multifunctional proinflammatory cytokine. Recently studies suggest that TNF gene is associated with narcolepsy. Some groups repeated that two SNPs, SNP C-857T in TNF promoter and rs1061622 in TNFR2, respectively, are associated with narcolepsy. We conducted a duplication study to see whether association is present in narcolepsy of Taiwan population. Subjects in our study were 111 EDS (excessive daytime sleepiness) patients and 200 normal controls. We found 4 SNPs in the TNF gene promoter region (including C-857T, C-863A, C-1031T) and rs1061622 in TNF receptor 2 gene. We found that there is a significant association between C-857T of TNF and Narcolepsy with Cataplexy, and rs1061622 of TNFR2 with EDS. So we concluded that there is a significant association between TNF, TNFR2 and narcolepsy in Taiwan.

P42

Identification of genes that regulate megakaryocytic differentiation

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The process of cell differentiation is regulated by the coordination of cell type-specific gene activation. Our previous studies indicated that anthrax lethal toxin (LT) could suppress TPA (12-O-tetrdecanoylphorbol-13 acetate) induced megakaryocytic differentiation in human erythroleukemia (HEL) cell line. Based on microarray data, BHLHB2, DACH1, LIMK1, RBP5, RIS1 genes were up-regulated after TPA treatments and down-regulated upon LT-pretreatments, so we hypothesized those genes may play important roles in normal and LT-suppressed megakaryocytic differentiation. Using flow cytometry, we found the differentiation abilities (specific surface marker expression and DNA polyploidy) of HEL cell were blocked when each of those genes was knockdown by shRNA. In this study, we have identified BHLHB2, DACH1, RBP5 genes play potential roles in megakaryocytic differentiation.

P43

Bovine Ephereral FeverVirus and it's Matrix Protein induce Caspase Activation through the Activation of Proapoptotic Signal by Linking Src to JNK

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The Bovine ephemeral fever virus (BEFV) belongs to the *Ephemerovirus* of *Rhabdoviridae* family. It causes the disease with the sudden onset of fever and lameness in bovine. Our previous results demonstrated that BEFV induces a caspase-dependent apoptosis, and, inhibition of Src and JNK releases this effect. The current work investigates the relationship between these signaling molecules in mediating BEFV-induced caspase-dependent apoptosis. BEFV induces Src, JNK phosphorylation and ICAD cleavage in a time- and dose-dependent manner. Inhibition of Src by SU6656 reduces JNK phosphorylation, caspase and ICAD cleavage simultaneously. Block JNK activation by SP600125 affects caspase and ICAD cleavage, but does not alter Src phosphorylation status. Therefore, BEFV activates a proapoptotic signal by linking Src to JNK. Ecotopic expression of BEFV matrix-GFP fusion protein resulted in the JNK phosphorylation, as well as caspase cleavage. The precise mechanisms by which how BEFV matrix protein affect apoptosis will be further elucidated.

P44

Genetic heterogeneity among *Vibrio vulnificus* strains isolated in Taiwan

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Vibrio vulnificus plays multiple roles when interacting with others. It is considered as a inhabitants of marine environment with ubiquitous distribution, yet it can cause severe systemic infection in fish and human. With molecular typing techniques and phylogenetic analysis, we are able to examine the evolutionary path led to this amazing diversity. Sequence heterogeneity was found in 11 functional genes, involved in virulence (*vvhA, vvpE*), stress response (*spot, relA*), metabolism (*trpD, purH*), outer membrane function (*viuB, vuuA, wciV, ompU, wza*), using SSCP analysis of PCR-amplified gene fragments. The human isolate YJ016 and tilapia isolate 86573B have identical SSCP pattern and gene sequence analyzed so far, suggesting a recent divergence of *V. vulnificus* into human and fish pathogens.

V. vulnificus strains have been separated into 3 genogroups using the multilocus sequence typing (MLST) analysis. Both human and fish pathogenic strains belong to MLST group 1, and MLST group 1 and group 2 were isolated from the marine environment in approximately equal amount. However, environmental strains demonstrated low virulence toward zebrafish, suggesting the presence of niche-specific adaption. Using the MLST tree as putative phylogenetic tree, we compared gene trees derived from each of the 7 functional genes (*ompN, ompR, vuiB, purH, trpD, ompU, wcvI*) among *V. vulnificus* strains isolated from various sources. Strain clustering patterns in *ompR, vvhA, trpD* gene trees revealed suspected niche-specific adaptation, and pattern in *ompU* may be the result of inter-MLST group recombination.

P45

Role of TRPA1 in Intermittent Hypoxia-induced Neuroplasticity of Pulmonary C Fibers

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Long-term exposure to intermittent hypoxia (IH), such as that occurring in association with obstructive sleep apnea, may generate systemic inflammation. Pulmonary C-fiber afferents, a major type of lung vagal sensory receptors, are known to be sensitive to chemical stimuli. Additionally, IH can cause increased release of various chemical mediators, which have been shown to be able to stimulate or sensitize PCFs and further eliciting respiratory reflexes. Transient receptor potential (TRP) A1 channels are cation channels found preferentially on nociceptive sensory neurons, including capsaicin-sensitive vagal pulmonary C fibers (PCFs), and are activated by various inflammatory mediators. In this study, we carried out using the single-fiber recording technique to determine the characteristics of PCF responses following IH challenge and to investigate the role of TRPA1 receptors in the afferent responses. Ten episodes (30 s of N_2 + 30 s of 21% O_2) of IH or room air (RA) were delivered via the respirator into the lungs, and afferent activity of PCFs was recorded in anesthetized, paralyzed, and artificially ventilated rats. In a separate group, we measured the sensitivity of PCFs to both mechanical (lung inflation) and chemical (capsaicin injection) stimuli before and after IH/RA challenge. We found that IH produced a stimulatory effect on PCFs. Indeed, stimulating PCFs with IH exhibited a long-term facilitation (LTF) of these afferents, a type of plasticity in which afferent activity persistently increases, even 45 min after termination of IH challenge. After IH challenge, the sensitivity of PCFs to capsaicin injection was markedly potentiated, whereas it failed to alter the afferent response to lung inflation. Pretreatment with HC-030031, a TRPA1 selective antagonist, attenuated the IH-induced stimulation, LTF, and hypersensitivity of PCFs. These results suggest that ten cycles of IH challenge evoked stimulation, LTF, and hypersensitivity of PCFs, all of which are mediated at least partly through activation of TRPA1 receptors.

