慈 濟 大 學

97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：97 年 5 月
研究成果發表地點：慈濟大學第一、二教學研討室

研究生暨大學生
看板論文展覽日期：97 年 5 月 12 日~97 年 5 月 16 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 97 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
5/7 (三)	12:30~ 12:35	林榮寵 (總)	TCIRP 95002	Hepatitis C Virus: Molecular Pathogenesis, Cellular and Immune Responses, and Antiviral Therapy	第二教學研討室	微生物學科
	12:35~ 12:50	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
	12:50~ 13:05	張新侯	TCIRP 95002-02	Characterizations of the association among viral hepatitis, anti-platelet autoantibody and thrombocytopenia		分子生物及細胞生物研究所
	13:05~ 13:20	張銘一	TCIRP 95002-03	Hepatitis C virus and Sjögren's syndrome: linking infection and autoimmunity		免疫學科
	13:20~ 13:35	曾英傑	TCIRP 95002-04	Construction of *in vivo* NS5A/NS5B-expressing systems for biological effect study and anti-HCV drug assay		分子生物及細胞生物研究所
	13:35~ 13:50	林榮寵	TCIRP 95002-05	Develop and Search for Antiviral Compounds Against Hepatitis C Virus: Study the Mode of Action		微生物學科
5/8 (四)	12:30~ 12:35	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
	12:35~ 12:50	劉哲文	TCIRP 96004-01	Atomic Force Microscopy of Hepatitis C Virus Proteins		生化學科
	12:50~ 13:05	李惠春	TCIRP 96004-02	Spectroscopic studies of structural proteins of HCV		生化學科
	13:05~ 13:20	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
	13:20~ 13:35	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
	13:35~ 13:50	羅時燕	TCIRP 96004-05	Study on the morphogenesis of hepatitis C virus		醫學檢驗生物技術學系
5/9 (五)	12:30~ 12:35	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
	12:35~ 12:50	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

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/9 (五)	12:50~ 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~ 13:20	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 (一)	12:30~ 12:35	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)
	12:35~ 12:50	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)
	12:50~ 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~ 13:20	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
	13:20~ 13:35	蔡勝國	TCIRP 95007-04	Molecular mechanism of inflammation and thrombosis involved in adriamycin induced cardiomyopathy		麻醉科--慈院(新店)
	13:35~ 13:50	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)
5/13 (二)	12:30~ 12:35	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	整合生理暨臨床科學研究所
	12:35~ 12:50	石明煌	TCIRP 95004-01	細胞色素 P450 與間歇性低氧之交互關係研究		麻醉科--慈院
	12:50~ 13:05	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		整合生理暨臨床科學研究所
	13:05~ 13:20	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
	13:20~ 13:35	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

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
	13:35~13:50	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
5/14(三)	12:30~12:35	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
	12:35~12:50	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
	12:50~13:05	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
	13:05~13:20	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		慈院內科
	13:20~13:35	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
5/15(四)	12:30~12:35	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
	12:35~12:50	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
	12:50~13:05	何翰蓁	TCIRP 95003-02	Ultrastructural studies on plasmalemma, organelles, and cytoskeleton involved in the compression-induced dendritic plasticity		解剖學科
	13:05~13:20	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
	13:20~13:35	劉培新	TCIRP 95003-04	An investigation of the compression-induced plasticity of cortical receiving neurons and thalamocortical inputs		解剖學科
5/16(五)	13:40~13:45	張景媛(總)	TCIRP 96001	正向心理的發展與實踐：科際整合研究	第一教學研討室	教育研究所
	13:45~14:00	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
	14:00~14:15	張景媛	TCIRP 96001-02	問題導向服務學習對師培生正向心理的影響		教育研究所
	14:15~14:30	陳畹蘭	TCIRP 96001-03	正向情緒在認知、壓力後的生理復原、以及適應力所扮演的角色		人類發展學系
	14:30~14:45	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
5/16 (五)	15:00~ 15:05	許木柱 (總)	TCIRP 95001	印尼紅溪河整治效應之科際整合研究	第一教學研討室	人類發展研究所
	15:05~ 15:20	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
	15:20~ 15:35	盧蕙馨	TCIRP 95001-02	雅加達大愛村的宗教會遇經驗		宗教與文化研究所
	15:35~ 15:50	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
	15:50~ 16:05	郭登聰	TCIRP 95001-04	社會福利需求		社會工作學系
	16:05~ 16:20	許木柱	TCIRP 95001-05	族群關係與文化發展		人類發展研究所
5/19 (一)	12:30~ 12:35	李哲夫 (總)	TCIRP 95005	Nicotinic acetylcholine receptor and neurovascular function	第二教學研討室	藥理暨毒理學研究所
	12:35~ 12:50	李哲夫	TCIRP 95005-01	Sympathetic nAChR and cerebral nitrergic neurogenic vasodilation		藥理暨毒理學研究所
	12:50~ 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		藥理暨毒理學研究所
	13:05~ 13:20	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
	13:20~ 13:35	許婷婷	TCIRP 95005-04	Effects of nAChR, Aβ and statins on glia cell function		免疫學科
5/20 (二)	12:30~ 12:35	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
	12:35~ 12:50	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
	12:50~ 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~ 13:20	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

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/21 (三)	12:30~ 12:35	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	微免暨分子醫學研究所
	12:35~ 12:50	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
	12:50~ 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
	13:05~ 13:20	李茹萍	TCIRP 95009-03	Development and application of *Helicobacter pylori*-infected Rat Model		護理學系
	13:20~ 13:35	林念璁	TCIRP 95009-04	Effects of *Helicobacter pylori* infection on mucin expression in gastric tissues of aborigines in Taiwan		微免暨分子醫學研究所
	13:35~ 13:50	伍超群	TCIRP 95009-05	The association between *Helicobacter pylori* and specific antigen express in gastric cancer		外科--慈院
5/22 (四)	12:30~ 12:35	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
	12:35~ 12:50	徐雪瑩	TCIRP 96005-01	Investigation of molecular mechanism on anti-tumor effect of *Momordica charantia*		生命科學系
	12:50~ 13:05	施玟玲	TCIRP 96005-02	Studies on Inhibition of Hepatitis B Virus Replication by *Momordica charantia*		生命科學系
	13:05~ 13:20	李政偉	TCIRP 96005-03	The Screening and Functional Study of Anti-HCV Infection Activity of Effective Integrants from *Momordica charantia*		生命科學系
	13:20~ 13:35	葉日弋	TCIRP 96005-04	A study on the antigluconeogenesis activity of *Momordica charantia*		人類遺傳研究所
	13:35~ 13:50	鄭靜明	TCIRP 96005-05	Isolation and characterization of terpenoid synthases and ribosome inactivating proteins from *Momordica charantia*		生命科學系
5/23 (五)	12:30~ 12:35	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
	12:35~ 12:50	陳俊堯	TCIRP 96003-01	Physiological Adaptation and Gene Regulation of *Vibrio* spp. to Chemical and Nutritional Changes		生命科學系
	12:50~ 13:05	林玲君	TCIRP 96003-02	Physiological Adaptation and Gene egulation of *Vibrio* spp. to Oxidative Stress and Oxygen Deprivation		微生物學科
	13:05~ 13:20	余美萱	TCIRP 96003-03	Physiological Adaptation and Gene Regulation of *Vibrio* spp. to Temperature		微生物學科
	13:20~ 13:35	林光慧	TCIRP 96003-04	Physiological Adaptation and Gene Regulation of *Vibrio* spp. to pH Fluctuation		微生物學科

【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
5/14 (三)	14:00~ 14:20	馬玉琴	TCMRC-P-95002	Effects of picture book group on the schizophrenia patients emotional intelligence	第二教學研討室	護理學系

【教師研究成果發表：個人型暨96年研究成果獎頒獎】

日期	時間	主持人	計畫編號	總計畫名稱	場地	所屬單位
5/16 (五)	10:00~ 10:20	96 年研究成果獎頒獎			第一教學研討室	全校
	10:20~ 10:40	張景媛	TCMRC 94003	時間的河慢慢流～慈濟小學統整課程的發展與生命教育的實踐		教育研究所
	10:40~ 11:00	鄭嫥嫥	TCMRC 94004	The Effect of Conflict Management on Marital Satisfaction: A study on Southern- Asia Brides in Taiwan		傳播學系
	11:00~ 11:20	程諾蘭	TCMRC 94019	佛教慈悲觀的理論基礎、特質及其倫理意義研究		通識教育中心
	11:20~ 11:40	周典芳	TCMRC 94026	素食推廣之有效傳播模式探究		傳播學系
	11:40~ 12:00	潘靖瑛	TCMRC 95006	應用「合作式自律學習法」於大學英文閱讀課程之成效研究		教育研究所
	13:40~ 14:00	劉培新	TCMRC 94017	The correlates of intra - and extra - cellular environmental alterations of facial motoneurons to functional recovery of reconnected facial nerve	第二教學研討室	解剖學科
	14:00~ 14:20	張銘一	TCMRC 94028	The trilogy of HCMV infection and autoimmunity		免疫學科
	14:20~ 14:40	莊育裡	TCMRC 94031	Analysis for the role of *Saccharomyces cerevisiae* B-type cyclins in cytokinesis		微生物學科
	15:00~ 15:20	胡正恆	TCMRC-P-95001	平埔祖先的獵鹿文化變遷與臺中盆地古代鹿群 mtDNA 之親緣研究		人類發展學系
	15:40~ 16:00	鄭靜明	TCMRC-P-95003	Metabolic engineering of terpenoid synthases, ribosome-inactivating proteins and p-insulin from *Cucurbita* spp		生命科學系
	16:00~ 16:20	蘇淑惠	TCMRC-P-95004	The study in the apoptosis control of human neutrophils by endogenous nitric oxide		醫學研究所
	16:20~ 16:40	王士廉	TCMRC-P-95005	Characterization of $NK1.1^{+}CD11c^{+}$ cells in murine *Listeria monocytogenes* infection		免疫學科

【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
5/16 (五)	08:30~ 09:05	簡位先	Identification of DNA copy-number aberrations by array-comparative genomic hybridization in patients with Autism	第二教學研討室	陳嘉祥 方菊雄
	09:05~ 09:40	謝維恭	Ethanol Phosporylation of NMDA NR1 and NR2B Subunits in Rat Sympathetic Preganglionic Neurons : Involvement in Acute Tolerance to Ethanol Inhibition of NMDA Receptor Function	第二教學研討室	賴志嘉 邱鐵雄 劉朝榮
	09:40~ 10:15	邱勝軍	In vitro studies of isochaihulactone in human prostate cancer LNCaP cells	第二教學研討室	馮清榮 王文柄 林欣榮
	10:30~ 11:05	陳穎信	Amiodarone Inhibits Epithelial to Mesenchymal Transformation and Causes Cardiac Valve Defect During Zebrafish Embryogenesis	第二教學研討室	胡勝川 蔡懷楨 韓鴻志 林欣榮 鄭景仁
	11:05~ 11:40	劉大瑋	FGF10 signaling controls the intestinal cell differentiation in zebrafish	第二教學研討室	王文柄 黃銓珍 翁慶豐 陳曜鴻 劉蕙雯
	11:40~ 12:15	黃欣儀	Molecular Mechanisms Underlying Urocortin-Induced Anti-proliferation In Neural Stem Cells	第二教學研討室	郭重雄 王文柄 王美人

96年度學術研究成果獎　得獎名單

序號	學院	單位	得獎人	職級	獎項
1	人社院	人類發展學系	陳堯峰	助理教授	論文獎
2	人社院	人類發展學系	胡正恆	助理教授	論文獎
3	人社院	英美語文學系	古添洪	教授	論文獎
4	人社院	英美語文學系	張堯欽	助理教授	論文獎
5	生科院	人類遺傳學研究所	孫德珊	助理教授	論文獎
6	生科院	分子生物及細胞生物研究所	張新侯	副教授	論文獎
7	生科院	生命科學系	彭致文	副教授	論文獎
8	生科院	生命科學系	施玟玲	副教授	論文獎
9	生科院	生命科學系	李政偉	助理教授	論文獎
10	生科院	生命科學系	劉嘉卿	助理教授	論文獎
11	生科院	神經科學研究所	李哲夫	教授	論文獎
12	生科院	神經科學研究所	謝坤叡	副教授	論文獎
13	教傳院	兒童發展與家庭教育學系	李雪菱	講師	論文獎
14	醫學院	公共衛生學系	朱正一	副教授	論文獎
15	醫學院	公共衛生學系	溫淑惠	助理教授	論文獎
16	醫學院	生化學科	林銘德	教授	論文獎
17	醫學院	原住民健康研究所	王豐裕	教授	論文獎
18	醫學院	解剖學科	何翰蓁	助理教授	論文獎
19	醫學院	整合生理暨臨床科學研究所	陳幸一	教授	論文獎
20	醫學院	醫學資訊系	劉瑞瓏	教授	論文獎
21	醫學院	醫學資訊系	陳信志	助理教授	論文獎
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33	醫學院	護理學系	李茹萍	副教授	論文獎
34	醫學院	護理學系	李麗蘭	講師	論文獎

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】

【博士生 看板論文摘要】

【研究生　看板論文摘要－醫學院】

【研究生　看板論文摘要－生命科學院】

【大學生　看板論文摘要】

O01

Hepatitis C Virus: Molecular Pathogenesis, Cellular and Immune Responses, and Antiviral Therapy

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Hepatitis C virus (HCV) infections are associated with acute and chronic liver diseases and hepatocellular carcinoma. To study the molecular and cellular mechanisms underlying the viral hepatitis, with the ultimate goal for developing therapeutic strategies to cure this liver diease highly prevalent in the Asian populations including Taiwan, we organize a Program Project focusing on the molecular pathogenesis, cellular immune responses, peptide-based vaccine development, antibody-targeted immunotherapy, and antiviral chemotherapy. To achieve these goals, we design a three-year Program Project, which represents an integrated effort involving five laboratories collaboratively working on a central theme. These five 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 five individuals combine their expertise as a task force working together on the daily basis.

O02

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.

The function of this cleaved peptide (a.a. 180-191) of HCV core protein is unknown. Using HLA peptide motif search, this cleaved peptide was found to bind HLA-A0201 specifically. Thus, we hypothesize that this peptide (homology with cytochrome P450 2A6 and 2A7) is responsible for the autoimmune hepatitis type 2. Relationship between this cleaved peptide and autoimmune response was studied using transgenic mice with HLA-A0201.

O03

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 severe thrombocytopenia patients from hospitals at Taiwan and found that a significantly higher percentage of thrombocytopenia observed in viral hepatitis patients compared to non-hepatitis patients. We found thrombocytopenia in viral hepatitis patients was specifically occurred during acute phase in associations with elevated aspartate aminotransferase and alanine aminotransferase (AST/ALT) levels and a higher anti-platelet titer. In addition, in our animal model, using chemicals such as chloroform to induce liver damages would induce thrombocytopenia and anti-platelet antibody. Our results indicate that thrombocytopenia and autoantibody might be part of the pathogenic mechanism to accelerate the hepatitis.

O04

Hepatitis C virus and Sjögren's syndrome: linking infection and autoimmunity

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Infection could break tolerance, aggravate or initiate autoimmunity through different mechanisms. Infection could not only induce chronic inflammation, but also activate immunological mechanisms and lead to autoreactivity. Genetics, on the other hand, also play a "ambiguous factor" in subsequent autoimmunity. Studies shown that HCV infected individuals frequently develop autoimmune-like symptoms, particularly Sjogren's syndrom, and hypotheses have been postulated. In this study we found that genetics may play a more critical role in the development of autoimmunity upon HCV infection than prior suggested. Two ethnic groups, Hakka and Min-nan, of HCV infected patients sera were collected in this study. Patients of both ethnic background developed equivalent humoral reactivity toward viral core antigen. Surprisingly, patients of Hakka background exhibited unequivocal elevated reactivity to viral NS5 antigen than Min-nan. Standard treatment of HCV infection involves cocktail of interferon-α/ribavirin, which elevated antibody responses to nuclear extract of Hela and Huh-7 cells by Hakka but not Min-nan patients. Regardless higher titers of autoantibody activity by ELISA, sera of Hakka patients showed less autoantibody activity to Hela extract than sera from Min-nan patients in immunoblot assays. This inconsistency, however, is insignificant if Huh-7 cell was used as immnoblot substrate. The clinical data revealed that Hakka patients often enjoy better outcomes following interferon-α/ribavirin treatment than Min-nan patents (82% vs. 55% recovery rate, respectively). In conclusion, HCV infection often induces autoactivity that share similarities to Sjogren's syndrome. This ethnicity specific autoreactivity, however, reflect the genetic complexity of immune responses upon infection and an edge in future tolerance study.