P46

Inhibition of Somatosensory Evoked Potential by Epidural Motor Cortex Stimulation in Rats.

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Motor cortex stimulation（MCS）was a promising clinical technique used to treat neuropathic pain which is resistant to other therapeutics approaches. Recently, the studies in animal models showed that MCS elicited antinociceptive effect in behavior test. However, mechanisms of MCS-induced pain relief were still unclear. The present study examined the effect of MCS on the response of primary somatosensory cortex (SI). The electrical stimulations were applied on the contralateral forepaws of both sides. The somatosensory evoked potentials (SEP) of both hemispheres could be recorded in SI to monitor the change of cortical activity. After the stable baseline cortical activities were obtained, the motor cortex stimulation was applied on the motor cortex of forepaw area. The different parameters of MCS in intensity, frequency and duration of MCS were tested. After cession of MCS, the SEP were recorded every 30 minutes and compared to baseline. The SEPs of SI ipsilateral to MCS were decreased after stimulations with higher intensity, frequency or longer duration applied on the motor cortex. The inhibition in these experiments with different parameters was dose-dependent. However, the SEPs of SI contralateral to MCS were not changed obviously. These results suggest that the function of SI was reduced by MCS and provide an electrophysiological evidence for the effect of motor cortex stimulation in the animal model of pain control.

P47

The effect of context pre-exposure on the neuronal activities of amygdala and medial prefrontal cortex in rat

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The amygdala plays an important role in the learning and memory processes of emotion events. Previous studies have indicated that medial prefrontal cortex (mPFC) would modulate the activity of amygdala. It had been shown that there was neuronal connection between amygdala and mPFC in the anatomical studies. This study examined how the pre-exposure of context affected the neuronal activities in amygdala and mPFC. The micro-wire electrodes were chronically implanted in the amygdala and mPFC of rats. The neuronal activities of amygdala and mPFC in conscious Long-Evans rats were recorded extracellularly during inhibitory avoidance task (IA). The animals of pre-exposure group (PE) were exposed to the context of IA for five days before training. The animals of non-pre-exposure group (NPE) were exposed on different apparatus. On the sixth day, the rats received foot-shock (0.4 mA , 1s) in the dark side of apparatus. The rats were tested on the next day of foot shock training. A shorter retention latency was shown in rats of the PE group than the NPE group. This result indicated that pre-exposure of context induced latent inhibition effect. Ensemble unit activities were recorded in the amygdala and mPFC during the task in both groups. Multiple single-unit activities in mPFC and amygdala were recorded simultaneously during IA. Many of recorded units changed their activities after foot-shock training. The pre-exposure of context affected the change of unit activities in amygdala and mPFC during IA. These results suggested that amygdala and mPFC might involve in the latent inhibition.

P48

Different Effects of Feeding Cue and Fasting on Expression of Circadian-Clock Genes and Their Related Genes in the Rat Colon

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Recent studies have shown that the circadian-clock genes present not only in the central nervous system but also in numerous peripheral organs, such as the liver, kidney and heart. The gastrointestinal tract exhibits 24-hour rhythms in many physiological functions including gut motility, mucosal enzyme activities, mucosal transporters, and proliferation rates. It is conceivable that circadian-clock genes in the gastrointestinal tract were related to its physiological functions and rhythms. Previous studies find while food is available only in the light-on period murine circadian-clock genes expression in the liver becomes phase-shifting. The main goal in this study is to investigate whether colon had daily patterns of the circadian-clock genes expression and whether this pattern was influenced by feeding cue and fasting. Real-time quantitative polymerase chain reaction (qPCR) to examine the expression levels of *Per1, Per2, Per3, Cry1, Cry 2, Bmal1, Clock, CK1ε, Dbp, Rev-erb α, Rev-erb β, Id2, PGC1α* and *PGC1β* genes in the colon of male adult Spraque-Dawley rat was used in this study. We found that expression of *Per1, Per2, Per3, Cry1, Bmal1, CK1ε, Dbp, Rev-erb α, Rev-erb β, Id2, PGC1α* and *PGC1β* exhibited daily rhythm in the colon. Feeding cue rested the expression patterns of *Per1, Per2, Per3, Cry1, Bmal1, CK1ε, Dbp, Rev-erb α, Rev-erb β, Id2 , PGC1α* and *PGC1β* genes. Fasting did not alter this rhythmic pattern but increased these circadian-clock genes expressions. These results indicate that not only colonic circadian-clock genes and their related genes have the patterns of daily rhythm but also feeding cue is an important cue to reset them. Moreover, fasting affects the expression levels of circadian-clock genes and their related genes, but not the daily pattern of them in the rat colon.

P49

受虐婦女對於加害人處遇計畫過程之主觀經驗探討---以高雄市為例

The Experience of Abused Women to The Process of Marital Batterer's Intervention Program---Kaohsiung City as An Example

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家暴防治服務中，多數屬於消極的阻隔、禁制，加害人處遇計畫(後簡稱加處)為其中屬於較積極減少暴力之服務，但目前國內的現況則是加處之核發率偏低、加處效能未定等。此外本研究經由文獻整理發現國內外在進行加處的重要差異，即美(杜魯斯模式)、英(尊重方案)兩國加處的服務對象不僅限於加害人，更把受虐婦女拉進服務網絡中，執行加處之專業人員與服務受虐婦女之社工彼此間是聯繫、互換訊息的。但國內加處的服務模式則將受虐婦女與加害人視為兩條平行線，兩方的工作人員亦是。因而本研究將加處之過程分為前、中、後三個部份，欲藉由質性研究之方式，了解受虐婦女對於加處過程之想法，進而形塑納入受虐婦女思維之加害人處遇計畫，並提供實務面與政策面之建議。