O05

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 Flaviridae family, is a positive-sense, single-stranded RNA virus with a genome size of ~9.4 kb. The gernome RNA encodes a polyprotein of 3,010 to 3,011 amino acid residues in the order of
NH2-C-E1-p7-NS2-NS-3-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/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 19 pups were identified to carry AB-NS5A transgene and among other set of 49 pups none were detected to be AB-NS5B carrier by using PCR analysis. The transgenic mouse lines of NS5A are under construction, while generation of NS5B transgenic founders is still developing. In case the NS5B transgenic mice will not be generated, a mouse RCAS-TVA system will be used as alternative strategy.

Once the mouse models will 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.

O06

Develop and Search for Antiviral Compounds Against Hepatitis C Virus: Study the Mode of Action

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Epstein-Barr virus (EBV)-encodes two small non-polyadenylated RNAs termed EBERs. EBER-1 and EBER-2 are strong protein binders and assume stem-loop structures analogous to hepatitis C virus (HCV) 5'-untranslated region (5'-UTR). Translation of HCV RNA is mediated by the interaction of ribosomes and cellular proteins with IRES located within the 5'-UTR. We investigated whether EBER-1, when introduced *in trans*, can bind to the cellular proteins and antagonize their binding to the viral IRES, thereby inhibiting HCV IRES-mediated translation. To make EBER-1 RNA *in vitro*, the *Eco*J fragment of EBV was digested by *Sau*3A resulting a fragment of 1.4 kb containing EBER-1, which was cloned into pGEM-3Z. For *in vitro* translation of HCV core, we subcloned an 830-bp *Hind*III-*Eco*RI DNA fragment into pGEM-4Z resulting the plasmid containing HCV 5'-UTR from nt 131 to 341 followed by the coding region of HCV core protein. Uncapped HCV core RNA transcripts were made and *in vitro* translation of core protein was carried out in the presence of various amount of EBER-1 RNA using rabbit reticulocyte lysate system. A dose-dependent inhibition of the expression of core protein by EBER was observed. Two expressing plasmids harboring one copy and ten tandem repeats of EBER were cloned into an expression vector (pcDNA3.1). The effects of these plasmids on cell proliferation of Huh 7, Huh 7.5, and Sg-PC1 were investigated. Our preliminary results indicated that EBER significantly retarded cell growth in cells harboring HCV replicon. These results provide a lead for developing therapeutic intervention of chronic HCV infection by targeting at the 5'UTR.

O07

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 several months, 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 4 and 5 are conducting the study on the NS3-interacting cellular proteins; Projects 2 and 4 are conducting the study on the fusion between HCV envelope proteins and cell membrane. Through this collaboration, we will understand more regarding structural information of HCV proteins.

O08

Atomic Force Microscopy of Hepatitis C Virus Proteins

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Hepatitis C virus (HCV), which causes severe liver disease, has become one of the major concerns in public health. Despite the seriousness of the impact to human 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), one of the most powerful research tools in nanotechnology, has become increasing important in biological and biomedical research recently. 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 show some of our approaches on applying the atomic force microscopy to the HCV protein studies. The first in the world direct visualisation of recombinant HCV core protein auto-assembled particles in various conditions, and the aggregation characterisation of different truncated virus envelope proteins using the AFM will be demonstrated.

O09

Spectroscopic studies of structural proteins of HCV

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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.

We have successfully expressed and purified E1 and E2 ectodomains. Circular dichroism analysis of these expressed proteins showed little pH induced secondary structural change. $E1_{260}$ can form oligomers and induce liposome fusion at acidic pH while $E1_{328}$ could not. Thus, we hypothesis that there is a segment in the $E1_{260}$ responsible for protein-protein interaction and liposome fusion while another region in the segment of $E1_{260\text{-}328}$ blocking these interactions. Truncated E1 mutants are being prepared to identify these regions.

O10

Relationship between assembled mechanism and structure of HCV core protein

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The hepatitis c virus core protein is the major component of hepatitis c virus nucleocapsid and also exhibits multiple functions involving in cellular growth, proliferation and other functions. The assembly and disassembly of core protein may play an important role in regulating the virus and cell functions. In this study, we have successfully established the core protein overexpression system in *E. coli*. and succeeded in purifying the large-scale core protein for different length of core proteins, including core protein 1-191, core protein 1-173, core protein 1-153 and core protein 1-116. Using TEM and AFM, we demonstrated that core protein 1-116 could assemble into a capsid-like particle *in vitro*. Further analyses of the structural properties of core protein 1-116 using circular dichroism spectroscopy showed that the core protein 1-116 contains about 23% α-helix, 34% β-sheet and 47% random coil. The ionic strength study indicated that core protein 1-116 would cause a 28→33% increase of helical content from 0 M NaCl to 0.5 M NaCl. The assembled core particle has been built up a capsid-like model to around 75 Å using negative stained TEM..

O11

A bioinformatic approach to study the viral entry and morphogenesis of HCV

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The hepatitis C virus (HCV) genome, of 9,400 nucleotides, comprises a single open reading frame (ORF) that codes a polyprotein of 3,000 amino acids. This polyprotein is further cleaved into three structural (C, E1, E2) and seven non-structural (NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins. Despite the progress that has been made in the characterization of HCV component proteins, the mechanisms of HCV replication and the pathogenesis of HCV-related liver disease are far from clear.

In general, the combined use of protein sequencing and structural analytic tools is helpful in mapping the potential important functional sites of a protein. In this work, the initial analysis focuses on two non-structural proteins, NS3 and NS5A. By anlalysing the conserved nature of sequence-conserved regions of the proteins, conserved sequences even conserved local structures existing in other proteins may be identified, which may in turn help to build protein sequence-structure-function relationships.

O12

Study on the morphogenesis of hepatitis C virus

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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. 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.

O13

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 host. 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

In the first phase of this integrated collaboration of EBV research, Dr. Chih-Wen Peng leads to work on the transcription model mediated by EBNA2 and EBNALP. His research team has identified three important repressors that are able to down regulate EBNA2 and EBNALP mediated transcription of EBV promoters, these three repressors appear as the potential therapeutic targets of EBV associated diseases and will be further addressed in the future work. Dr. Lee-Fong Lin specifically works on EBNA1 mediated transcription and has completed the proteomic analysis of EBNA1 associated proteins using large scale immunoprecipitation pull down protocol. She identified three cellular proteins that are associated with EBNA1, suggesting these three proteins play a role in EBNA1 mediated cellular processes. She will be working on the advanced studies of which these factors contribute to EBNA1 mediated transcription and episomal maintenance. Dr. Hong-Chi Chen tended to investigate the role of LMP1 in Cox 2 activation. Also, a HEK293T cell based high throughput assay system is under construction.

With the close collaboration, we look forward to uncovering the important knowledge not only for basic science but also for clinical application on EBV-associated diseases. We have made an important progress toward understanding the EBV latent proteins mediated processes.

O14

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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EBNA2 mediated transcription of EBV promoters which is coactivated by EBNALP is implicated in B cell transformation outgrowth by EBV infection. The interaction between EBNALP and p53 suggested that p53 may have a role in cell defense to EBV infection although such event is likely masked by the presence of EBV. As we expected, the expression levels of p53 were extremely low or nearly undetectable in the presence of EBV among all three types of EBV latent infection, whereas the expression levels of p53 were elevated in the absence of EBV. We next investigated whether overexpression of p53 would affect EBNA2 and EBNALP mediated transcription of EBV LMP1 promoter reporter or endogenous LMP1 expression from EBV genome in the context of an EBV latency infected type I cells, Akata (EBV+). Our results revealed that transiently expressed p53 strongly down-regulated EBV promoters transactivated by EBNA2 and coactivated by EBNALP. The N-terminal AD and C-terminal Olg domains of p53 triggered the robust repressive effects of EBNA2 mediated transcription, whereas Olg and Pro domains triggered the p53 down-regulation of EBNALP coactivation. These results suggested p53 down-regulation of EBNA2 and EBNALP is mechanistically different.

Hsp72 strongly associates with EBNALP in B lymphoblast and up-regulates EBNALP coactivation with EBNA2, suggesting hsp72 associated cochaperones could have a role in modulation of EBNALP coactivation. To test this possibility, we investigated whether overexpression of the well known cochaperones affected EBNALP coactivation with EBNA2 followed a protocol as described previously. In this study, we identified two of BAG family proteins, BAG3 and BAG4, both efficiently down-regulated EBNALP coactivation while possess very limited up-regulating effects on EBNA2 response to LMP1 promoter. The conserved BAG domain and PXXP were essential for maintenance of BAG3 repressing activity to EBNALP, whereas WW domain and serine-rich were dispensable. Distinct from that of BAG3, a truncated deletion mutant of BAG4 BAG domain remained efficiently down-regulated EBNALP coactivation, indicating down-regulation of EBNALP by BAG4 did not require the BAG domain. To gain further insight of these two scenarios by which BAG3 and BAG4 down-regulated EBNALP coactivation, we further tested the down-regulating activity of both BAG proteins to EBNALP coactivation with p300/CBP using a p300/CBP responsive reporter. Strikingly, only BAG3 was shown efficiently down-regulated EBNALP coactivation p300/CBP, whereas BAG4 did not possess any repressing activity on EBNALP coactivation with p300/CBP. Our current data suggested the models by which BAG3 and BAG4 down-regulated EBNALP coactivation are mechanistically distinct.

O15

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

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EBNA1 was previously reported to be involved in the viral episome maintenance and DNA replication in EBV latent infections. Although EBNA1 lacks any enzymatic activities, cellular proteins were proposed to be associated with EBNA1 to carry out its transcriptional activation from episome.

In this study, EBNA1 stably expressed clones were generated. Large scale immunoprecipitation assays using M2 mouse monoclonal antibody and the lysates from these stable clones were performed in order to search for cellular proteins interacting with EBNA1. Three specific proteins, Nucleolin, 60s ribosomal protein L4 (RL4), and Histone 1.5 were identified by LC-MS-MS analyses (Protec Inc.). The role of each cellular protein in modulating the EBNA1-associated viral processes will be further analyzed.

In addition, a reporter system, ori-P-Luc, containing dyad symmetry (DS) and family of repeats components (FR) sequences located upstream of the simian virus (SV40) mini-promoter, as well as an open reading frame of luciferase was constructed. EBNA1 was shown to efficiently activate oriP-Luc about 50-60 fold compared with the background levels using transient reporter assays. Arg-Gly rich region, aa325-376, of EBNA1 was proposed to be the methylation target for protein arginine methyltransferase 1 & 5 (PRMT1 & PRMT5). Furthermore, four serine residues within aa325-376 region maybe the potential targets for phosphorylation. Mutational analysis is currently under way to shed a light on the biological functions of these sites/region.

O16

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

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Epstein-Barr virus (EBV) is found in more than 95% adults world wide. While it is predominately latent in its associated malignancy, more than 70% of the undifferentiated form of nasopharyngeal carcinoma is associated with EBV. Among all 9 EBV latent proteins expressed during latency latent membrane protein 1 (LMP1) appears to be the major transforming protein of EBV. Therefore, LMP1 has been 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. In addition, NF-κB has been shown to play a critical role in the 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. Therefore, we intend to investigate the molecular mechanism of LMP1 and Cox-2 interaction, as well as the potential effects of catechins on LMP1 signaling. Furthermore, high throughput assay system will be developed and utilized for screening potential anti-EBV drugs.

During transient transfection, the expression of full length LMP1 in HEK293T cells induced the activation of NF-κB-luciferase reporter (3XκBL) construct. In contrast, the expression of LMP1 mutant had similar background activity as control vector. However, the expression of LMP1was shown to reduce Cox-2(-327 to +59)-luciferase activity in HEK293T cells, suggestion possible negative regulation of LMP1 on this promoter region. In addition, since the expression of LMP1 appears to affect the TK promoter of the normalizing vector, it might be necessary to generate stable Cox-2 promoter/luciferase clone for future study. To further study the promoter of Cox-2 in response to LMP1 stimulation, a (-1500 to +133) and a (-254 to +133) regions of human Cox-2 promoter were inserted into pGL3-enhancer vector, respectively. Upon confirming their responses to LMP1, these vectors will be used to generate more mutations for promoter study and to generate stable HEK293T clone for high throughput assay system.

O17

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 5 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: Molecular mechanism of inflammation and thrombosis involved in adriamycin induced cardiomyopathy. Project 5: 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 5 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; in vitro studies of adriamycin induced cardiomyopathy were used in project 3 and 4; 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; neonatal rat cardiomyocyte culture in project 3 and 4; 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 and 5. The first 4 projects (1-4) were basic research in elucidating either molecular mechanism or establishing disease animal model, while project 5 may hopefully will be the extension of our conclusion resulted from *in vitro* and *in vivo* animal studies (projects 1-4) with further clinical application.

O18

G-CSF induce inflammatory-dependent cardiac thrombosis in iron overload heart in mice

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Summary. *Background:* It was known that coagulation cascade and inflammatory molecules work as partners during thrombotic and inflammatory process. However, the molecular mechanisms to trigger thrombus formation and its connection to the inflammatory process in cardiovascular disease are still unclear. *Objectives:* We investigate inflammation-thrombosis circuit in cardiovascular disease by using iron loading and G-CSF supplement. We hypothesize that iron loading can increase oxidative stress to cardiac endothelium and with the administration of G-CSF can recruit leukocyte and increased coagulability thus result in inflammation-dependent thrombus formation. *Methods and results:* We demonstrated that seven of ten iron and G-CSF treated mice (I+G group) showed impaired cardiac diastolic function and mural thrombi formation in the left ventricular chamber, while no mice showed abnormality in other experimental groups. Endothelial fibrosis, macrophage infiltration and cellular thrombogenesis were observed in I+G hearts. Quantitative-PCR studies indicated higher levels of inflammatory coagulants, including ICAM-1, MCP-1, tissue factor, and TNF-α, in the affected myocardium of I+G mice than those in other experimental groups. The recruitment of monocytes and neutrophils was obvious in the site of cardiac thrombus in I + G mice. Supplement of simvastatin to I+G mice abrogated such thrombus formation by attenuating inflammatory profiles, which was most likely due to the activation of the pAKT signaling pathway. *Conclusion:* Iron loading and G-CSF supplement can induce inflammation-dependent cardiac thrombosis in mice, in which the above condition can be attenuated by simvastatin treatment. We provided here a novel in vivo disease model to study inflammation-thrombosis circuit in cardiovascular diseases.

Keywords: G-CSF, iron loading, thrombosis, inflammation, tissue factor, cardiomyocyte.

O19

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 have established the techniques of recombinant adiponectin production and purification, and have constructed and amplified liver-specific driven promoter Adv-albumin-adiponectin. Mice hepatocytes subjected to adiponectin treatment increased PPAR-α-enhancer luciferase activity by approximately 50%. The increased PPAR-α activity is accompanied by HO-1 induction. We observed that adiponectin induced HO-1 induction was decreased using a PPAR-α antagonist, suggesting its dependency on the PPAR-α activation. Using synthesized putative PPAR-α binding site in the HO-1 promoter region, EMSA provided an evidence of increased DNA-binding activity of PPAR-α in response to adiponectin treatment. Adiponectin treatment decreased hemin-induced inflammation and apoptosis such as reverse of hemin-induced NF-kb-p65 at 2 hr and Cox-2 at 6 hr, and reverse of hemin-induced decrease in Bcl-xL at 6 hr. In addition, adiponectin decreased hemin-induced iron deposition in hepatocyte *in vitro* as compared to control group. *In vivo* study has shown that mice subjected to in intraperitoneal iron dextran challenge cause apoptosis and iron deposition in liver. Whether iron-induced apoptosis and iron deposition in liver is reversed by adiponectin gene therapy is still under investigation.

O20

The role of adiponectin in ROS-related cardiomyopathy induced by doxorubicin or iron overloading

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Iron overload is a less frequent condition, but high contents of tissue iron have been associated with several pathological conditions, including liver and heart disease. Our preliminary studies demonstrated that G-CSF will augment iron-overload cardiac dysfunction and promotes endomyocardial fibrosis in mice with upregulation of some inflammatory cytokine factors like TNFα, IL-1β, IL-6, and MCP-1. In addition, intracardiac thrombus in left ventricular chamber seen in G-CSF and iron supplement revealed accumulation of monocytes and leukocytes. In agreement with the pathology findings, echocardiography data showed that fractional shortening (FS), inter-ventricular septal thickness (IVS) and LV posterior wall thickness (LVPW) in both diastolic and systolic phases were significantly thinner in G-CSF combined iron-treated than control. In heart, there are many anti ROS or anti inflammatory protect mechanism which can protect cardiomyocyte from ROS or inflammatory these anti-ROS or anti-inflammatory factor like adiponectin, which is secretes from adipocyte and except for anti-inflammatory effect adiponectin is a protein acting as an anti-obesity protein and suppresses DM. Therefore, we intend to elaborate whether adiponectin can reduce GCSF+iron induced cardiomyopathy. From the results indicated that AAV8 containing adiponectin was largely expressed in heart after 14 days i.v injection in mices in addition, over-express adiponectin can attenuated the numbers of WBC, leukocytes and monocytes. In agreement with the blood numbers findings, many inflammatory cytokine factors like TNFα. IL-1β, IL-6, MCP-1 all were reduced by adiponectin treatment in mice. We then intend to utilize echocardiography to reappraise the heart function and appearance level of thrombus after adiponectin treatment. Echocardiographic data showed that fractional shortening (FS), inter-ventricular septal thickness (IVS) and LV posterior wall thickness (LVPW) in both diastolic and systolic phases were significantly thinner in G-CSF+iron-treated than control and normal cardiac contractility and ventricular wall thickness were found to be preserved in the adiponectin treated group. Even more, the appearance of G-CSF+iron induced cardiofibrosis and iron accumulation in heart can be eliminated by adiponectin i.v injection. Hence, we conclude that G-CSF aggravated iron-induced cardiomyopathy can be attenuated by adiponectin treatment via anti-inflammatory pathway.

O21

Molecular mechanism of inflammation and thrombosis involved in adriamycin induced cardiomyopathy

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Previous studies showed that the mice given adriamycin has high incidence of atrial thrombosis and increased concentrations of plasma C-reactive protein (CRP), matrix metalloproteinase (MMP), IL-1β cytokine and fibrinogen indicated severe inflammation in the adriamycin-treated rats. Those results indicated that adriamycin-induced cardiomyopathy was involved inflammation and thrombosis. IL-1β cytokine production is mediated caspase-1activation. This study investigated the role of caspase-1 and the prevention effects of carvediol and biobalide on adriamycin-induced cardiomyopathy.

Pretreatment with a broad caspase inhibitor and caspase-1 inhibitor could eliminate adriamycin-induced endothelial cell apoptosis by flowcytometry analysis and DNA ladder assay. Analysis using RT-PCR, Western blot and caspase-fluorescent assay revealed that pretreatment with a caspase-1 inhibitor also prevented blocked caspase-3 activation and PARP cleavage in adriamycin-treated endothelial cells.

With pretreatment carvediol and bilobalide on adriamycin-treated rat, we also find these two drugs can block the cytotoxicity effect of adriamycin by hemodynamic data and Echo examination. Pretreatment with carvediol (25.6± $9/10^6$ cell) and bilobalide (40.6± $16.2/10^6$ cell) can reduce adriamycin-induced myocardium apoptosis (269.7± $20.3/10^6$ cell) by TUNEL assay ($P<0.01$ *vs* adriamycin group). Down-regulated caspase-1expression is showed with pretreatment carvediol and bilobalide on adriamycin-treated rat by immunohistochemetry staining.

In conclusion, this study demonstrated a novel caspase-1 dominant apoptotic signal pathway that is important in adriamycin-induced cell apoptosis. With pretreatment carvediol and bilobalide can prevent adriamycin-induced myocardium apoptosis by blocking caspase-1 activation.

O22

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

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Serum C reaction protein (CRP) level, an inflammatory marker that predicts future cardiovascular events, is a heritable trait with genetic contribution of 27-40%. The aim of the investigation was to test the statistical association between genetic variations on the CRP gene and serum CRP levels in a Taiwanese population with interactive analysis. A sample population of 617 Taiwanese subjects was enrolled for the study. Five CRP single nucleotide polymorphisms (SNPs) previously reported to be associated with CRP levels and with reasonable coverage of the CRP gene region were analyzed by polymerase chain reaction and restriction enzyme digestion or by TaqMan SNP Genotyping Assays. After adjustment of clinical covariates, 3 of the 5 SNPs were associated with CRP levels. Minor alleles of SNPs rs3091244 and rs1205 polymorphisms were associated with increased CRP levels ($p=0.001$ and $p<0.001$, respectively), while minor allele of SNP rs1800947 was associated with decreased CRP levels ($p=0.001$). Two haplotypes inferred from 5 SNPs (CCGCG and TAGCG) were associated with increased CRP levels and one haplotype (TCCCA) was associated with decreased CRP levels. Interactive analysis revealed interaction of obesity with CRP genotypes on CRP levels ($p=0.022$ and $p=0.017$ for SNPs rs2794521 and rs1800947, respectively). Interaction with obesity on CRP levels was also noted in haplotype interaction analysis with the association occurred predominantly in obese subjects ($p=0.034$). In conclusion, analysis of our data revealed an independent association between CRP polymorphisms and CRP levels in the Taiwanese. The data suggests that CRP genotypes/haplotypes interact with obesity on CRP levels. These findings have implications for the prediction of atherosclerotic cardiovascular disease.

O23

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。至於心臟血管疾病患者於臨床用藥上，更需注意藥物代謝途徑是否受到影響之臨床課題。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升壓中樞(RVLM)麩胺酸 NMDA 受體，導致交感活性輸出增加；而對同處抑制升壓之延腦交感神經抑制區（CVLM 或 intermediated NTS），其上之 NMDA 受體活性卻無明顯地影響。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，即可明顯地增強交感神經活性及平均動脈血壓，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。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，隨著間歇性低氧時間增加其細胞壞死的比率亦隨之增加，且自發性高血壓大鼠細胞壞死比率約為正常血壓大鼠的兩倍。至於，影響藥物代謝的肝臟細胞色素 P450，亦發現間歇性低氧處理二十天後，自發性高血壓大鼠肝臟細胞的 P450 細胞色素 1A2 的蛋白質量有顯著的增加，但在正常血壓大鼠則無明顯的變化。另外，我們比較間歇性低氧處理之自發性高血壓大鼠與正常血壓大鼠，發現自發性高血壓大鼠心肌細胞的脂質過氧化程度及抗氧化物的活性皆較高。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初期急性低氧化學反射活性、交感神經活性興奮與血壓上升之反應，但對於上述之後期反應則無明顯地抑制效果。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；其中超氧陰離子基之產生可能只參與間歇性低氧引發初期血壓上升之過程，至於其他可能之影響因素仍有待我們進一步探討。

O24

細胞色素 P450 與間歇性低氧之交互關係研究

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長期暴露於間歇性低氧之環境可能引起呼吸，心血管，及代謝生理之改變，以適應此環境。這些生理學上的改變，有些是保護性，能夠避免發生疾病，也能改善選手的運動效能，暴露時間過長，則可能引發高血壓，腦血管及冠狀血管等問題，也可能產生發育，神經認知以及神經退化等蓄積作用。此外，間歇性低氧狀況也如同缺血再灌流作用，可釋放大量的自由基，包括活性含氧物與活性含氮物，且此大量自由基之產生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亦較高，甚而對於低氧刺激引發交感神經興奮之反應亦更為明顯；並且也發現於正常情況下其體內可產生較強的氧化壓力。P450 細胞色素是肝臟藥物代謝，以及腎臟內生性血管作用物質產生主要途徑，與高血壓大鼠生成高血壓有關。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，可能因氧化壓力作用，內生性血管作用物質產生導致心臟血管系統調控失常。然而，其中產生之連續性生理變化及相關生理機轉，至今仍尚未有進一步的探討。本實驗設計是探討自發性高血壓大鼠與正常血壓大鼠暴露於間歇性低氧時，測量其 P450 細胞色素之含量變化。實驗結果發現，正常血壓大鼠在給予間歇性低氧處理 10、20 及 30 天後，其肝臟細胞的 P450 細胞色素中 1A1，1A2 及 2B1 的蛋白質表現量與正常氧分壓處理組(RA)之間都沒有顯著增加的現象；但自發性高血壓大鼠在給予間歇性低氧處理 20 及 30 天後，其肝臟細胞的 P450 細胞色素中 1A2 的蛋白質量與 RA 相比，有顯著的增加；但 1A1 及 2B 則無顯著的差異。根據以上結果推測，在高血壓的情況下合併有間歇性低氧的情況發生，可能會導致肝臟細胞 P450 細胞色素中的 1A2 量有增加的現象，而此蛋白質的增加對於生理現象的影響則是我們下一步探討的目標。

O25

(計畫名稱：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)

自由基對於間歇性低氧引發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。一般而言，周邊化學接受器中的頸動脈體為主要持續地偵測動脈血液氧氣分壓之變化，而當長期間歇性低氧過程中，更可能因頸動脈體反覆地被刺激，反射性地引發交感神經活性增加，進而導致持續性高血壓現象。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。並且，於另外一組動物每天事先給予腹腔注射超氧陰離子基驅除物 (MnTMPyP, a scavenger for superoxide anion)，比較間歇性低氧所引起之心肺反應是否有所差異。本實驗結果顯示，暴露於間歇性低氧約於第九天開始可明顯地增強心率變異性中低頻比值（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。相反地，無論是間歇性低氧或空氣暴露，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。而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初期急性低氧化學反射活性、交感神經活性興奮與血壓上升之反應，但對於上述之後期反應則無明顯地抑制效果。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，而其中可能與過度增強的化學反射反應引發交感神經活性興奮有關；其中超氧陰離子基之產生可能只參與間歇性低氧引發初期血壓上升之過程。

O26

(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)

間歇性低氧對自發性高血壓與正常血壓大鼠中樞交感相關神經核上 NMDA 受體次單位表達之影響

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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。而此 IH 所造成的高血壓被認為與中樞交感神經活性輸出增強具密切相關。位於中樞前腹外側延腦(rostral ventrolateral medulla, RVLM)之神經元，已知可輸出交感活性，維持一定血管張力，因而被稱為前交感血管運動神經元。此外，研究顯示：周邊的感壓接受體可因血壓驟升而將興奮訊息傳至延腦孤獨核(nucleus tractus solitarius, NTS)中段，稱為 intermediated NTS 神經元，再投射至延腦後腹外側區(caudal ventrolateral medulla, CVLM)，活化 CVLM 的 GABAergic 神經元，接著再傳導至 RVLM，使該區受抑制，則減弱交感活性之輸出，調降血壓。目前已知上述 RVLM、CVLM 與 NTS 各區之神經元活性皆與其上麩胺酸離子通道型受體活化狀態有關。本研究目前進行之方向為，將自發性高血壓大鼠(SHR)與對照之正常血壓大鼠(WKY)，各別給予每天持續 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，於第八天犧牲動物，取其 RVLM、CVLM 與 NTS 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。目前獲致之結果顯示：於 SHR 之 RVLM 區上，NMDA 受體次單位蛋白 NR1 與 NR2B，在經 IH 處理後較其正常通氣之 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，RVLM 上 NR1 與 NR2B 表現量並無統計上之差異。CVLM 與 NTS 兩區之結果則恰與 RVLM 相反：在 SHR 之 CVLM 區，NR1 及 NR2B 之表現量並不因 IH 處理而產生有意義之改變；然於 WKY 大鼠，該區之 NR1 表現量可因 IH 而顯著提升，而 NR2B 表現量之改變雖然未具統計意義，亦有增加之趨勢，若比之 SHR 之 IH 組別，則有顯著增高之意義。就 NTS 而言，IH 處理與否並不影響 SHR 該區 NR1 與 NR2B 之表現量；然對於 WKY 大鼠，IH 處理則顯著增加此二蛋白之表達。由本子計畫現有之數據，配合子計畫二之現有結果，可推估：SHR 經慢性 IH 處理後引發之顯著增高血壓現象，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；而對同處延腦之交感神經抑制區 CVLM 或 intermediated NTS，其上之 NMDA 受體活性則較無影響。有關 WKY 大鼠血壓指數與 NMDA 受體活性之相關性，則待進一步研究結果彙整後方能評估。

O27

間歇性低氧對於凝血功能以及動脈血管的影響

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為了解間歇低氧對於血液組成及凝血功能的影響，我們將 9 週大的 WKY 大鼠分成兩組，每天給予間歇性低氧或是正常空氣處理 6 小時，在 2、5 及 20 天後抽血測量血球數目、血容比、血漿凝血時間、血中血紅素、血漿中紅血球生成素(erythropoietin)及多種 cytokine (IL-2, IL-6, IL-10, TNF-α, TNF-β) 的含量。結果顯示，經過 2 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有顯著的上升。隨著間歇性低氧處理天數的增加，血中紅血球、血紅素、血容比及紅血球生成素上升的情況更顯著。但白血球、血小板、IL-2、IL-6、IL-10、TNF-α、TNF-β、aPTT 及 PT 凝血時間在間歇性低氧處理 20 天後卻沒有顯著變化。這些結果顯示大鼠在面對間歇性低氧時體內很快就會有所因應，時間越久，體內的變化就越大，特別是血中紅血球、血紅素、血容比及紅血球生成素的上升。最近的研究顯示紅血球、血紅素、血容比及紅血球生成素過度的上升可能會促進心血管疾病及增加血栓的機會。基於上述的結果，我們認為長期暴露在間歇性低氧可能會增加血管疾病及血栓形成的機會。至於紅血球、血紅素、血容比及紅血球生成素的上升如何促進心血管疾病及增加血栓之形成，值得我們進一步探討。

O28

間歇性低氧造成心肌細胞死亡機制之探討

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間歇性低氧(intermittent hypoxia)是一種反覆性低氧再重新恢復氧氣(hypoxia-reoxygenation)的現象，常見於一些呼吸有關的生理及病理狀態，包括夜間換氣不足及阻塞型睡眠呼吸中止症(obstructive sleep apneas syndrome, OSA)。在臨床上，睡眠呼吸中止症的病患常伴隨心血管方面的問題產生，包括高血壓、左心室功能降低、心臟功能的喪失，但目前對於其引發的機制仍未清楚；先前的研究發現間歇性低氧造成心肌細胞的死亡類似缺血再灌流的傷害(ischemia reperfusion, IR injury)，缺血再灌流會造成細胞內大量自由基的產生及堆積，並引起細胞內離子調控失衡，使細胞走向死亡。為了探討間歇性低氧引發心肌細胞死亡的機制，本研究利用自發性高血壓大鼠(spontaneously hypertensive rats, SHR)及與 SHR 源至相同動物種群的正常血壓 WKY 大鼠，給予反覆性低氧再重新恢復氧氣連續 10、20、30 天的處理，另外一組則給予反覆性正常氧分壓(room air, RA)做為對照組，來探討間歇性低氧造成心肌細胞死亡的機制，並釐清高血壓是否增強間歇性低氧造成的心肌細胞死亡；我們利用脂質過氧化(lipid-peroxidation)及分析 superoxide dismutase (SOD)的活性來評估氧化壓力的程度；以 propidium iodide (PI)偵測細胞膜的完整性作為細胞壞死(necrosis)指標，細胞凋亡(apoptosis)則以 DNA ladder 來確認胞核內 DNA 的斷裂，並利用西方點墨法來探討其活化的路徑。由實驗結果發現 SHR 及 WKY 大鼠在連續處理 10、20 及 30 天間歇性低氧後，隨著處理時間增加其細胞壞死的比率有增加的現象，且自發性高血壓大鼠 SHR 其細胞壞死比率約為 WKY 的兩倍，偵測與細胞壞死有關的 PARP 的表現量及活化並無顯著的差異；雖然 WKY 大鼠心肌細胞壞死的比率較低，但發現其心肌細胞可偵測到凋亡的現象，並有 cytochrome C 的釋放及 caspase-3 的活化，而 SHR 則無細胞凋亡表現；進一步分析不同組別在處理間歇性低氧之後氧化壓力的差異，SHR 的心肌細胞其脂質過氧化程度及 SOD 的活性都比 WKY 的高，因此推測間歇性低氧造成的心肌細胞死亡與自由基的增加量有關，若增加量較低會引發細胞凋亡，而高血壓老鼠在給予間歇性低氧後會造成較大的氧化壓力並導致心肌細胞壞死，而導致心臟功能的喪失。