研究結果發現，受虐婦女的婚暴歷程與加處之內容相關。而其與正式支持系統互動之經驗，在社政系統部分，受虐婦女與社工對於社工角色之期待有所落差；在警察系統部分，受訪者分別經歷了三種不同的警察化身，有嚇阻加害人暴力行為的、有勸阻被害人的通報的，亦有藉由與加害人之同事關係進行勸說的；在法院系統部分，受虐婦女們分別認為司法流程過於冗長、複雜。另外，受虐婦女對於加處之經驗，多為法官告知，其所持之期待與實際實施目的之間有所落差，加處對於暴力行為之影響有限，甚至有可能對於雙方關係有負面影響，因而受虐婦女認為應增加加處執行人員與其之互動頻率與內容，而其自身亦需要接受接受與家暴相關之教育團體，亦即加處之服務對象，必須含括加害人及受虐婦女。

依據受訪者之訪談結果亦發現，其對於加處之建議所描繪出的加處，與英國正在實施的尊重方案相當類似，建議如下：當受虐婦女進入家暴防治系統、或聲請保護令之際(即加處進行之前)，社工員應主動將加處之效應及可能正負面效益等告知案主；在加處進行中，應使加處執行人員與受虐婦女之聯繫保持暢通，同時，加處執行人員應主動告知受虐婦女該加害人在團體中之表現，以作為受虐婦女相關判斷之依據，而受虐婦女之主責社工亦需與加處執行人員針對該加害人與受虐婦女之狀況互相告知相關訊息；而在加處結束之後，仍應就受虐婦女部份追蹤六個月，以確認其是否還有相關需求未被滿足。

P50

TTC1138 from *Thermus thermophilus* HB8 and HB27 Involved in Biofilm Formation

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Bacteria exposed to changeable environments can elicit adaptive responses by triggering the differential expression of genes via two-component signal transduction systems. This study describes the *TTC1138* signal transduction systems in *Thermus thermophilus* HB27. *T. thermophilus* HB27 not only prefers to grow at 65 ℃ but also has ability to develop biofilm. The aim of the study is to identify the relationship between the *TTC1138* and biofilm formation in *T. thermophilus* HB27. In genetic approach, a *TTC1138* (response regulator) deletion mutant was constructed. The *TTC1138* gene was mutated by kanamycin resistant gene and the result was confirmed by western blot analysis and polymerase chain reaction. The quantity of biofilm was test by 96 well microplates that revealed TTC1138 mutants of *T. thermophilus* HB27 were inhibiting the quantity of biofilm formation compare to the wildtype. Interestingly, the results revealed that TTC1138 mutants of *Thermus thermophilus* HB8 were both prompting and inhibiting the quantity of biofilm formation compare to the wildtype. The relationship of strains and biofilm formation behavior will be studied in the future. In biochemical approach, the gene encoding TTC1138 in *E.coli* was overexpressed and purified by the Ni^{2+} affinity chromatography for antibody preparation and protein crystallization. In addition to identify the period of protein expression via the western blot analysis, we identified the difference of TTC1138 expression between the planktonic cell and sessile cells at 6, 12, 15 and 18 hours. The result revealed that TTC1138 expression in biofilm cell was more than in planktonic cell at 6, 12, 15, 18 hours. The differences of protein expressions profiles between sessile cells and planktonic cells of wildtype and *TTC1138* mutant will be studied by the two-dimension electrophoresis. Moreover, we will also find out what promoter was regulated by TTC1138 with modified chromatin immunoprecipitation assay.

P51

Proteomic change in *Vibrio parahaemolyticus lux*S mutant

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In both gram-negative and gram-positive bacteria, the LuxS quorum sensing system is reported to regulate a wide variety of cellular activities, such as flagellar motility, metabolic regulation, toxin production and pathogenicity. LuxS (S- ribosylhomocysteinelyase) is responsible for the production of quorums sensing molecular, autoinducer-2 (AI-2). In our previous study, a *luxS* mutant of *Vibrio parahaemolyticus* was constructed and checked by sequencing. We compared biofilm formation, swimming and swarming ability of mutant with those of wild-type strain. The results showed there are no significant differences. In this study, we aim to analyze changes in proteome of *luxS* mutant and wild type and identify proteins that are involved in LuxS quorum sensing systems. Several significant protein spots were observed on 2-DE gels of mutant and wild type, respectively. Those spots were collected and analyzed by MALDI-TOF-MS. The result showed that LuxS (S- ribosylhomocysteinase), GroEL (stress-shock protein), and VPA1023 (putative exonuclease) were expressed in wild-type strain, but not in mutant strain. However, a protein involving in amino acid biosynthesis, cysteine synthase A, was significant expressed in *luxS* mutant. In the future, we will exam the expression of those candidate proteins at RNA level by RT-PCR.

P52

Electronic Electrocardiogram (ECG) Data Recovery by Applying Image Processing on ECG Paper Charts

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Introduction - Medical imaging plays a very important role on bioinformatics. Most of imaging processing technologies is focused on the identification of the locations of diseases such as MRI, CT, PET, and SPECT to assist the physicians for medical diagnosis. However, fewer researches focus on signal recovery from old clinical paper charts to reconstruct electronic signals for helping biomedical engineers to develop computer aid diagnosis (CAD) tools. Unlike ECG paper chats, not all the hospital bioinformatics systems keep the original ECG data. Hence, there is the need to recover electronic ECG data by applying image processing technologies on ECG paper charts to be able to make further CAD analysis and modeling simulation possible. The aim of this research is to recognize ECG phantom, to recovery raw ECG signal, and to evaluate the performance of each imaging processing skills.

Methods - In this project, there are three methods for processing electrocardiogram chart images, mainly including 2D Fourier transform, Laplace transform, and color thresholds with interpolation. The first step is to remove the unnecessary grids and lines with different methods. Second, the missing pixels or dots are refilled and then the 2D images are converted to 1D signals. Third, the linear interpolation is applied to make sampling frequency at 500 sps. Finally, the performances are evaluated by percentage of root mean square (PRD) to calculate the waveform similarity.

Results- There are three examples from MIT/BIH sudden cardiac database for performance evaluation. The results are listed in the following table:

Methods Samples no.	PRD values by using RGB imaging processing	PRD values by using 2D Fourier Transform
1	43.94%	69.85%
2	62.66%	121.18%
3	24.7%	52.92%

Discussions – The system still has the room to improve. To compare with original raw data, the recovered signal with displacement condition makes PRD to increase. The RGB imaging processing method provided the better performances if the RGB colors in chart are kept.