O29

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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失血性休克是創傷病人常見的問題，大部分病人的死亡發生在創傷後 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。輸液治療是臨床最常用的處置方式，對於大量失血的病人給予快速加溫的大量輸液，是護理人員執行的主要措施。本研究中從保健到醫療的觀點，提供急重症單位醫護人員在面對大失血病患時，給予既安全又有效的最佳醫護處置與建議。整體目標是希望藉由實驗研究，探討保健食品與行為對大失血狀況是否具保護效果，並找出較佳的輸液給予及治療模式，以提供醫護人員執行大失血病患的輸液措施與治療時的參考指引。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(子計畫一)以及規律運動的保健行為(子計畫二)，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，最常出現肝腎衰竭的現象，可能導致體內不平衡及代謝異常，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，本研究中就大失血後肝腎衰竭的發生與治療做相關探討(子計畫一、三)；目前臨床處置上，對於急性期的失血性休克病患，多數是遵循高級創傷救命術的建議，以大量、快速的回溫輸液灌注為治療導向，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，也有人提出不宜將輸液加溫，反倒是低溫療法可促進病人存活率，因此在輸液處理上仍無定論，本研究在輸液的速度調控、體溫變化與輸液回溫上做了相關探討(子計畫四)；而近年來備受重視的抗發炎藥物 Fluvastatin 在失血性休克中是否具有器官保護的作用，也在本整合型研究中一併做相關探討(子計畫三)。整合目前兩年的研究結果發現：失血對肝臟確實造成嚴重損傷，而蜆萃取物對失血後的肝臟損傷有保護作用；有規律運動的各項血液生化數值、肺泡支氣管灌洗液中顆粒球比例均低於無運動組，且肺、肝、腎臟病理學損傷程度較輕，48 小時的存活率也較高；而 Fluvastatin 對於嚴重失血下有保護器官免於損傷的功效；慢速輸液組呈現較長時間的低體溫、對於器官的損傷較輕微，而快速輸液時若合併 Fluvastatin 治療，可明顯降低 CPK、LDH、GOT、GPT 的數值。目前研究結果顯示蜆萃取物、規律運動、Fluvastatin 對嚴重失血下有器官保護的功效，而慢速輸液方式對肝臟、肺臟、腸道損傷較輕，若使用快速輸液時，合併 Fluvastatin 治療可明顯降低器官的損傷。第三年計畫將探討輸液加溫對個體的影響，並進一步針對各項保護機轉做相關的探討。

O30

探討蜆萃取物對失血性休克下肝臟的保健作用

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臨床上護理人員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。大部份創傷病人在創傷發生後 24 小時內死亡，急救存活之病患常面臨多重器官衰竭之問題。失血性休克之生理病理反應與系統性發炎反應有關，可能會造成器官功能喪失，最後形成包括肝臟等器官衰竭，這種現象與高死亡率有密切的關係。近年來由於國人對於具有生理機能調節作用的食品越來越重視，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，然而蜆的功能機制仍未經醫學完全證實。因此，本研究主要在探討蜆萃取物對失血性休克下肝臟是否具有保健作用及其機轉。第一年研究中，探討出血性休克模式下引起的急性肝衰竭的生理、病理層面的機轉，第二年研究中，觀察蜆萃取物對於失血前肝臟保護之生理表現機制與保護功效。研究中實驗動物為 WKY 大鼠，第一年研究將其進行大失血並觀察肝臟損傷情形，第二年再分別於失血前給予蜆萃取物，實驗共分五組：蜆萃取物 CE150 mg/kg、 CE75 mg/kg、失血 BL40 %、BL 40 %合併 CE150 mg/kg、BL40 %合併 CE75 mg/kg，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 aspartate aminotransferase (AST)、alanine aminotransferase (ALT)、lactate dehydrogenase (LDH)之變化，並在第 48 小時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。綜合兩年的研究結果顯示：急性大失血除了導致平均動脈壓下降之外，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。這些結果顯示蜆萃取物能有效減輕急性失血所引發之肝損傷。在第三年的研究中，將嘗試在失血後才給予蜆萃取物，測試蜆萃取物除了在保健功能外，是否也具有治療的功效，以提供臨床醫護人員參考的實證。

O31

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，在失血性休克早期產生的炎症物質與後續的多重器官損傷有關。有研究指出運動訓練與炎症反應有一定的相關性，強度高的運動訓練會增加感染的易感性，但也有研究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。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，第二年比較有無運動下經歷大失血時的免疫功能反應、體重變化、活動力變化與存活率。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 20%及 60%的失血，將實驗動物分為運動合併 20%失血(EHS20%)或 60%失血(EHS60%)、無運動合併 20%(HS20%)或 60%(HS60%)失血共四組，使用 8 週齡的大鼠隨機分配到各組，運動組給予每天 5 分鐘 10m/min 的跑步，接著給予 25 分鐘 15m/min 的跑步(相當於中等強度)，並監測每日體重與活動力的變化。在運動四週後進行失血實驗，給予股動靜脈插管，由股動脈監測血壓與心跳，股靜脈導管則連接在輸液幫浦上進行失血模式，在執行失血前、失血後 1、3、6、9、12、18、24 與 48 小時各採集血液 0.5 ml，檢測血中 GOT、GPT、血液尿素氮、肌酸酐、LDH、CK-MB、血糖、乳酸和血液酸鹼值，實驗動物在失血後 48 小時給予大劑量的 pentobarbital 犧牲，進行肺泡支氣管灌洗檢測顆粒球之比例，並留取肺、肝、腎等器官做病理學檢測，並紀錄失血後 48 小時的存活率。兩年的研究結果統整如下：有運動組的體重增加較無運動組少，且活動力較高；EHS60%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 HS60%組，且肺、肝、腎臟病理學損傷程度較輕，48 小時的存活率也高於 HS60%組。目前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，臨床上需注意對規律運動者勿低估了其失血的嚴重性。在第三年的研究中，將針對熱休克蛋白以及急性炎症反應路徑進行探討，以了解其保護器官免於損傷的機制。

O32

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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因大量出血引起的出血性休克常造成包含腎臟損傷的多重器官衰竭，其中引起的急性腎衰竭之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及多重器官衰竭的表現，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。本實驗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機轉。研究發現，在 20%、40%、60%出血性休克下均會引起 GOT、GPT、BUN、CPK、LDH 的上升，但 Cre 的變化則發生在 40%及 60%出血性休克，而其中以 60%出血的變化最明顯。進一步取 Fluvastatin 以 40%及 60%出血性休克引起急性腎衰竭，看能否有保護作用，研究發現 Fluvastatin 可減少 40%出血性休克引起的 GOT、GPT、BUN、Cre、CPK 上升，在 60%出血性休克下可降低 GOT、GPT、BUN、Cre、CPK、LDH、Lactate 的量及 PH、bicarbonate、PCO2、TCH 的下降，另可增加 TG 的上升，及減少大鼠的死亡率。第二年實驗探討在 60%出血性休克中，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，60%出血性休克後快速輸液下，有給予 Fluvastatin 更可改善 GOT、GPT、CPK、LDH、TG，但對 BUN、Glucose、TCH 較無反應。在 60%出血性休克後慢速輸液下，有給 Fluvastatin 更可改善 GOT、Glucose、TG，但對 GPT、BUN、Cre、CPK、LDH、Lactate、TCH 較無正向反應。在第一年的實驗病理組織上，證實 Fluvastatin 可減少 60%出血性休克造成的肝、腎、肺及小腸傷害，第二年的病理組織染色及腎臟免疫組織染色仍持續進行中，以了解腎臟的損傷狀況是否改善及評估造成損傷的機轉，目前推測 Fluvastatin 可改善 60%出血性休克所造成的腎小管 E-cadherin 的流失，及減少因 60%出血性休克造成的腎小管 NF-κB 的活性。第三年的研究將探討大失血下 Fluvastatin 在不同溫度的輸液治療中，對腎臟是否也具有保護的作用，是否因輸液溫度不同而有作用的差異。

O33

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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失血性休克是創傷病患常見的死亡原因，而低體溫為失血性休克後常見的合併症。高級創傷救命術中將創傷早期大量的輸液治療及輸液回溫視為一項重要的急救指標，這也成為急重症護理人員行輸液復甦治療時的依據。過去有研究發現，當創傷後身體呈現輕度至中度的低體溫狀態時，能抑制癌症反應所活化的細胞毒素濃度，進而減少後續性的傷害，但也有研究提到大量輸液造成低體溫可能導致死亡率上升。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快速與慢速及不同溫度下，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。第一年的研究是探討失血性休克期間不同輸液速度對器官損傷及發炎物質的反應，第二年探討失血後快速與慢速輸液復甦，對失血性休克後體溫的影響與器官損傷的表現。研究中將大鼠分為三組：純失血組、失血後立即輸液及失血後慢速輸液12小時，在清醒鼠模式下進行全血量40%的失血，出血時間維持30分鐘，並經由股動脈導管監測血壓與心跳，股靜脈導管則連接輸液幫浦進行失血模式，於失血後給予失血量1:1之輸液量，速度分為10分鐘立即灌注完成及12小時緩慢灌注完成。在執行失血前、失血後1、3、6、9、12、18、24與48小時各採集血液0.5 ml，檢測血中全血球數值、GOT、GPT、血液尿素氮、肌酸酐、LDH等，在失血後48小時給予大劑量的pentobarbital犧牲，並留取肺、肝、腸等器官做病理學檢測。另外於第二年研究時，在失血前開始以肛溫監測器記錄，監控體溫每分鐘的改變，持續監控48小時，實驗動物在失血48小時犧牲後，進行肺泡支氣管灌洗檢測灌洗液中TNF-α與IL-10的量。兩年的研究結果發現：失血後慢速輸液12小時組，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，而在肺、肝、腸病理學損傷程度上也較輕。依據研究結果推論：慢速輸液治療使個體處在輕微低體溫的表現下，對於失血後的器官傷害程度較快速輸液組輕微。第三年的研究將於失血性休克後模擬臨床輸液加溫處置，給予加溫與不加溫兩種的處置，探討體溫的變化、各器官損傷的狀況及發炎反應，是否受到液體加溫處置所調控。

O34

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

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Trauma and meningioma that compress cerebral cortex often cause discrete abnormalities. Changes of the underlying cortical neurons although essential to understanding the pathophysiology and treatment of the diseases remain largely unexplored. This integrative project aims at investigating how cortical neurons can be remodeled when subjected to compression. We approached this issue using a rat epidural bead implantation model that effectively compressed the underlying sensorimotor cortex without causing chemical interaction (Chen et al., 2003). The implanted bead can also be removed easily thus offers the capacity to study the effect of decompression (Chen et al., 2004). The reproducibility of this in vivo cortical compression/decompression model allows us to study thing such as the structural remodeling of cortical neurons under compression systematically and is a big improvement over previous studies that resorted to studying culture neurons to address process elongation and retraction. In addition, the thick and relatively straight and almost uniformly aligned apical dendritic trunks of the cortical pyramidal neurons made possible the evaluation of many aspects of the dendritic reconfiguration since CNS neuronal dendrites are usually thin and long that meander 3-directionally thus elude examination. The first project of this integrative series studies the structural changes of cortical pyramidal neurons and its associated mechanism. The second project studies the ultrastructural aspect of the compression-induced instantaneous distortion of dendrites and the subsequent remodeling of dendrites and retrieval of dendritic spines, including how plasma membrane and intracellular organelles, microtubules and neurofilaments accommodate to these changes. The third project addresses whether and how cholinergic innervation and NGF regulate the densities of dendritic spines on cortical pyramidal neurons. The last project aims at understanding whether and how cortical receiving neurons, mainly layer IV stellate neurons, are affected by the compression.

As presented in detail in each project, we are proceeding as proposed and the results obtained are encouraging and support our hypothesis. The results obtained thus far demonstrated unequivocally that in the CNS neuronal processes could undergo large-scale structural remodeling swiftly in areas far from their terminals. The swiftness of the compression-induced cortical neuronal reconfiguration and the fact that these showed slow and marginal recovery if prolonged argue strongly for early clinical intervention of abnormalities that compress the cortex for not only patients holdup seeking medical help until clinical manifestation but also that the evaluation and determination of treatment strategy in clinics regularly delay decompression.

O35

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

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Compression compromises cortical functions. Compressed cortical pyramidal neurons underwent swift structural remodeling including changes of dendritic arbors, microtubule cytoskeleton and dendritic spines in 3 days. Here we explored how cortical pyramidal neurons could be instantly deformed upon compression and how subsequent plasmalemmal and cytoskeletal changes returned the deformed dendrites to normal profiles in 3 days. We found that compression shortened dendritic length by reducing the number of terminal dendritic branches rather than dendritic trunks. The apical dendritic trunks of these neurons that received direct compression due to their orientation were twisted instantly and became straight again in 3 days, suggesting that in addition to retracting terminals, the thicker intermediate part of dendrites might also be reconfigured. Cytoskeleton-wise, compression compacted the loose spiraling microtubules typical of normal dendritic trunk instantaneously into tighter coil. This could be reversed instantly upon immediate decompression; or returned to the loose spiraling configuration characteristic of normal neurons in 3 days as compression persisted presumably via active mechanisms. Compression also deformed the plasmalemma instantly so that loose spirals and bends occurred. These deformations were largely removed within a day of compression indicating the involvement of an active plasmalemmal remodeling perhaps via mechanisms different from that mediating the reconfiguration of the microtubules due to the discrepancy in time courses. Microtubule reorganization is likely to involve the severing of longer microtubules into short pieces for rapid transportation. To find out whether the compression-induced dendritic remodeling involved large-scale microtubule depolymerization, micortubules were stabilized with taxol. It affected the restoration but not the early immediate part of the compression-induced dendritic responses. Taxol completely suspended the restoration of microtubule morphology but partially affected the smoothing of plasmalemma. We then looked at the changes of the microtubule-associated protein tau since hyperphosphorylation changes its affinity for microtubules and destabilizes microtubules. Results show that compression swiftly downregulated the phosphatase PP2A activity and transiently increased the activities of a few cytoskeleton-related kinases. The time courses of these changes coincided well with those of the hyperphosphorylation of tau and the dendritic remodeling of the compressed neurons, thus were likely responsible for tipping the tau protein toward hyperphosphorylation and resulted in the dissociation of tau from microtubules. These rendered microtubule reconfiguration by constitutively active microtubule-severing proteins such as katanin.

O36

Ultrastructural studies on plasmalemma, organelles, and cytoskeleton involved in the compression-induced dendritic plasticity

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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. Although prominent changes in dendritic microtubule arrangement and the distortion of dendritic contour were found under light microscopic level in pyramidal neurons subjected to compression, the ultrastructural changes of these organelles remain unknown. Since microtubules and plasma membrane were restored to normal appearance after 3-day compression, active membrane and cytoskeleton remodeling are speculated to happen after compression; making the apical dendritic trunk regain the smooth contour and regular microtubule arrangement similar to the normal control neurons. In this study, we used transmission electron microscopy to examine microtubule arrangement of apical dendrites of pyramidal neurons in rat somatomotor cortex. Microtubule densities within apical dendritic trunks decreased significantly ($p < 0.05$) and arranged irregularly following compression for 30 min to 24 h. However, microtubule densities gradually restored to control level after long-term compression ($\geqq$ 36 h). Interestingly, apparent endocytosis represented by increased number of endocytotic vesicles occurred along the apical dendritic membrane in neurons subjected to 36-h compression. These results suggested that active membrane and microtubule remodeling are correlated and both affect the apical dendritic morphology after compression. Studies on effects of decompression on neuroplasticity is undertaken to elucidate the effects of surgical benefits on neuronal recovery.