P53

篩選腸炎弧菌啟動基因庫中與氧化壓力相關的基因

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腸炎弧菌是一株嗜鹽的格蘭氏陰性菌，生長於海水之中，亦可感染人類造成食物中毒。為了研究腸炎弧菌的氧化反應機制，本實驗利用 *Vibrio parahaemolyticus* 93 建構啟動子基因庫，並分析對雙氧水刺激有反應之啟動子。經酵素切割之腸炎弧菌基因體片段接入至 pSA19CP_*luxAB* 質體後，轉殖至大腸桿菌中，篩選出含嵌入基因片段的菌株後，進而測試雙氧水對菌株的冷光反應。另外將腸炎弧菌質體上的 VPA0453 基因啟動子片段接至 pSA19CP_*luxAB* 中，再轉殖至大腸桿菌，做為正控制組。此外，利用正控制組測試冷光表現的分析條件。正控制組培養 4.5 小時，分別加入長鏈醛類及雙氧水之後放置不同時間，觀察其冷光表現情形。由實驗結果得知，加入長鏈醛類之後在 2 分鐘時冷光表現情形最佳，而加入雙氧水之後則在 10 分鐘時冷光表現情形明顯上升。

P54

Characterization of *TTC1137* from *Thermus thermophilus* HB27

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In pathogenic bacterium, they always produce virulence factors to facilitate the infection to their host. During these pathogenicities, the tissue-degrading enzymes, hemolysin, is processed by *Staphylococcus aureus* and some *E. coli* strains to induce the lysis of erythrocyte. In our study, we find out the operon *TTC1134* to *TTC1139* of *Thermus thermophilus* HB27 shows a putative hemolysin possibility. This gene is called *TTC1137*. It shares a conserved domain with Hemolysin-III superfamily, which is origined from *Bacillus cereus.* In the study of *B. cereus*, researchers found that the Hly-III is a pore-forming hemolysin and showed at least three steps on interacting the erythrocyte. It gives rise to our curiosity why this non-pathogenic bacterium has a putative hemolysin gene and how did it be regulated. Here, we put our focus on the TTC1137 expression. We got a clone of dN*TTC1137*/pET30a(+)/BL21 and observed its protein expression. Somehow, we hypothesis it is also a pore-forming protein and it might be expressed on the bacterial membrane, as well as the inner membrane, outer membrane and the periplasm fraction. Following, membrane fractionations were performed to ensure the location of the TTC1137. Results showed that TTC1137 located in cytoplasmic and membrane fractions. In genetic approach, we use the homologous recombination to get the mutant of the *TTC1137*. In the future, we will use the *TTC1136*-kanamycin resistant gene-*TTC1138*/pGEM3Zf(-) to truncate the *TTC1137* of *T. thermophilus* HB27. Then, we will utilize the truncated *TTC1138* strain of *T. thermophilus* HB27 to observe the *TTC1137* mRNA transcription to figure out their relationship. Moreover, we will check the hemolysis activity of *Thermus thermophilus* HB27.

P55

Grouping of *Streptococcus agalactiae* (GBS) by Random Amplication Polymorphism DNA-Polymerase Chain Reaction (RAPD-PCR)

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Group B streptococcus (GBS) is a significant human pathogen, especially in newborns and pregnant women. The relationship between genotype and virulence factor has accentuated the importance of efficient and sensitive typing methods. The objective of this study was to determine if DNA polymorphisms generated by Random Amplification of Polymorphism DNA PCR (RAPD-PCR) could be utilized to characterize GBS for epidemiology purposes. A rapid and convenient method for detecting the DNA polymorphism of GBS was generated by PCR (RAPD-PCR) with a 10-mers primer, GBS-2. This primer was used in this assay to achieve the polymorphic amplicons based on their ability to differentiate GBS genotypes. Only a primer GBS-2 was generated in RAPD-PCR which showed DNA polymorphic patterns which were then analyzed in electrophoresis. The isolates were clustered into six major groups with their respective polymorphic patterns. The grouping patterns were then compared to previous report of the serotypic patterns to observe the correlation between the genotypes and serotypes. Intragroupic variations were also detected indicates the heterogenous nature of individual GBS strains. This assay also appears to be easier to perform than conventional serotyping methods. In conclusion, the RAPD-PCR is an useful assay for the characterization of GBS isolates as it is able to discriminate between the DNA polymorphism of GBS which correlates with virulence factors.

P56

Gene Regulation of Cold Shock Response and Transposon Mutant Library Construction of *Vibrio* spp.

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In this study, we mainly studied how *Vibrio* spp. faces the temperature variation and cold adaptation of *Vibrio* spp. at 4 ℃. We also studied if *Vibrio* spp. has cross protection dealing with different stresses after the cold shock. We found that YJ016 and 93U204 have no adaptation at 4 ℃ after treated with 16 ℃ for half hour in log phase or stationary phase. But the survival rate of Vp93 no matter with or without 16 ℃ pre-treatment are both 90%. Therefore, 16 ℃ pre-treatment did have effect for 4 ℃ survival in log phase. As for cross protection, Vp 93 has cross protection for 7% sodium chloride or 1 mM hydrogen peroxide treatment following 16 ℃ cold adaptation. But Vp 93 has no cross protection for pH3, 2.5 mM hydrogen peroxide, and 4 ℃ treatment following 16 ℃ cold adaptation. However, 14AVP11 has no cross protection for 1 mM hydrogen peroxide and pH3 treatment following 16 ℃ cold adaptations. We also cloned *csdA* of YJ016 and expressed this protein but have no extra band have been induced. Meanwhile, western blotting also showed no His-taq protein have been expressed. It seems that CsdA was not induced in *E. coli*. We will mutate the *csdA* to find out the relationship of *csdA* and cold tolerance. We also construct a transposon mutant library to observe the phenotype and screen for survival in 37 ℃ and in 4 ℃. We selected about three thousand colonies and choose about thirty mutants what we are interested.