O37

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 in 3 days (the shortest that we have examined) and decompression failed to reverse this suggesting that compression could cause permanent changes of the cortex in a matter of days. NGF added immediately following decompression resulted in an increase of the dendritic spines of these neurons and thickened the cholinergic axon bundle at the border of motor and cingulate cortices at the same time. 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. We first developed a rat sensorimotor cortical cholinergic denervation model by injecting the immunotoxin, 192 IgG-saporin directly into the nucleus basalis magnocellularis (NBM) and substantia innominata (SI) unilaterally to eliminate sensorimotor cortical cholinergic innervation. Immunotoxin injection destroyed NBM and SI cholinergic neurons and over 90% of the sensorimotor cortical cholinergic fibers in 5 weeks as revealed by ChAT immunohistochemistry and immunoblotting. In normal animals, cholinergic fibers and terminals dispersed in all layers and were particularly rich in the layer I of the cortex. Layer V pyramidal neurons of the denervated cortex were revealed with intracellular dye injection and their dendritic spines analyzed. Spine densities were reduced by half in layers I-III and 25% in layer V, but unchanged in layer IV. Loss of dendritic spines on the pyramidal neurons of the sensorimotor cortex was accompanied by specific decreases of the postsynaptic marker protein PSD-95 and the dendritic spine protein spinophilin suggesting that these represent reduction of excitatory synapses, thus cortical functions. Since pyramidal neurons are the key output neurons of the cerebral cortex and dendritic spines are the main excitatory input-receiving structures, our results suggest that basal forebrain cholinergic projection to the cortex may play a significant role in regulating cortical function through alterations of cortical neuronal morphology. In addition, cholinergic inputs appear to 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. We are currently investigating whether NGF applied intraventricularly promotes the spine genesis of cortical pyramidal neurons of the sensorimotor cortical cholinergic denervation animals.

O38

An investigation of the compression-induced plasticity of cortical receiving neurons and thalamocortical inputs

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Compression of cerebral cortex is usually induced by brain tumor, epidural or subdural hematoma, intracranial hemorrhage, hemangioma, head trauma, or large-scale arteriovenous malformations. 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 through destruction of blood-brain barrier (BBB) and/or thalamocortical connections. After S1 compression, male SD rats were allowed to survive for 1d, 2d, 3d, 14d, and 3m. Assessed by measuring Evans Blue extravasation, BBB permeability began to increase in compressed S1 at 1d and peaked at 3d, but recovered at 3m. Nevertheless, the expressions of tight junction proteins occludin, claudin-5, and ZO-1 increased at 1-3d following compression. The touch sensitivity of whisker pads, forepaws, and hindpaws of S1-compressed animals were reduced at 1d-3m, evidenced by von Frey behavioral tests. The amplitudes of somatosensory evoked potentials (SSEPs) of compressed S1 also decreased. Using BDA anterograde tracing, thalamocortical projections to layer IV of compressed S1 were observed to decrease.

These findings suggest that physical compression of S1 will cause disruption of BBB, loss of thalamocortical connections, and impairment of somatic sensation. The correlation of these changes remains to be further investigated.

O39

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。第一年初步結果已搜集資料，正在進行資料分析的工作。

O40

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（Positive psychology）是探討個人的正向經驗與品格長處的科學研究（Duckworth, Steen, & Seligman, 2005），主要目標在提昇人們的基本能力，如樂觀、勇氣、誠實、自我了解與人際互動的技巧，幫助個人找到內在的心理能量，以作為對抗挫折的緩衝、掌控逆境，使得個體在遇到困難時不會輕易落入憂鬱的狀態中（Seligman & Csikszentmihalyi, 2000）。本研究旨在運用正向心理學觀點，進行大學生的品格長處、情緒與行為之關係調查，以及介入課程的成效評估。本研究分為三個階段，預計三年完成：第一年為文獻探討與量表試題編擬；第二年編制正式量表並進行介入課程的試探性分析；第三年進行正式課程的介入研究與追蹤評估，並彙整所有資料完成研究報告。

Peterson與Seligman（2004）出版之《Character Strengths and Virtues: A Handbook and Classification》（簡稱CSV）一書中，列出經跨文化研究所發現的六項美德：智慧、勇氣、人道關懷、正義、修養、心靈的超越。每一項美德之下，又找出符合下列標準的24項長處：(1)普遍性；(2)能滿足個人發展；(3)道德價值；(4)不會減弱其他特質；(5)不幸福的反面；(6)是一種特質；(7)可測量的；(8)獨特的；(9)模範的；(10)非凡的；(11)不會因具備該項長處而讓人忌妒的；(12)社會想透過學校或機構培養的特質。不過，西方心理學著重幫助人發展健康的自我，讓人擁有自我效能的感覺；而東方心理學一方面知道人必須建立健康的自我才能超脫，同時也注重個我(self)如何超越自私的我(ego)（何縕琪，2007）。由於因文化與環境的差異，轉譯西方理論並應用在不同群體上的研究，應透過更多方案的設計與研究來加以檢核與修正（Snyder & Lopez, 2007）。因而本年度之重點在於分析現有量表之內容，並請專家審定後，預擬出量表之初步架構和題目。同時開始聯繫預試對象，徵得該系與同學的同意後，於2007年9月起進行預試量表的施測。

在「正向心理學」課程的擬定，本年度已完成收集美國各大學開設「正向心理學」課程之大綱與活動設計，加以彙整後擬定出初步的課程設計大綱，而方案之實施原則包括：1、由於時間的限制，教學方案中宜先著重幾個較為重要的品格特質，如勇氣、正義、公平、人道與關懷都等共通與不變的核心價值；2、重視方案的外在效度，強調真實世界研究（real-world research），在方案中應包括「了解正向特質」、「培養正向情緒」、以及「實踐正向行為」，讓學生展現積極的自我；3、除量化研究外，同時結合質性或民族誌的研究；4、評估方案預期的與非預期的結果，例如減少大學生的不良適應行為、方案的實用性與費用等。

O41

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。

關鍵詞：問題導向服務學習、正向心理學

O42

(計畫名稱：正向情緒在認知、壓力事件後的生理復原、以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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這幾年所興起的正向心理學理論顯示：正向情緒對個體不管是在復原力、身心健康、或是認知功能上，皆有著關鍵性的影響。但目前僅有數量極少的實證研究可以提供理論上的之支持。本研究是以正向情緒的擴展-建構理論 (Broaden-and-build theory of positive emotion)作為參考的架構，以多重的研究法(multi- method approach)來探討正向情緒對於個體的生理、認知、和社會適應的影響。第一年的研究是以實驗室法探討以下的研究問題：正向情緒是否會影響個體在壓力事件之後，各項壓力反應的生理指標回復至基準線的速度；此外，實驗中也將採用受試者的自陳式報告(self- report) 作為測量情緒狀態的另一項指標。研究結果對於仍處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。另外在臨床心理學的運用方面，本研究的結果可以對適應的機制，提供較周延的解釋。此外對於心理衛生的預防和介入策略的討論，也會具有相當重要的價值。

O43

慈濟志工的正向心理研究

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正向心理學研究旨在探討正向心理特質退人類社會的重要影響。1990 年代開始積極倡議的正向心理學，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」；在 24 項特質中，感恩、知足、包容、善解、助人、正直等，都是慈濟人文的重要內涵。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。本報告為第一年度計畫進度報告，重點在說明本計畫近半年來資料收集概況，主要為：(1)已出版的文獻資料，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。

O44

印尼紅溪河整治效應之科際整合研究

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印尼雅加達的紅溪河整治計畫於 2002 年開始整治，這個由印尼慈濟人主動發起進行的遷建計畫，不僅受到印尼政府及當地回教居民的高度肯定，而且成為國際救援組織矚目的案例。但是這個規模龐大的援助計畫實施成效如何？特別是被援助者對於這個遷居計畫的主觀感受，遷居後的適應狀況，特別是基本生活需求(如營養、健康)和較高層次的需求(如未來期望與自我實現)，是否獲得相當程度的滿足？援助計畫是否還有需要增強之處？這些問題都需要透過客觀的科學實證研究資料予以確定。本計畫的目的在透過科際整合的研究範式，探討紅溪河整治計畫對居民的健康、文化、教育、宗教和心理層面的效應。研究期間自民國 95 年 8 月起至民國 98 年 7 月止，預定三年完成。本計畫以大愛一村為具體的研究對象，探討住戶在遷移前與遷移後適應狀況的差異，以及遷移後的社區組織與文化特色。參與本計畫的子計畫共五個，涵蓋的面向包括：(1)健康適應；(2)跨文化能力、學習投入與利他表現；(3)宗教會遇；(4)社會福利需求；(5)族群關係與文化發展。本報告將分別說明至 97 年四月為止，大愛村遷移後的概況，以及村民在前述五個面向的適應狀況。

子計畫一的初步分析結果顯示：當地居民最重要及最需要優先處理的兩大健康問題皆為傳染性疾病，分別為肺結核病及皮膚病。因此本年度本團隊與印尼大愛醫院共同設計完成肺結核防治的知識、態度、行為的問卷，並分在學校及社區進行前測、介入及後測。

子計畫二的初步分析結果顯示：(1)雖然學生的學習方法與意志力仍待增強，但教育援助對學生「知與自我實現」的成長需求及希望感的增強，似乎相當明顯。(2)大愛慈悲跨越宗教國界，伊斯蘭教學生呈現出跨文化能力，能包容佛教與華人，並理解人類群體也具有共通性。(3)感恩慈濟志工的助人情懷，但學生的利他行為尚未深化。

子計畫三的初步分析結果顯示：慈濟對「大愛一村」的居民而言，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。村民和佛教的慈濟團體的互動，在宗教信仰方面保持禮敬的距離。村裡受訪的多位宗教教師視慈濟的價值觀和伊斯蘭教理相同，如幫助別人，保持和平，尊重其他宗教。他們在道德教育的基礎上肯定「靜思語錄」之為實際的品行指導，亦即以各文化共同關懷的德行主題，產生宗教會通與交流。

子計畫四的研究結果尚待處理。

子計畫五的初步分析結果顯示：(1)在族群距離與刻板印象方面，發現遷入大愛村的印尼民眾對一般華人的社會關係仍然維持相當程度的距離；(2)接受華人成為鄰居、工作伙伴及領導人的意願，和年齡顯著的成正比，也就是年齡越大者，越有意願和華人成為鄰居、工作伙伴，也比較願意接受華人的領導。(3)在社會文化發展方面，我們歸納出五個和社會行為及文化發展有關的問題：(1)高度依賴外來補助；(2)有些居民想將房子出脫，賺取權利金；(3)潛在衝突；(4)社區參與不足；(5)不安全感。這些問題又與貧窮、社區能量不足及居民缺乏合作精神有關。

O45

(計畫名稱：紅溪河整治方案之公共衛生影響評估)

環境改變對健康的影響：以印尼大愛村居民肺結核防治為例

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本計劃目的在了解紅溪河整治工作及大愛村居民在遷村後環境的改變對健康的影響，透過有系統的社區健康評估，以健康促進介入方式，改變大愛村民對健康知識、態度及行為的改變。

研究方法：透過實地考察、印尼慈濟中小學師長座談及大愛醫院團隊醫師座談會，以了解當地居民最優先處理的問題。並設計介入方案整合大愛醫療資源及志工系統進行介入措施。

研究進度及結果：當地居民最重要及最需要優先處理的兩大健康問題皆為傳染性疾病，分別為肺結核病及皮膚病。因此本年度本團隊與印尼大愛醫院共同設計完成肺結核防治的知識、態度、行為的問卷，並分在學校及社區進行前測、介入及後測。

目前在印尼慈濟中小學以健康促進學校的概念進行充能及觀念的溝通，預計 5-6 月完成在校三年級以上學生約 400 人前測，暑假過後開始進行肺結核防治嵌入式教學計畫；並設計家庭聯絡簿，一週一次將每週在學校的肺結核知識宣導重點抄在聯絡簿給家長簽閱，一學期結束後進行後測。而大愛一村社區 18 歲以上民眾約 400 人，則是透過肺結核篩檢時請訪員進行訪視調查，訪視結束後由訓練志工對篩檢出有病的肺結核病人進行「送藥到手、服藥入口、吃完再走」的肺結核直接觀察療法，並進行防治宣導。針對沒有罹病者也透過社區志工宣導及人際傳播舉辦活動讓居民了解肺結核的防治，以增加民眾對肺結核防治的知能。

預期結果：本計劃透過當地居民的共同合作，整合醫師、學校老師、家長、社區民眾及慈濟志業，透過介入性的活動，以期提高不同年齡層對肺結核健康知能的提升，以期增加肺結核完治率及治癒率、減少肺結核二次感染率、未來可能整體降低大愛村民肺結核罹患率。

O46

雅加達大愛村的宗教會遇經驗

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本研究為「紅溪河整治效應研究」整合計畫的子計畫「宗教會遇經驗」，研究第一階段重點為：伊斯蘭教的災民如何接納慈濟之為佛教團體的援助？他們的宗教生活和信仰是否因和慈濟的接觸產生變化？從他們信仰的角度，如何看待解讀慈濟的助人行為，以及慈濟志工所介紹的 證嚴法師印尼文的「靜思語錄」？

研究的初步結論是，慈濟對「大愛一村」的居民而言，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。村民和佛教的慈濟團體的互動，在宗教信仰方面保持禮敬的距離。村裡受訪的多位宗教教師視慈濟的價值觀和伊斯蘭教理相同，如幫助別人，保持和平，尊重其他宗教。他們在道德教育的基礎上肯定「靜思語錄」之為實際的品行指導，亦即以各文化共同關懷的德行主題，產生宗教會通與交流。參與慈濟志工服務的伊斯蘭教婦女，較從提升自我價值和精神品質的角度談心得，甚且認為服務更堅定他們的信仰。

本研究也訪問慈濟援助米糧的雅加達奴魯亞伊曼習經院院長和師生，觀察其和慈濟志工互動的情形，發現他們亦在道德關懷上和慈濟和諧互動，甚至共事。本研究據此進行助人行為牽涉的概念之宗教間初步比較，期望進一步建立宗教對話的模式。

O47

跨文化能力、學習投入與利他表現

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本研究第一年的研究重點在於了解研究場域，並與受訪者建立互信基礎。初步的資料顯示：1、雖然學生的學習方法與意志力仍待增強，但教育援助對學生「知與自我實現」的成長需求及希望感的增強，似乎相當明顯。2、大愛慈悲跨越宗教國界，伊斯蘭教學生呈現出跨文化能力，能包容佛教與華人，並理解人類群體也具有共通性。3、感恩慈濟志工的助人情懷，但學生的利他行為尚未深化。

2007 年 7 月起，印尼慈濟中小學為了提升學生的競爭力，開始招收村外學生，並從高職部甄選了 8 名表現優異的學生擔任夜間課業輔導小老師，此外，還自台灣聘請了 4 位教師負責中文教學。基於上述學校措施的改變，再加上原定之目的，第二年研究探討的重點包括：1、了解村外學生的適應與感受；2、訪問擔任課輔小老師的輔導經驗；3、了解印尼慈濟中小學的行政措施與教師教學感受，作為訪談與量表編製的依據；4、拜訪紅溪河邊學校，探詢量表施測的可能性與對象；5、訪問分會負責教育援助的同仁，了解印尼教育援助的類型與成效。

在田野實察部分，共計訪問 25 位村外學生（10 位高職學生、15 位國中生）、8 位高職課業輔導小老師、7 位印尼籍教師、4 位中文教師、2 位印尼基金會負責教育援助的同仁。從村外學生的訪談資料，再次印證慈濟中小學在多數人心目中的正面感受：環境設備好、教師教學認真、重視品格教育以及有紀律。教師和學生對夜間課業輔導均持肯定態度，不僅改善村內部分學生不寫功課的問題，也因而減少師生衝突；而課輔經驗對這些小老師來說，不僅營造善的循環，也是「助人助己」的好方式，學生表示「不同的經驗就多了一種可能性」、也印證了可蘭經的教義：「ApaGila ada orang yang bertanga kepach kita maka apaGila kitd tahu, kita wajib untuk memberitahunya.」（如果別人問問題而你知道答案時，應當竭盡所能告訴他）。