P57

Physiological response of *Vibrio* spp.by acid treatment and role of *cadA* in *Vibrio parahaemolyticus* under acidic condition

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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 understand the mechanism of acid adaptation of *Vibrio* spp. in human gastrointestinal. 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. has higher resistance towards acidic environment after treated with acid adaptation in log phase compared to stationery phase. On the other hand, we are also interested to investigate the effect of cross protection as there are also other stresses from the environment. We found that *Vibrio parahaemolyticus* 93 does not show cross protection in high salt concentration (7% NaCl) environment after acid treatment (pH5). As for genetic approach, we chose to investigate *cadA* since it is an important acid resistance gene. In the low external pH, *cadA* gene will transcript CadA protein to induce the degradation of lysine by lysine decarboxylase. Cadaverine will be produce as a result of decarboxylation. Another resistance gene *cadB* which encodes lysine-cadaverine antiporter is responsible for the transportation of these substrates. We will clone *cadA* in *Escherichia coli* to observe the acid tolerance of the gene in the absence of CadB and mutate *cadA* gene to studt its role of acid resistance in *Vibrio* spp. On the other hand, we use Tn*10-kan-ori* to generate a mutant library. The main purpose of generate this mutant library was to discover acid resistance related genes.

P58

Response of *Vibrio vulnificus* to hyperosmotic stress

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Vibrio vulnificus is a Gram-negative halophilic bacterium ubiquitous in the seawater. It is also an opportunistic human or animal pathogen, and cause primary septicemia. Vibrios must face environmental changes, such as temperature shift, starvation, osmotic change and alternant pH. In this study we examine the response of *Vibrio vulnificus* to hyperosmotic stress. Hyperosmotic stress can reduce or inhibit the growth of *V. vulnificus*. Tolerance to hyperosmotic stress was affected by pre-treatment culture condition, and apparent inter-strain variation was observed. The tolerance limit for *V. vulnificus* is 6% NaCl (equivalent to 9.227 ~ 9.916 Osm/kg), however a pretreatment of culturing in 4.5% NaCl TSB medium can shift the limit to 9% NaCl (10.606 Osm/ kg).

Electron microscopic examination revealed morphological adaptation of *V. vulnificus*. Bacterial cells became elongated under hyperosmotic stress, and the average cell length of biotype 2 strains is longer than biotype 1 strains tested. Expression of osmotic adaptation-related genes *ompR* and *envZ*, cell division-related gene *zipA* and a RpoS-regulated gene *bolA* were followed, and the patterns are generally similar to other bacterial species.

P59

Characterization of *luxS* in *Thermus thermophilus* HB27 involved in biofilm formation

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So far the scientists studied in thermophilic bacteria in the molecular and physiological levels for a long time. We are interested in biofilm formation of *Thermus thermophilus* HB27. Biofilm formation which could help bacteria to defend the toxins in environment is produced by bacterial secreting polysaccharide. In previous study, quorum sensing is an important factor in biofilm formation. Quorum sensing (QS) is a bacterial communication system that controls the expression of multiple genes in response to population density. The LuxS related quorum sensing system regulates the expression of several virulence factors in a variety of pathogens. The goal of this study is to find out the relationship between *luxS* and biofilm formation. *Vibrio harveyi* MM32 carries the receptor of AI-2 and is a *luxS* mutant. The feature of the reportor strain is useful to measure the luciferase activity in the condition medium of *Thermus thermophilus* HB27. Result showed that the luciferase activity in the condition medium which is from *Thermus thermophilus* HB27 incubate after 6 hours reveals that 60 folds more activity than negative control. After *luxS* junction genes were amplified by polymerase chain reaction, the products are cloned in *kan*^{r}/pGEM3zf(-) for the mutant construction. We would like to know the different protein expression between *Thermus thermophilus* HB27 and *luxS* mutant by two-dimension electrophoresis. Further we could ensure the relationship between *luxS* and biofilm formation and define the luxS regulon in *Thermus thermophilus* HB27.

P60

Characterization of three translocon components, Tic40, Tic55 and Tic62, located in the inner envelope membrane of chloroplasts

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The majority of proteins therefore have to be imported post-translationally from the cytoplasm, which is most generally performed via two translocation machineries present in the outer and inner envelope membrane, called Toc (translocon at the outer envelope of chloroplasts) and Tic (translocon at the inner envelope of chloroplasts) complexes, respectively. The main function of both complexes is to provide a protein-selective channel through the envelope membrane and to exert the necessary driving force for the translocation. In previous works, our molecular and biochemical data support that Tic40 functions as a co-chaperone in the stromal chaperone complex that facilitates protein translocation across the inner membrane. To identify new component associated with Tic40, we use co-immunoprecipitation, proteomic tools and further confirmed by LC/MS mass spectrometry analysis. Our data show that another inner membrane protein, pTAC4 (plastid transcriptionally active chromosome protein 4), physically associated with Tic40. The function of pTAC gene family may require for plastid gene expression so far. Through yeast two-hybrid analysis, Tic40 and Tic55 can be interact through its TPR domain. Genetic and phenotypic analysis of the T-DNA-tagged *tic55* mutants in *Arabidopsis thaliana*, knockout mutant plants were indistinguishable from wild type. Because Tic55 and Tic62 involved in Redox regulation play a prominent role in the chloroplast metabolism, the truncated Tic55 protein with His-tag and truncated Tic62 protein with GST-tag were expressed in *E.coli* and purified from the soluble phase on a nickel column and glutathione-conjugated Sepharose for specific antibody against Tic55 and Tic62. We will further study the functional significance of these interactions among Tic40, Tic55 and pTAC4. Knowledge obtained from this study will help us understand the composition and functional mechanism of chloroplast protein import machinery and also advance our understanding of chloroplast biogenesis in general.

P61

Molecular cloning and functional analysis of genes regulating the floral formation in *Lilium formosanum* and *Lilium* Elite

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Mechanism of floral development is under the control of a complex genetic system. The ABCDE model for floral development was proposed and since then many studies have been performed on model species, such as *Arabidopsis thaliana*, *Antirrhinum majus*, and many other species in order to confirm this hypothesis. Floral formation involves the development of four whorls of organs, sepals, petals, stamens, and pistils, that are specified by a set of major flower organ identity genes that include *APETALA1* (*AP1*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), *AGAMOUS* (*AG*) and several *AGAMOUS- LIKE* (*AGL*) genes. Base on the sequence and functional similarity, the MADS box genes play a central role involved in floral development. Lily is a monocot related to the lily family (Liliaceae). It is one of the most popular horticultural plant species in Taiwan, but little research on MADS-box genes regulating the process of floral formation in *Lilium formosanum* and *Lilium* Elite. Here we isolate many MADS-box genes from Formosan lily and *Lilium* Elite, a strategy combining RT-PCR with degenerate primer and 5'-RACE or 3'-RACE was used. The deduced amino acid sequence show that full-length *LFAG1* and *LEAG1* gene revealed the MIKC structure and a high homology in the C-function genes among AG and other orthologues. Partial sequence of *LFGLO1* gene is homologus to B-function gene *GLO1*by blast analysis. In addition, *LFAGL2* and *LFSEP* genes showed sequence homology to E-function gene individually. Spatial expression data showed *LFAG1* and *LEAG1* transcripts exclusively in floral organ by RT-PCR. Functional analysis was carried out in Arabidopsis by overexpression of *LFAG1* and *LEAG1*driven by the CaMV35S promoter. Phenotypic and expression analysis in these transformed plants will be discussed. Further study the functional significance of these interactions among MADS-box genes during the stage of floral development genetically and biochemically. Knowledge obtained from this study will help us understanding of the function of lily MADS-box genes and those of orthologs from other plant species would contribute to the elucidation of molecular regulation during floral transition and floral formation.

P62

TChi-2 suppresses LPS-induced iNOS gene expression and NO release via reducing NF-κB activation in BV-2 microglia

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Microglia, resident innate immune cells in the CNS, is implicated in neurodegenerative diseases. Overproduction of inflammatory factors, such as tumor necrosis factor-α, superoxide or nitric oxide (NO), by activated microglia causes neuronal death in the CNS. In the present study, we investigated whether and how TChi-2, a compound extracted from a Chinese herb (*Scutellaria Baicalensis*), would inhibit the release of inflammatory factors from the activated microglia. Lipopolysaccharide (LPS), a component of Gram-negative bacteria, concentration-dependently induced NO production in BV-2 microglia (EC_{50} = 107 ng/ml). Both purified TChi-2 extracts from the herb and chemically synthesized TChi-2 concentration-dependently inhibited LPS-induced NO production in BV-2 microglia with IC_{50} values of 34 μM and 38 μM, respectively. NF-κB, an important transcription factor of proinflammatory cytokines, mediates gene expression of inducible nitric oxide synthase (iNOS) which is responsible for the large increase in NO production following LPS challenge. Our preliminary results indicated that TChi-2 inhibited NF-κB activation and iNOS gene expression in 264.7 macrophages. Thus, we hypothesize that TChi-2 inhibits LPS-induced NO production in BV-2 microglia via blocking NF-κB and therefore iNOS gene expression. Results of this study suggest that TChi-2 is beneficial in alleviation of neuronal injury caused by LPS.

P63

Impact of cellulose supplement on the composition of wild rodents gut microbiota

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Bacteria colonizing animal large intestines have only access to dietary residues that evade digestion by host enzymes in the upper gut. Different amount of such 'non-digestible' carbohydrates in daily diet can influence the composition and metabolic rate in gut microbial community. Increased cellulose intake is expected to facilitate hydrogen production by polysaccharide degraders, and influence the balance among hydrogen-utilizing groups such as methanogens, acetogens and sulfate reducers.

We assessed impact of cellulose supplementation on microbial community composition, using both *in vivo* feeding experiment and *in vitro* feces-inoculated culture approaches, as monitored with DGGE profiling. The dominant fecal microbiota were identified as *Lachnospiraceae* and *Bacteroidales*. The 13 unique *Lachnospiraceae* sequences have only limited similarities to known taxa. Cellulose supplementation led to short-term fluctuation in community composition but returned to pre-treatment condition rapidly. Using specific *apsA* PCR-DGGE analysis, we monitored the dynamics of hydrogen-utilizing sulfate reducer population in mouse intestine. The composition of sulfate reducer population remained unchanged during the experiment period. The major *apsA* fragments amplified from fecal DNA are novel and have 92% similarity to both *Desulfovibrio piger* and *Desulfomonas pigra*. We used *in vitro* fermentor culture to study the effect of carbohydrate supplements on microbial composition. When carbon mixture (animal-derived mucin and plant-derived cellulose, xylan, pectin, starch) was added into the medium, certain members of the community were enriched within 6 hrs, but it took 48 hrs for cellulose-specific members to become dominant. This suggests the observed stability might be due to the slow appearance of cellulose-utilizer.

P64

The Effect of Mibefradil Infusion into The 3rd Ventricle on Contextual Fear Conditioning

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Voltage-dependent calcium channels (VDCC) are a group of voltage-gated ion channel which is very abundant in human. They are involved in muscle contraction, gene expression, hormonal secretion, neuronal excitation and etc. At resting membrane potential, VDCCs are usually closed. However, they will be activated (opened) at depolarized membrane potential. There are many different types of VDCCs: L-type, N-type, P/Q-type and R-type. Of all the VDCCs, the α_1H T-type Ca^{2+} channel ($Ca_v3.2$) is highly expressed on the hippocampus. In addition, the hippocampus plays an important role in contextual learning and spatial memory, thus we infer that α_1H T-type Ca^{2+} channel is crucial for contextual learning and spatial memory formation. From previous studies, we understand that $\alpha_1H^{-/-}$ mice are impaired in contextual fear learning, step-down and step-through tasks. Besides, wild-type mice infused with T-type Ca^{2+} channel blockers (mibefradil and ethosuximide) locally at the hippocampus displayed deficient in contextual fear learning. It is well known that the process of learning and memory formation can be divided into a few stages, which is information learning (acquisition) and information storing (consolidation). Information is later being recalled (retrieved) from present existing memory. From our previous studies, we have proved that α_1H T-type Ca^{2+} channel is not required for memory acquisition. However, it remains unclear whether consolidation or retrieval of memory is dependent on α_1H T-type Ca^{2+} channel. In order to investigate whether consolidation or retrieval of memory is dependent on α_1H T-type Ca^{2+} channel, we infused mibefradil into the 3rd ventricle near the hippocampus of wild-type mice to knock down the expression of T-type Ca^{2+} channel and performed contextual fear learning task on the mice. Our preliminary data showed that memory retrieval is not dependent on α_1H T-type Ca^{2+} channel. My future work should focus on the role of α_1H T-type Ca^{2+} channel in memory consolidation.