系統化的中文教學以及「過新年」主題週的設計，都讓印尼籍學生和教師對華人文化有更多的熟悉和認識。到紅溪河邊拜訪過去學生就讀的學校，已取得相關人員的首肯協助問卷的填寫。訪談印尼分會負責教育援助的同仁後發現，金融風暴後需要援助的學生已擴及至當地華人，而分會在這個部分的經費支出每年都超過原訂比例。但由於天災頻仍，慈善與醫療援助佔去分會多數人力與時間，無法定期關懷受援助學生，加上部分家長仍將孩子視為家庭的勞動人口，導致社會階級複製的情形仍無法避免，原先的教育援助成效也就大打折扣。由此也對映出印尼慈濟中小學這樣的教育援助方案，比較可能藉由長期的經營，達到協助學生脫貧與發揮潛能的教育功效。

O48

社會福利需求

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承襲本研究在期初研究的成果與發現，在第二年的研究重點，除了繼續對第一期的討論做資料的蒐集及佐證之外，另者是對整個研究的工作進一步探詢做問卷的思考和設計。雖然在這一次的跨國研究中，是否能夠在文化及語言的差異之下，同時加上諸多未來調查工作進行上的各種不便和限制，在在都影響著本研究在思索利用何種研究方法或工具上的困擾。但為了配合研究發展的進行，因此，初步仍以問卷的調查方式為主要的考量，而再搭配若干關鍵人物的深度訪談，同時再配合對當地相關文獻資料的蒐尋作為整個研究資料的彙集和整理。

因此在第二期工作中的進行上，是思考整個問卷設計上的面向和問項上的討論，而目前的進度則是將問卷的雛型做一呈現（如附件），此乃一個簡單和基本的開始。而本研究小組成員目前則是繼續對此問卷的完整性做意見的提供和完整，並企盼在今年的五月底之前能有一個完整的成品，屆時再透過翻譯的過程，將問卷改為符合印尼的語言，接著再依研究的步驟做相關的後續工作。希冀能在今年的六月至七月期間能有到印尼做調查的相關研究作業，以為本研究的第二年工作做一階段性的完成。

藉由第二年的研究發現以為第三年工作的鋪陳，屆時將提出對問題的發現及對策的提供，同時也為此等國際援助塑造一個工作模式或？型，以為後續相關研究的參考。

O49

族群關係與文化發展

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本計畫以遷居大愛村的民眾為對象，主要目的在探討：遷居至大愛村這樣重大的環境改變，對原先居住在紅溪河民眾的社會文化適應有何影響？特別是在族群關係與社區文化的發展方面，本計畫著重的主要問題包括：家庭成員的互動模式有何顯著的改變？居民的自我概念與未來的期望為何？社區文化（如社區成員的互動、人際關係與傳統文化等）展現何種特色？居民的族群刻板印象有無顯著改變？與華人的族群互動是否明顯增加？本計畫第一階段的資料分析，顯示下列幾點主要研究發現：(一)在族群距離與刻板印象方面，發現遷入大愛村的印尼民眾對一般華人的社會關係仍然維持相當程度的距離：在 10 種社會關係中，僅對「成為工作伙伴」有比較明顯的正向傾向，在其餘 9 種關係上則不願意和華人太過接近；但是對華人的刻板印象有相當正向的評價，依頻率高低排名，華人獲得較高分數的前十名（包括 13 種特質）依序如下：(1)渴望進步、(2)工作勤奮、(3)聰明、(4)整潔、(5)家庭聯繫強、(6)誠實、(7)有禮、(8)喜捨等；接下來的評價包括：(9)多疑、(10)值得信任、(11)好心腸、(12)井然有序、(13)友善。其中僅有多疑為負向評價，顯示他們對華人大多保持正向的刻板印象。另一項相當有趣的發現是：接受華人成為鄰居、工作伙伴及領導人的意願，和年齡顯著的成正比，也就是年齡越大者，越有意願和華人成為鄰居、工作伙伴，也比較願意接受華人的領導。我們認為導致這個結果的主要原因是年長者在遷移過程中，和華人的接觸與互動較多，對印尼慈濟人所代表的華人意象也比較正向。年輕人則因在外工作時間較長，與華人的接觸較少，因而保留較多主流社會的刻板印象及族群互動模式。(二)在社會文化發展方面，我們根據在村內的訪問與觀察，歸納出五個和社會行為及文化發展有關的問題：(1)高度依賴外來補助；(2)有些居民想將房子出脫，賺取權利金；(3)潛在衝突；(4)社區參與不足；(5)不安全感。這些問題又與貧窮、社區能量不足及居民缺乏合作精神有關。

O50

Nicotinic acetylcholine receptor and neurovascular function

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The main purpose of this proposed program project is to develop a core research group in the area of neuro-vascular function at Tzu Chi University. This proposal includes two senior professors, 1 associate professor, and 1 junior assistant professor as principle investigator at the time of application. The scientific theme for this project has been to study the role of nicotinic receptors in mediating neurotransmission in the central and peripheral nervous system, and the glia, and its influence by beta-amyloid (Aβ) peptides and statins in regulating vascular function. Aβs have been shown to play a key role in pathogenesis of Alzheimer disease (AD), while epidemiological studies have reported that statins, the lipid-lowering drugs, are beneficial in treating this devastating disease, probably not by lipid lowering effects of these drugs. These projects has been supported by three cores; an in vitro tissue bath technique core for measuring vascular reactivity, an in vivo experimentation core for measuring blood flow and systemic blood pressure, and tissue culture core. This program project includes 4 subproject: project #1, Sympathetic nAChR and cerebral nitrergic neurogenic vasodilation; Project #2, Control of common carotid arterial blood flow by nicotinic, glutamatergic, and nitrergic actions in the medulla of cats; Project #3, The effects of amyloid beta-peptides on the function of nicotinic and glutamatergic receptors in central sympathetic neurons of rats; Project #4, Role of nAChR and its effects by Aβ and statins on glia cell function. Each project has made significant progress during the past year and half, and has generated interesting information for advancing our understanding of the nature of neuronal nicotinic receptors and their roles in regulating neurovascular transmission and vascular functions in health and disease.

O51

Sympathetic nAChR and cerebral nitrergic neurogenic vasodilation

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Cerebral blood vessels from several species receive both vasoconstrictor and dilator nerves. The close apposition of the sympathetic adrenergic and the parasympathetic nitrergic nerve terminals is a unique feature in large cerebral arteries of different species, allowing axo-axonal interaction between both types of nerve terminals in regulating the vascular smooth muscle tone. We have demonstrated in the pigs in USA that functional α7-nicotinic ACh receptors (α7-nAChRs) are present on the cerebral perivascular sympathetic neurons. Stimulation of these receptors on the sympathetic nerves by nicotinic agonists causes release of norepinephrine (NE), resulting in facilitation of nitric oxide (NO) release from the neighboring nitrergic nerve terminals and vasodilation. This α7-nAChR-mediated relaxation was blocked by β-amyloid (Aβ, a key factor in pathogenesis of Alzheimer's disease/AD). Preliminary results indicated that similar axo-axonal interaction in nicotinic agonist-induced neurogenic nitrergic vasodilation in cerebral arteries of the pigs in Taiwan was observed. This nicotine-induced vasodilation, however, was not affected by Aβ, questioning that the nAChR on the sympathetic neurons is of an atypical α7- or a non-α7-nAChR-subtype. We therefore examined the nature of the cerebral sympathetic nAChR in the pigs in Taiwan. Using in vitro tissue bath technique, we demonstrated that nicotine-induced, nAChR-mediated dilation of the basilar arteries was not blocked by α-bungarotoxin, a selective α7-nAChR antagonist, at the concentrations up to 3 μM. The nAChR-mediated vasodilation, however, was inhibited by tropane and tropinone (nonselective α3 and α6 subunit-containing nAChRs antagonists). Furthermore, the vasodilation was inhibited by α-conotoxin MII (0.3 μM, a selective α3β2-nAChR antagonist). Furthermore, using reverse transcription polymerase chain reaction, we found that the superior cervical ganglions (SCGs), the origin of perivascular sympathetic nerves, contained α3 and α7 subunits. These data indicate that the predominant functional nAChRs located on the perivascular sympathetic nerves of the pigs in Taiwan are of α3β2 subtype. This may explain the failure of Aβ in blocking nicotinic agonist-induced cerebral nitrergic vasodilation.

O52

Control of common carotid arterial blood flow by nicotinic, glutamatergic, and nitrergic actions in the medulla of cats

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Nitric oxide (NO) or glutamate stimulation of dorsal facial area (DFA) increases blood flow of common carotid artery (CCA) that supplies intra- and extra cranial tissues. Nitrergic fibers and neurons as well as preganglionic cholinergic neurons are present in the DFA. We hypothesized the presence of nitrergic-glutamatergic fibers and preganglionic nitrergic-cholinergic neurons in the DFA for regulation of the CCA blood flow. In microdialysis studies, perfusion in the DFA of S-nitroso-N-acetylpenicillamine (SNAP, an NO donor) increased glutamate concentration in the dialysate. This effect was abolished by co-perfusion of methylene blue (a guanylyl cyclase inhibitor). Intra-DFA injections of L-arginine (an NO precursor) or glutamate increased the CCA blood flow. L-arginine-induced flow increases were reduced by prior administrations of N^G-nitro-arginine methyl ester (L-NAME, a non-specific NO synthase inhibitor), 7-nitroindazole (7-NI, a relatively selective neuronal NO synthase inhibitor) as well as D-2-amino-5- phosphonopentanoate (D-AP5, a competitive N-methyl-D-aspartate (NMDA) receptor antagonist), and glutamate diethylester (GDEE, a competitive α-amino-3-hydroxy-5- methylisoxazole-4-propionic acid (AMPA) receptor antagonist). Glutamate-induced flow increase was reduced by prior administration of L-NAME, 7-NI and methylene blue. The induced increases in the CCA blood flow, however, were not affected by endothelial NO synthase inhibitor. Findings indicate that in presynaptic nitrergic-glutamatergic fibers, the activated neuronal NO synthase and guanylyl cyclase might cause release of glutamate; glutamate activates NMDA and AMPA receptors on preganglionic nitrergic-cholinergic neurons to activate neuronal NO synthase and guanylyl cyclase in the neurons, leading to an increase in the CCA flow. In conclusion, nitrergic-glutamatergic fibers and preganglionic nitrergic-cholinergic neurons may be present in the DFA for regulation of the CCA blood flow.

O53

The effects of amyloid beta-peptides on the function of nicotinic and glutamatergic receptors in central sympathetic neurons of rats

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The abnormal accumulation of amyloid beta-peptides (Aβ) has been considered one of the main characteristics of Alzheimer's disease (AD). Cerebro- and cardiovascular diseases are known to be risk factors for developing AD. Aβ has been shown to induce vascular dysfunction. The effect of Aβ on central control of cardiovascular function, however, is poorly understood. Rostral ventrolateral medulla (RVLM) neurons and sympathetic preganglionic neurons (SPNs), located in the intermediolateral (IML) column of thoracolumbar spinal cord, are key neurons involved in the regulation of sympathetic activity and cardiovascular function. Acute application of Aβ recently has been found to produce profound effects on the function of neurotransmitter receptors and the underlying synaptic transmission in several neuronal preparations. Whether Aβ affects the activation of neurotransmitter receptors in central sympathetic neurons remains unclear. The present study was undertaken to examine effects of Aβ on the function of neurotransmitter receptors especially NMDA receptors in rat RVLM neurons and SPNs. Repeated microinjections of NMDA (0.14nmol) into the RVLM every 30 min caused reproducible increases in mean arterial pressure in urethane-anesthetized rats. Microinjection of lower doses of Aβ1-40 (20 pmol) into RVLM 10 min pre-injection of NMDA significantly potentiated NMDA-induced pressor effects. In the *in vitro* electrophysiological study, consecutive applications of NMDA every 5 min induced reproducible membrane depolarizations in SPNs of neonatal rat spinal cord slice preparation. Superfusion of Aβ1-40 (0.1 and 0.3 μM) for 5 min significantly potentiated NMDA-mediated depolarizations in a reversible manner. Aβ (0.3 μM) had no significant effects on AMPA-induced depolarizations or GABA-induced hyperpolarizations. Western blot analysis showed increases in the levels of phosphoserine 897 (regulated by protein kinase A) and phosphoserine 896 (regulated by protein kinase C) on NMDA NR1 subunit subunits in IML areas of thoracic segment spinal cord slices at 10, 30 and 60 min following incubation of Aβ (0.1, 0.3 μM). These results suggest that acute Aβ selectively increases NMDA receptor function in central sympathetic neurons and kinase-dependent mechanisms are involved in Aβ sensitivity of NMDA receptors.

O54

Effects of nAChR, Aβ and statins on glia cell function

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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 two types of glia cells, astrocytes and microglilal cells, 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). These results indicate that nicotinic agonist-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.

O55

(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 Diabetes Mellitus

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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, the biomedical studies about the progress and concurrent comorbid conditions of DM are important. In this study, we had focused the influence and clinic applications, such as inhalation anesthetics and hyperbaric oxygenation in the streptozotocin induced type I DM of adult male Spraque-Dawley rats. Using RT-qPCR, the circadian rhythm-related genes, such as *period1 (Per1)*, *Per2*, and *Per3*, still showed the patterns of diurnal rhythmicity in the liver, kidney and spleen of control groups. However, expression levels of these genes in the type I DM groups were significantly influenced. In addition, the frequency-domain analysis of telemetric systemic arterial pressures (SAP) and pulse-pulse intervals (PP) were applied to quantify the parameters of blood pressure variability (BPV) and heart rate variability (HRV) as the functional indices of autonomic nervous system. We found that SAP and vascular sympathetic modulation were significantly decreased and PP, cardiac vagal modulation, and total autonomic cardiovascular function increased by hyperbaric oxygenation. Furthermore, mean blood pressure and cardiac sympathetic modulation did not be changed during three inhalation anesthetics (halothane, desflurane and sevoflurane) exposure in the type I DM groups. Vascular sympathetic modulation was significantly decreased at 1 and 1.3 MAC of desflurane and sevoflurane but halothane just 1 MAC when compared to rat awake. In conclusion, these findings of the integrated study are invaluable and may provide benefit at the clinical application in DM patients in the future.

O56

(計畫名稱：Relationships between rhythm-related genes and type I and II diabetes mellitus)

Expression of Circadian Rhythm-related Genes Influenced by Streptozotocin-induced Type I Diabetes Mellitus in Rats

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In past the studies of circadian rhythm-related genes were focused on the relationships with the circadian generator and the biological clock. Recently Turek et al. (2005) showed the *Clock*, one of circadian rhythm-related genes, mutant mice were easy to be obese and have the metabolic syndromes. These studies support that the circadian rhythm-related genes are important to maintain not only the biological clock but also the peripheral physiological functions. Adult male Spraque-Dawley rats were intraperitoneally injected streptozotocin (60 mg/kg) and monitored the levels of blood sugar and urine sugar after three days to examine whether they were induced as diabetes mellitus. The control group with vehicle injection received the same examination. Animals were sacrificed two or four weeks after treatment at two or nine hours after light-on. The expression levels of circadian rhythm-related genes, such as *period1 (Per1)*, *Per2*, and *Per3* in the liver, kidney and spleen were examined by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR). The gene expression of *Per1*, *Per2* and *Per3* showed the diurnal rhythm in the liver, kidney and spleen at the control groups. The expression of circadian rhythm related-genes in streptozotocin -induced diabetes mellitus groups were changed in deed, and showed the different trends. In conclusion, these changes of circadian rhythm-related gene expression in peripheral tissues are related to the functioning or daily pattern of metabolic processes. The causal relations between these are needed to approach by the further studies.