P65

如何以少量突變小鼠繁殖出足以進行實驗所需的群體：由三種不同品系所得到的經驗分析

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近年使用各式突變小鼠的研究日益增加，然各品系的總使用量低，研究者多需要自行繁殖出足以進行實驗所需的群體，常造成實驗動物設施飼育空間無法因應，此現象源於使用者無法在繁殖前估算出可信的種原數量及空間需求，故本研究藉三種不同品系突變小鼠 (抖抖鼠、DB、OB) 的計畫性繁殖及 (或) 育種，探討如何使用最少種原族群及有限飼育空間繁殖出合乎實驗需求的動物數量與規格。

本報告彙整三種不同品系突變小鼠的繁殖經驗，分析小鼠生理層面以及人為操作層面中，各種可能與生產效率相關的因子，在將配種條件盡量規格化的操作情形下，比較三次繁殖經驗中生產效率差異與各因子間的相關性，並歸納其結果，期望能供後人作為繁殖配種時之參考。

P66

Characterization of streptococci isolated from aquaculture species in Taiwan, with emphasis on *Streptococcus iniae*

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Infection of fish by streptocci has plagued the aquaculture industry for decades. The major *Streptoccus* species isolated in Taiwan are *S. iniae* and *S. agalactiae*, affecting both freshwater and saltwater species such as tilapia, barramundi and grouper. These two species can be specifically identified by PCR primers. Although *S. agalactiae* demonstrated more severe histological damages in tilapia, the prevalence of *S. iniae* seems to be higher. The host ranges of these two pathogens were compared.

We tested the resolution of 16S rRNA-23S rRNA intergenic spacer (ITS), enterobacterial repetitive intergenic consensus (ERIC) and repetitive extragenic palindromic (Rep)-PCR typing approach to separate fish streptococcal isolates, both ITS- and ERIC-PCR are sensitive enough to identify intraspecific diversity. Among the fish isolates of *S. iniae*, we identified at least 4 ITS patterns, and variation in their virulence to zebrafish were found among the pattern groups. Variation in known virulence genes were then examined using SSCP approach.

A recent increase in repeated streptococcal infection was found in barramundi farms in Kaohsiung area. The causative agent were found to be *S. iniae*, and they have identical ITS pattern. These isolates are highly virulent to zebrafish after intraperitoneal injection. We will further examine the phylogenetic relationship of these strains to other *S. iniae* isolates.

P67

Site-directed mutagenesis of HCV E1 protein

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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 three structural proteins: a capsid protein and two envelope glycoproteins, E1 and E2. E1 and E2 are thought to play pivotal roles at different steps of the HCV replicative cycle. There is now strong evidence that they are essential for host-cell entry, binding to receptor(s), inducing fusion with the host-cell membrane as well as in viral particle assembly.

E1 and E2 are type I transmembrane (TM) glycoproteins, with N-terminal ectodomains and a short C-terminal TM domain. These proteins interact with each other and assemble as noncovalent heterodimers. Like other viral envelope proteins involved in host-cell entry, HCV envelope proteins are thought to induce fusion between the viral envelope and a host-cell membrane. The HCV envelope glycoproteins E1 and E2 are thought to be class II fusion proteins because the putative fusion peptide is supposedly localized in an internal sequence linked by antiparallel β-sheets.

From our previous study (Tsai, 2008) that only E1 260 fragment can induce liposome fusion under low pH but not pH 7 environment while other E1 and E2 protein fragments can not. Thus, we propose that E1 has the ability to induce membrane fusion by pH-sensitive conformational change. We will use site-directed florescence labeling to test this hypothesis. The eight cysteines of E1 are changed to alanine to create a cysteine-null and eight single-cysteine mutants. Then fluorescence label are attached to the specific Cys for detection under different pH environment.

Chia-hao Tsai（2008）. Structural Characterization of HCV E1 and E2 Proteins. Master thesis from Tzu chi university

P68

Culture and genetic characterization of green microbial mat in a local alkalinic hydrothermal environment

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Extensive appearance of green microbial mat was discovered in local hydrothermal environment. The sampling site is a pond with continuous input of hot spring water, and the temperature fluctuating within 50 to 65°C and pH at 8.8. The mats were found either floating in water column or loosely attached to the bottom substrates. We were able to maintain the green mat in laboratory condition with proper illumination. Culturing efforts yield enrichments that are macroscopically either green, dark green or pink in color. The two green enrichments composed of green chains of cocci with auto-fluorescence, and are presumable considered phototroph. Result of 16S rRNA gene sequence analysis indicates that the enrichment was dominated by *Tepedimonas taiwanensis* and a novel sequence 91% similar to *Trupera radiovictrix*. However, none of these is known phototroph, so the major component in the enrichments are still remain unclear.

We performed 16S rRNA gene-based PCR-DGGE analysis to reveal the genetic composition of the microbial community. The floating mat and the attached mat have similar microbial compositions, which is distinct from that of the sediments. The communities are dominated by novel sequence 95% similar to uncultured thermophilic phototroph candidatus" Chloracidobacterium thermophilum", or sequence 95% similar to known *Chloroflexus* species. The sediment community is dominated by *Acinetobacter calcoaceticus* and *Thermus brockianus*.

P69

Traditional Chinese medicine influence after Listeria monocytogenes infect in mice

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Murine Listeria monocytogenes (LM) infection is an important model about cellular immunity research. We can study the relation between the immune system and Listeria for this model. However, Chinese medicine becomes more popular recently, therefore, we want to test whether Chinese herbal medicine can enhance the resistance to LM infection. In this experiment, we infect the mice with a dose that will kill more than 80% of them, and give the mice Chinese herbal Medicine orally. Survival rate is recorded daily until day 14 post-infection. So far, we have tested 20 Chinese herbal medicines, but find no one is consistently effective against LM in vivo.

P70

Biofilm Formation regulated by *TTHA1483* of *Thermus thermophilus* HB8

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Thermus thermophilus HB8 was isolated from Izu-Mine Hot Springs. It is viable at 65 ～ 85 ℃. *T. thermophilus* HB8 has ability to form biofilm. The proteomic differences between biofilm cells and planktonic cells were observed in the 2-DE, and one of them is the *TTHA1483* encoding protein. The aim of this experiment is to characterize the property of *TTHA1483* from *T. thermophilus* HB8 involved in biofilm formation. *T. thermophilus* HB8 expressed more abundant of TTHA1483 of in biofilm cells than in plaktonic cells at eighteenth hours by western blot analysis. Therefore, we would construct *TTHA1483* mutant to study the effect toward biofilm formation. The gene *TTHA1482* and *TTHA1484*, which near by *TTHA1483*, were cloned to upstream and downstream of *kan*^{r}/pGEM-3Z, respectively.*TTHA1483* mutant would be generated by nature transformation by using previous plasmid. In biochemical approach, according to the data base of NCBI, function of TTHA1483 might be phosphoesterase. Phosphodiesterase, which is one of phosphoesterases, is related to biofilm formation formation in *Pseudomonas aeruginasa*. TTHA1483 will be purified to identify whether phosphoesterase related to biofilm formation.

P71

社工系學生死亡恐懼之研究

A Study on Fear of Death among Social Work Students

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本研究旨在瞭解社工系學生不同背景因素與修習死亡學相關課程狀況，於死亡恐懼上之差異性。透過文獻探討以及基於社會工作專業關係為助人者與案主或案家的雙向互動過程，本研究自擬以恐懼自己的瀕死與死亡、恐懼他人的瀕死與死亡兩面向為主之死亡恐懼量表問卷，作為研究工具，並自九十六學年度第一學期國內大專院校社會工作學系，其校內或系上於九十五全學年度、九十六學年度第一學期死亡學相關課程開設的情形為「校內及系上均開設」、「僅系上開設」和「僅校內開設」的大三與大四學生抽樣進行問卷調查，共取得 545 位有效樣本，以獨立樣本 t 考驗、單因子變異數分析、Scheffe 事後比較法，和皮爾遜積差相關等統計方法進行分析。研究發現，摘要如下：

一、社工系學生的死亡恐懼呈現稍有恐懼的情形。
二、社工系學生修習死亡學相關課程的情形，以尚在考慮的學生最多(62.2%)，其次為不考慮修習(20.6%)，已經修習過(13.9%)和正在修習(3.3%)的學生則佔少數。
三、社工系學生的死亡恐懼，會因性別、宗教虔誠度、接觸瀕死與死亡之經驗、對瀕死與死亡議題的興趣，以及自殺意念等個人背景變項的不同而有顯著差異性。
四、社工系學生的死亡恐懼，會因家庭中討論瀕死與死亡情形、家庭中討論瀕死與死亡時個人的自身感受、朋友間討論瀕死與死亡時個人的自身感受、閱讀瀕死與死亡的書籍文章等環境背景變項的不同而有顯著差異性。
五、社工系學生的死亡恐懼，不因有無修習死亡學課程而有所顯著差異性。

關鍵字：社工系學生、死亡恐懼、死亡學

P72

課後方案對花蓮弱勢家庭青少年自我效能之影響

The Effect of After-School Program to Disadvantaged Family's Adolescent in Hualien

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在課後方案評鑑的過程中，常看到許多描述統計量表述說著方案輸送的成效，指標往往以滿意度、觀察記錄等作為服務效果的證據，卻無法進一步說明案主內在動力在參與方案後有何改變。在檢視相關研究後，發現課後方案對於參與者自我效能提升有良好的成效，特別是針對低收入戶等弱勢家庭子女。

本研究採用量化研究方式，針對花蓮地區承接內政部兒童局補助「96 年社區照顧與弱勢家庭外展服務方案」及「兒童少年社區照顧輔導支持系統計畫」之機構，其方案內服務案主之學區進行調查研究，希望藉此瞭解課後方案對於弱勢家庭青少年之自我效能有何影響，研究探究的焦點在於參與者之人口學變項影響、參與因素、參與情況以及中介變項自我調節，對其自我效能的影響。

研究結果發現，隨著年級越大，青少年的自我效能越低，顯示課後方案的服務應盡早介入弱勢家庭，並且應提供不同的方案內容以因應不同年級參與者的需求。而參與動機是最重要的影響因素，參與動機越高則自我效能越好，其中同儕吸引力是重要的因子，如何藉由同儕關係提高參與動機是重要的策略。最後，參與者投入程度越高、參與時間越長者，其自我效能越好，因此如何辦理更多元、適切性高的方案以提升參與者投入程度與參與時間，也是重要的策略之一。

P73

The superinfection immunity of filamentous bacteriophage cf

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Bacteriophage cf is a single-stranded DNA phage that infects bacterium *Xanthomonas campestris* pv. *citri*. It has been shown to establish a stable lysogenic state with its genome integrated into the host chromosome. Unlike the Ff phages that contain ten putative genes, the genome of cf (7.3 kb) encodes an additional PT gene. The PT gene is expressed from the negative strand of the phage replicative form and has been reported to responsible for the determination of plaque turbidity.

To understand the immunity determinants of cf, we applied the DNA shuffling technique to mutate the cf PT gene for isolation of possible cf immunity mutants. After the superinfection screening, an immunity mutant cf-LS was obtained with a superinfection ability 10^4-fold greater than the wild type cf.

Two putative immunity determinants have been proposed in accordance with the previous study by Cheng et al. (1999). This report suggests that both RNA-RNA interaction and repressor protein inhibition are involved in the regulatory mechanism of cf immunity. In this study, we further focused on the generation of a small double-strand RNA of 64 nucleotides and on a hypothetical repressor protein from a 165 open-reading-frame, the potential product of the PT gene.

Memo