O57

(計畫名稱：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)

To evaluate the effects of different inhalation anesthetics on the cardiovascular neural regulation of autonomic nervous system in the type I and type II diabetic rat

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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 insulin-dependent diabetes mellitus is associated with alternations in autonomic nervous system control of cardiovascular function. The specific aim in this study is to elucidate the effects of different inhalation anesthetics on cardiovascular autonomic functions in diabetic rats. We measured daily blood pressure variability (BPV) and heart rate variability (HRV) of streptozotocin (STZ, 60 mg/kg, ip) induced diabetic Sprague-Dawley rats and vehicle control groups exposure the different inhalation anesthetics (halothane, desflurane and sevoflurane) and recorded to recovery stage. Frequency-domain analysis of telemetric systemic arterial pressures (SAP) and pulse-pulse intervals (PP) were applied to quantify the parameters of BPV and HRV. High frequency power of HRV (HHF) was regarded as the cardiac vagal modulation. Low frequency power of BPV (BLF) was referred to the vascular sympathetic modulation. Normalized low-frequency power (LF %) of the RR spectrogram was regarded as the cardiac sympathetic modulation.

Our results show that STZ-induced diabetes was associated with significant reduction of HR and higher HHF among the three inhalation anesthetics. Mean blood pressure (MBP) and LF% were not changed after STZ injection as compared with vehicle controls during three inhalation anesthetics exposure. BLF was significantly decreased at 1 and 1.3 MAC of desflurane and sevoflurane but halothane just 1 MAC when compared to rat awake.

In conclusion, dose effects of desflurane and sevoflurane but halothane significantly depressed the vascular sympathetic modulation. STZ-induced diabetic rats have higher cardiac vagal modulation when used the inhalation anesthetics. Our results may provide partial benefit at the clinical anesthesia at diabetes patients.

O58

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

Hyperbaric Oxygenation Augments the Cardiovascular Autonomic Neural Regulation in Streptozotocin Induced Type I Diabetic Rats

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Our previous publication demonstrated that the cardiac neural dysfunctions in diabetic foot patients are attenuated by programmed hyperbaric oxygen therapy. The specific aim in this study is to elucidate the effect of hyperbaric oxygenation on cardiovascular autonomic functions in diabetic rats. We measured daily blood pressure variability (BPV) and heart rate variability (HRV) of streptozotocin (STZ, 60 mg/kg, ip) induced diabetic Sprague-Dawley rats and vehicle control groups during the duration of ten 60-minute treatments with either 1 ATA air, 3 ATA air or 3 ATA oxygen. Frequency-domain analysis of telemetric systemic arterial pressures (SAP) and pulse-pulse intervals (PP) were applied to quantify the parameters of BPV and HRV. High frequency power of HRV (HHF) was regarded as the cardiac vagal modulation. Low frequency power of BPV (BLF) was referred to the vascular sympathetic modulation. Variance of HRV (HVar) was regarded as the total autonomic cardiovascular function. Our results show that hyperglycemia and glucosuria are evident in STZ treated animals. SAP and HVar were not changed after STZ injection as compared with vehicle controls. SAP, BLF were significantly decreased and PP, HHF, HVar significantly increased by hyperbaric oxygenation in diabetic rats. HHF was not changed, however BLF was significantly decreased, in the hyperbaric air group.

In conclusion, hyperbaric oxygen therapy, 3 ATA, 100% oxygen, 10 dives, augments the cardiovascular autonomic regulation of STZ induced diabetic rats by increasing the cardiac vagal modulation and decreasing the vascular sympathetic activities. Oxygenation plays a major role on regulation of cardiac vagal functions and pressurization contributes, at least in part, to vascular sympathetic modulations in our experimental model.

O59

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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東台灣因先天地理環境的阻隔，族群的多樣化，且原住民人口為全國比例最高之區域，幽門桿菌的致病性也有地緣與種族的特異性，因此是個研究幽門桿菌基因型別與胃癌發生自然史之關係的獨特且優良的場域，而慈濟醫院又是花東地區唯一的醫學中心，扮演解除疾病的威脅、守護東台灣住民健康與生命的重要角色，因此發展東台灣胃癌及幽門桿菌之研究中心，並建立胃癌基因診斷、生成機制及預警系統是為本研究團隊的首要任務與目標。為達成此工作目標，本研究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」。在三年的研究計畫中，我們將利用分子流行病學、實驗動物模式、基因體系統性方法探討台灣東部原住民幽門桿菌感染與胃癌發生之關係，致力於胃癌發生和發展過程中，幽門桿菌基因型、環境及宿主基因等影響因子之研究，並藉由蛋白質體學(proteomics)及轉錄體學(transcriptomics)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。

各子計畫均按照預定進度在執行，並有相關的研究成果呈現，在這近一年半的研究成果中，已可見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(子計畫一)；以免疫組織化學染色法發現原住民患者之胃黏液醣蛋白基因 MUC2 與 Ki-67 的表達皆較漢人患者來的明顯(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，此動物模式已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)。

O60

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

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Gastric cancer (GC) is the second commonest malignancy in the world. In Taiwan, there is definite geographic variation in GC incidence and death, with the higher rates seen in the aboriginal populations than Han's group. It was assumed that there were different susceptible factors existed between these two groups, such as genotypes of the virulent genes of *H. pylori*, host genetic factors, and environmental factors. Therefore, to evaluate the epidemiology of GC-related risk factors is important to clarify the mechanism of gastric carcinogenesis. The specific aims of this study include (1) to determine the prevalence of *Helicobacter pylori* infection and the genotypes of virulence-related genes (2) to investigate genetic polymorphisms of carcinogenesis- related genes to explain individual variability in gastrocarcinogenesis. We used the PCR-RFLP to determine the genotypes of *H. pylori* virulent genes and the host's gene polymorphism of immunoregulatory molecules. The results shown the prevalence of *H. pylori* was higher and the age was younger in aboriginal population than Hans'. The distribution of virulent factors, *vacA* and *iceA*, were significantly different between aboriginal population and Han. Moreover, the SNPs of *ICAM-1* K469E were associated with the susceptibility to the naïve immunity of aborigines but not Han's group.

O61

Isolation of virulence genes in *Helicobacter pylori* from eastern 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 in male the most common cancer sites of Taiwan Aborigines were stomach, liver and lung. In female, the most common cancer sites of Taiwan Aborigines were cervix uteri, stomach and lung. Therefore, the aim of our study is to examine the special virulence factor of *Helicobacter pylori* from Taiwan Aborigines. 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. To identify genes involved in virulence, we began to construct a mutant /or expression library derive from the most virulent strain A699. Furthermore, we use the A699 induces elongation responses in gastric epithelial cells. In the future, we want to find out which signaling pathways were important in the A699 induced cell elongation phenotype. Thus, maybe we will find some new virulence associated genes out in *Helicobacter pylori*. Then, these virulence associated genes will be further analyzed.

O62

Development and application of *Helicobacter pylori*-infected Rat Model

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Infection of human stomach by *Helicobacter pylori* has been thought to induce acute and chronic gastritis, and to be associated with peptic ulcers. Nowadays, although the mechanism is still not well known, *H. pylori* has been thought to be an independent risk factor for gastric cancer from the data of epidemiological studies. In addition, the results obtained with an animal model, the Mongolian gerbil, mimicking the gastric carcinogenesis steps after *H. pylori* infection, is another strong argument. In our country, *H. pylori* infection was found in one half of general population, and gastric carcinoma was also an important fatal disease. The complexity of host-*Helicobacter* relationships with large variations in the severity of disease in infected population suggests that it is dependent on uncharacterized host factors, and the potentially specific disease related bacterial virulence factors. This study was designed to determine whether *H. pylori* will colonize the gastrointestinal tract of rats. The preliminary results showed (1) the best conditions of infection were *cagA*-positive *H. pylori* after cultured 24 hr, 1 x 10^9 CFU/ml feeding at least every time; (2) six to eight-week-old male Wistar rats were the suitable animal model for *H. pylori* infection; (3) active chronic gastritis observed in rats 26 weeks after *H. pylori* inoculation; (4) expression of cyclooxygenase-2 (COX-2) mRNA in gastric mucosa was significantly increased in H. pylori-infected rats; (5) immunohistochemistry showed increased expression of inducible nitric oxide synthase (iNOS) and COX-2 genes in *H. pylori*-infected rats.

O63

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

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Helicobacter pylori, the major etiological agent of chronic gastritis and peptic ulcer disease, is found both in the mucous gel layer of the stomach and attached to the gastric surface mucous cells. One of the possible mechanisms through which *H. pylori* may injure the gastric mucosa may be by impairing the effectiveness of the protective function of the mucins. Our goal was to determine the distribution of mucins (MUC1, MUC2, MUC5AC, MUC6) and Ki-67, a proliferation-associated nuclear antigen, in the gastric biopsy specimens, which were enrolled from the patients who undergoing upper gastrointestinal endoscopy at Buddhist Tzu Chi General Hospital, with relation to the *H. pylori* status by the immunohistochemical analysis. So far, the results reveal that differential expression of MUC2 and Ki-67 between *H. pylori*-infected nonaboriginal and aboriginal group. In addition, the secretion of mucous in *H. pylori*-infected aboriginal patients was much lower than the *H. pylori*-infected nonaboriginal patients. In conclusion, the present study indicates that the MUC2 mucin and Ki-67 antigen expression pattern is likely a reliable marker, which appears in the context of *H. pylori* infected aboriginal individuals. The relationship among the aboriginal genetic factors involved the incidence of gastric carcinoma, MUC2 mucin, and Ki-67 antigen expression will be studied in the future.

O64

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

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Gastric cancer (GC) is not only the second most frequent form of cancer worldwide, but also represents the fourth leading cause of cancer death in Taiwan. 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 diagnosis, therapy and vaccine development and to investigate potential associations between specific immune responses and manifestations of disease. We performed comprehensive proteome analysis of tumor and nontumor tissues, and identified several proteins of which the expression levels are commonly altered in clinical cases. In addition, we utilized the RT-PCR to verify the genes that are differentially expressed in gastric adenocarcinoma in comparison to non-tumor mucosa. The results showed that all these 9 genes (*ATF3*, *FOSL1*, *CYCS*, *COPEB*, *SERTAD1*, *FOS*, *DNAJB1*, *GADD45A* and *HSPA1A*) except ATF3 and CYCS were expressed in *H. pylori*-positive gastric cancer mucosa and matching non-tumor mucosal tissue. Only the transcripts of c-Fos, COPEB and HSPA1A were detected in the *H. pylori*-negative gastric mucosal tissue. In conclusion, we propose that this "proteome" approach for identification of previously unknown proteins will be useful in examining regulation of *H. pylori* gene expression and protein localization in the development. This approach will also be useful for identifying potential targets for antimicrobial or vaccine development.

O65

(計畫名稱：苦瓜對肝細胞病生理影響之研究)

The Pathophysiological Effects on Liver Cells by *Momordica charantia*

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Liver diseases are considered to be an important health problem that result in serious health problems and exhaust lots of medical resources. It is becoming increasingly evident that chronic viral or metabolic liver diseases are at risk for the development of hepatocellular carcinoma. Obesity, a rapidly growing health issue, is a risk factor for cardiovascular, metabolic, neoplastic and sleep-disorder complications. Numerous efforts have been directed at the development of effective liver-specific therapeutic strategies by natural products. Diets rich in bioactive phytochemicals are used for both prevention and treatment of liver diseases recently. *Momordica charantia* was reported to have some biomedical activities such as anti-inflammation, anti-virus, anti-tumor and anti-diabetes. Although our previous study had shown that some species of *Momordica charantia* have a significant growth inhibitory effect on colon cancer cells, the effect of this diet on liver diseases, including hepatocellular carcinoma in chronic viral or other metabolic liver diseases has not been investigated. The aims of this integrated study are to investigate the anti-virus, anti-diabetes and anti-tumor effect of *Momordica charantia* on hepatocellular carcinoma cells or other assay systems through the analysis of sequence of extracted bioactive phytochemical components.

O66

Investigation of the molecular mechanism on anti-tumor effect of *Mormodica charantia*

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Momordica charantia, commonly called bitter gourd, was recently reported to have some biomedical activities such as anti-tummor and anti-virus effects. Our previous investigations revealed that ethanol crude extracts of *Momordica charantia* showed significantly different effects on inhibiting the growth of some tumor cells. Hepatocellular carcinoma cells is one of the tumor cell lines observed to be sensitive to the crude extract of *Momordica charantia*. Hepatocellular carcinoma is known to be closely associated with the infection of hepatitis B virus (HBV). It is important to realize the different efficacy of the crude extract on hepatocellular carcinoma cells with or without HBV. To further evaluate the anti-tumor potential of *Momordica charantia* on hepatocellular carcinoma, this study is designed to use Hep G2, Hep 3B, Huh 7, MSG2 and 2-2-15 cells to comparatively evaluate the differential anti-proliferation activities of *Momordica charantia* at doses from 0.1 to 2mg/ml. Among all the crude extracts, methanol extract of whole fruits was found to be more effective on the growth inhibition of hepatocellular carcinoma cells. The effective doses for cell growth inhibition of these tested cells are similar. To elucidate the possible mechanism for *Momordica charantia* to inhibit the cell growth of hepatocellular carcinoma cells, DAPI/PI staining and Annexin/PI analysis were used to comparatively evaluate the apoptosis induced in these cells by *Momordica charantia*.

O67

(計畫名稱：Studies on Inhibition of Hepatitis B Virus Replication by *Momordica charantia*)

Effects of crude extracts from *Momordica charantia* on Hepatitis B virus replication

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The *Momordica charantia* is the popular vegetable in Asia. The up-to date scientific research confirms proteins and metabolites appearing in *Momordica charantia* having efficiency of lowering blood sugar, anti-tumor and anti-virus effects. This investigation would like to evaluate the possible anti-HBV effect of crude extracts from *Momordica charantia*. Utilization of 2.2.15 cells that secret hepatitis B virions, HBV surface antigen and e antigen were measured by using ELISA in the absence or presence of various crude extracts from *Momordica charantia*. Crude extracts 6-1, 8-4, 9-9 and 9-12 reveals the suppression effect of HBV surfaces antigen synthesis after treatment for 4 to 7 days. Extracts 6-1 and 8-4 shows the reduction effect of HBV e antigen production after treatment for 4 to 10 days. Efforts trying to purify active components and analyze their anti-HBV ability effects will be further accomplished.

O68

The Screening and Functional Study of Anti-HCV Infection Activity of Effective Integrants from *Momordica charantia*

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HCV infection is one of etiological factors of hepatocellular carcinoma (HCC). HCV E1/E2 complex is required for virus entry into hepatocytes through interacted with cell surface proteins such as CD81, low-density lipoprotein receptor, scavenger receptor class-B-type-I (SR-BI), L-SIGN and DC-SIGN. However, the critical determined cellular receptor for virus infection is largely unknown. Recently, a number of studies reveal that claudin-1 plays as an essential co-receptor for HCV entry. These evidences provide an opportunity for anti-virus drug discovery and suggested a critical role of claudin-1 in virus infection. To demonstrate our viewpoint, we sough to ask whether extracts from *Momordica charantia* has anti-HCV infection ability based on blockaded interaction between E1/E2 complex and claudin-1. By utilized blot overlay method, we screened extracts from *Momordica charantia* and obtained some results. Consistent with our purpose, of interest, we also displayed an anti-apoptotic activity of claudin-1. Therefore, in addition to establish an *in vitro* system to screen herbs extracts, we showed that antagonized apoptosis ability of claudin-1 might provide an advantage for virus entry.

O69

(計畫名稱：A study on the antigluconeogenesis activity of *Momordica charantia*)

Mormordica Charantia Fruit Extracts Promote Adipogenesis and Insulin Response in NIH/3T3 Adipocytes

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The hypoglycemic effect of bitter gourd was noticed since more than 600 years ago. The bitter gourd ingredients with this effect are potential anti-diabetic drugs. To identify the compounds with this desirable effect, we used cell culture systems that can be assayed for the glucose uptake and gluconeogenesis response to insulin stimulation. Liver and several hepatoma cell lines were assayed for their gluconeogenesis activity in culture. We did not screen bitter gourd extracts with these cell lines as planned because the gluconeogenesis activities in these cell lines were low so that inhibition of these activities by insulin could not be reliably detected.

Confluent NIH/3T3 cells can be induced into insulin-responsive adipocytes with a cocktail containing dexamethasone and methylisobutylxanthine. The induction time can be shortened by adding rosiglitazone to the inducing cocktail and the adipocytes formed under this condition respond better to insulin stimulation. Earlier work in our lab showed that knocking down genes involved in adipogenesis in adipocytes often reduced insulin response so the effect on the degree of differentiation can serve as a surrogate assay for effects on insulin response. The degree of differentiation can usually be assayed by oil red O staining. We screened the extracts of bitter gourd on insulin response by replacing rosiglitazone with the extracts in the differentiation cocktail and assayed the degree of differentiation. A crude extract from the leaves and a partially purified extract from the fruit were able to partly substitute the function of rosiglitazone in the cocktail. The adipocytes formed in the presence of these extracts also responded better to insulin stimulation. Future work will focus on purifying the active principle in these extracts and their effects on insulin response in rodent models of diabetes.

O70

Isolation and characterization of terpenoid synthases and ribosome inactivating proteins from *Momordica charantia*

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In order to obtain further insights into the development and application of *Momordica charantia* (bitter gourd) as functional foods, ribosome-inactivating proteins (RIPs, EC 3.2.2.22) that represent possible pharmacological effects were focused in this study. This approach combines the research resources between five laboratories of the Life Science Department of Tzu-Chi University and the Hualien District Agricultural Research and Extension Station to study bitter gourd as an economical crop and to develop good agricultural practices (GAP) guidelines, respectively.

PCR-based cDNA libraries were constructed from different lines and tissues of bitter gourd. Degenerate primers designed from the conserved motifs of functional genes including ribosome-inactivating proteins and terpene synthases were used to amplify possible candidate clones. Isolating from the seed of bitter gourd, a cDNA fragment RIP2-12 was transformed into bacteria vector and sub-cloned into mammalian expression vector pcDNA3.1/His for further functional studies.

The appealing feature of this study is to isolated novel genes from bitter gourd with pharmaceutical importance. PCR profiling used in this approach is in principle not restricted to known genes. Once novel genes with medicinal activities are isolated, they will admit unpredictable value of both academic and commercial potential.

O71

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

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Vibrios are highly diversified in life styles, ranged from free living plankton, symbiotic partners to infectious pathogens. Certain coherent forces confine these bacterial species within one genus, and these forces might exert their power through regulatory mechanisms of bacterial responses to environmental changes. We set out to find the conserved regulation and unique feature among vibrios. The main goal of the first year is to evaluate and establish standard methods for future studies, as well as familiarize and prepare tools needed in the future.

Standard testing procedures have been tested and established for starvation, hyperosmolarity, oxidative stress, cold treatment and pH downshift. Cross protection effects were tested among combinations of stress conditions. Differential responses between log phase cells and stationary phase cells were found under several stress conditions. Different induction pattern of catalase to combat oxidative stress was seen between log and stationary phase *V. parahaemolyticus*. The discrepancy between cell number estimates derived from optical density and direct plate count were noted in both *V. parahaemolyticus* and *V. vulnificus*.

Fosmid library for both species has been constructed. Promoter library and mutant library construction have been started with some progresses. Techniques to perform zymographic analysis and in-frame deletion mutant have also been established. Collaboration among laboratories and interaction among participants were frequent, with routine group meetings held monthly.

O72

Physiological Adaptation and Gene Regulation of *Vibrio* spp. to Chemical and Nutritional Changes

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In natural marine environment, vibrios constantly face hyperosmotic stress and nutritional starvation problem. We started our study with *Vibrio vulnificus*, an environmental bacterial species as well as an infamous human and fish pathogen. Strain 93U204 selected for further study was isolated from local infected tilapia. Our multiulocus sequence typing analysis result reveal its close relatedness to local clinical isolates.

93U204 is able to grow in tryptic soy broth supplemented with 0 to 6.5% NaCl. Growth at medium supplemented with 3% NaCl or higher resulted in reduced growth rate, and a growth lag were observed after osmotic upshift treatment. Apparent discrepancy between optical density measurement and plate counting results was found when tracking the bacterial growth in broth culture, and this can lead to misidentification of growth phase. These phenomena were also seen in *V. parahaemolyticus* VP93 strain.

Log phase 93U204 greatly reduced viability after being transferred into phosphate buffer saline (starvation) or artificial seawater (starvation and hyperosmolarity) within minutes. No loss of viability in stationary phase culture under same conditions. Oxygen content in the environment may influence bacterial survival in prolonged culture, with higher survival in environment with little oxygen. Promoter library and genomic fosmid library of *V. vulnificus* strain 93U204 have been constructed and are ready for future analysis.

O73

Physiological Adaptation and Gene Regulation of *Vibrio* spp. to Oxidative Stress and Oxygen Deprivation

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Oxidative stress has been shown to be a critical problem that V*ibrio* spp. need to overcome when adapted themselves to the natural habitats. Genomic surveillance of the genes involved in detoxification of hydrogen peroxide showed that more than one copy of bifunctional catalase (KatG) and monofunctional catalase (KatE) in *V. parahaemolyticus* genome. The context of genome organization corresponding to both catalases showed arrangement different from that of *V. vulnificus*. In contrast to this, AhpC, another important antioxidant species, revealed more conserved pattern. This suggests that these two species might have evolved unique solution against oxidative stress to adapt themselves in their special niche. In order to establish a standard protocol to analyze the contributors involved in detoxification of reactive oxygen species during aerobic growth or a variety of stress conditions, overall antioxidant activity response to hydrogen peroxide in *V. parahaemolyticus* was examined during growth by zymographic analysis. Specific activity of catalase was also measured to evaluate the decomposition rate of hydrogen peroxide at different growth stages. Both results demonstrated different antioxidants were expressed. When cells were treated with hydrogen peroxide, it also showed different induction patterns. One species can be induced specifically during stationary phase. At least three major antioxidants can be recognized in native gel. MS-MS analysis was performed to verify the identity of these proteins. To clarify the role of the stationary phase-induced antioxidant, nutrient starvation and stress conditions such as temperature shift or osmolarity changes will be performed to test the possible relationship. One of the goals of this subproject is to construct promoter libraries of *V. parahaemolyticus* and *V. vulnificus.* At present, a shuttle vector containing *luxAB* reporter, designated as pSA19CP_luxAB, was constructed for that purpose. Restriction enzyme-digested genomic fragments will be ligated with pSA19CP_luxAB and used to transform *Escherichia coli* first for screening. Every member in this group project would get benefit on a better-defined assay condition and share resource established.

In addition, for better understanding of the relationship among different stress conditions, we also performed a series of experiments to study the cross-protection effects among diverse stress conditions. When cells were subject to a sublethal dose of hydrogen peroxide pretreatment, a better tolerance to increased hydrogen peroxide resulted. Same condition was applied and measured the effect on higher osmolarity, extreme pH or temperature shift. Pre-adapted cells showed a mildly increased survival than cells directly applied a high osmolarity shock. Extreme pH or temperature shift showed no significant protection. Taken together, how the bacterial responses to the two stressful conditions applied still needs further studies. A modified procedure and treatment condition will be test to gain further insight in the future.

O74

Physiological Adaptation and Gene Regulation of *Vibrio* spp. to Temperature

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Vibrios are widely distributed in marine environments worldwide. Some of them are pathogenic to human and marine animals. Vibrios encounter temperature fluctuation under certain circumstances, such as seasonal change, host invasion or seafood refrigeration. In our study, *Vibrio parahaemolyticus* and *V. vulnificus* were used as model organisms to exam the response against temperature changes. Cold treatments were performed under 4^{o}C in different liquid media. Bacteria were grown at optimal temperature to log or stationary phase, then were transferred to 4^{o}C for different length of time. Results showed that *V. parhaemlyticus* VP93 (clinical strain) and 14A VP11 (environmental strain) could survive cold condition and remained culturable for respectively 7 and 8 days,. The stationary-phase bacteria seem more cold tolerance than exponential-phase bacteria, and 14A-VP11 could survive one day longer than VP93 did. Meanwhile, the stationary- phase *V. vulnificus* YJ016,survived 4 ^{o}C for 15 days. In contrast, the exponential phase bacteria could only survive for 10 days. Cross-protection effects of cold adaptation to other stresses at 16 ^{o}C for 30 min were examined. Results showed that there is no cross-protection effect for oxidative stress, acid shock or osmotic shock after cold adaptation. On the other hand, the survival rate of 4 ^{o}C cold treatment after 16 ^{o}C adaptation is 10- fold higher than non adapted bacteria. This indicates that 16 ^{o}C pre-treatment only provide protection for further cold treatment but not other stress conditions. Besides, we tested and established standard procedures the use of suicide vectors, shuttle vectors and some *E. coli* strains for conjugation in vibrios. The optimized methods, including gene disrupted by overlapping PCR, transfer of shuttle vector into vibrios by electroporation, and suicide vector conjugation, were established successfully. Additionally, a mini-*Tn*10-based mutant library is constructed in progress that will be a comprehensive resource for us to screen mutants under indicated stress. Those materials and techniques are provided as efficient tools for other group members for genetic manupilation of various vibrio species.

O75

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

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Most *Vibrio* species are natural inhabitant of estuaries and sea water, and can cause foodborne disease and wound infection. In response to a variety of environmental conditions, vibrios should equip themselves 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*-related studies. In this study, we will focus on the effect of pH fluctuation on *Vibrio* species. First of all, physiological responses to pH fluctuations of *Vibrio* species were analyzed to establish the basic knowledge of those bacteria. Disk diffusion assays showed that *V. parahaemolytiucs*VP93 survived in different media with board range of pH variation, especially in alkaline conditions. Viable count analysis revealed that bacteria in exponential phase are more acid resistant than bacteria in stationary phase. Meanwhile, *V. parahaemolytiucs* 14A VP11 has similar pattern as VP93. Proteomic analysis of total cellular protein after mild acid treatment (pH 4~5) will be performed to verify protein expression profile after acid adaptation. According to the studies of the stress responses in other bacteria, cross-protection might provide evidence to clarify the gene regulatory networks of stress response of *Vibrio* species. After knowing the basic physiological response toward different stresses, we are wondering whether acid adaptation might provide any effect for *Vibrio* species. to the other stresses and vice versa. Results indicated that acid adaptation of *Vibrio* species under mild condition did not provide any cross-protection effect to the other stress such as oxidative, temperature and osmotic stresses. Indicating that acid fluctuation might not share the same regulatory pathway with the other stress response or acid treatment only promote a basic responses instate of a global regulatory networks in *Vibrio* species. On the other hand, the other stress treatments provoked cross-protection effect according to the data of the others in this group. Finally, fosmid library of *V. parahaemolytiucs* were constructed by the EPICENTRE system and at 3,000 fosmid clones were stored for future studies.

Key words: *Vibrio* spp., pH fluctuation, proteomic, fosmid library

O76

時間的河慢慢流～慈濟小學統整課程的發展與生命教育的實踐

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本研究主要目的在將慈濟人文精神落實在慈濟小學主題統整課程中。研究對象包括全校一年級到六年級的教師及全體學生。研究共分三大階段，第一階段進行各項研習（包括生命教育及主題統整課程等）；第二階段進行統整課程的設計與實施；第三階段則是檢討與省思，瞭解主題統整課程實施的情形及未來改進的方向。研究結果：主要是以六年級的主題統整課程進行分析，從探索夢想、建構夢想、發現大家的夢想、肯定自己的夢想，最後要成為夢想實踐家，必須用心實踐、克服困難、享受實踐後的喜悅。評估學生學習的情形則是透過學習單、心得寫作、發表分享、影像影音及創作等方式。本研究只是一個初探，未來還需要繼續深入的探究主題統整課程及慈濟人文精神落實的情形。

關鍵詞:主題統整課程

O77

(計畫名稱：The Effect of Conflict Management on Marital Satisfaction: A study on Southern-Asia Brides in Taiwan)

Inter-racial Marital Conflictt and Marital Satisfaction: A study of foreign spouses in Taiwan

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Research indicated that marital communication could predict marital satisfactory. Research found that particular patterns of interaction separate satisfied from dissatisfied couples. Prolonged negative conflict spirals prompt poor physical and mental health in family members. However, human conflicts and conflict resolutions are cultural phenomena; the ways that conflicts are perceived and handled reflect a culturally shared set of attitudes and beliefs. In the past two decades, interracial marriage, in Taiwan, has been dramatically increasing, particularly those foreign spouses from Southeast Asia. Those new immigrants' marital communication has drawn a great deal of attention from conflict researchers. This study attempted to study how foreign spouses and their husbands manage their marital conflict, and the way they manage conflict effects their marital satisfaction.

Data for this study were provided by 246 couples of interracial marriage in Taiwan. Marital satisfaction was measured by using the scale, Marital Satisfaction Inventory developed by Hendrick (1988). A self-report scale on how participants select conflict coping strategies during their marital conflict was developed based on Rahim's (1983) Rahim Organizational Conflict Inventory II (ROCI-II), and used in this study.

Data in this study showed a positive relationship between both wives' and husbands' marital satisfaction and conflict coping strategies of integrating, accommodating, avoiding, and compromising. There ass no relationship between wives' marital satisfaction and conflict coping strategies of competing and third party. A negative relationship was found between husbands' marital satisfaction and conflict coping strategies of competing and third party. There was a significant difference between wives and husbands' use of integrating and avoiding conflict strategies; wives used integrating strategy more often, and husband used avoiding strategy more often. A significant difference was found between the uses of integrating and compromising strategies among wives from different racial; Filipino spouses used more these two strategies, and then following by Indonesian spouses.

O78

佛教慈悲觀的理論基礎、特質及其倫理意義研究

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本研究嘗試對佛教「慈悲」概念進行理論基礎之探討與倫理特質之比較分析。研究發現,「慈悲」觀念為釋迦摩尼佛傳法時期思想上的重要理念,佛陀常藉由身體力行表現慈悲情懷,在教理上以慈悲法現眾生弟子。受到傳統婆羅門教部分教義影響,在「早期佛教」階段,「功德論」與「福田說」形成其重要理論基礎,慈悲實踐含攝利己特質與利害衡量,與有利於推廣於大眾信徒有關,思想特質上為他律理論時期。自大乘佛教理論發揚之後,慈悲實踐建立在「自他不二」、「無我」、「空行」與利他的「無緣慈」理論上,在「六度」戒持中形成慈悲最高善。自此之後,大乘菩薩道、「佛性」、「如來真常心」等理論成為慈悲實踐核心思想,成就「三輪體空」最高境界,為自律倫理的開展。慈悲之愛是無眾生差別,為平等之愛,甚至可以超越愛。在義理上,慈悲與「四聖諦」、「六道輪迴」、「業力果報」、「涅槃解脫」等理論取得一致。慈悲行為的動力包含自因與他因,慈悲可以為倫理行為目的,亦可為手段。在因緣法的世界裡,慈悲具有轉動因緣業力的功能,慈悲的最終目的仍在脫離輪迴機轉。相較於中西倫理思想,佛教慈悲觀明顯表現出社會目的,行為功能與超脫特質。

關鍵詞:慈悲,佛教倫理,業力果報,空行,倫理特質

O79

素食推廣之有效傳播模式探究

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本研究目的在於探討素食推廣的有效說服傳播模式。本研究以素食行為的選擇與認知為研究主題，探究素食者在轉變飲食型態的過程中，面對素食推廣訊息的接受過程與意義的自我詮釋，以期解構素食者在與訊息的互動過程中，選擇性的接觸、認知、記憶與理解等知覺訊息的過程，以及其轉變態度的關鍵原因。藉著素食者處理訊息過程的類別化，探討素食推廣訊息在被詮釋的過程中，影響說服傳播效果的三大環節：說服者、訊息呈現的方式與其和被說服者三者之間的互動型態。

本研究利用質性研究法中的深度訪談法，以素食者為研究對象，探討其素食行為的選擇與態度形成的原因，以及素食推廣訊息對其之影響。本研究除了是對說服理論做一實證性與本土性的研究之外，更期望利用本研究所歸納的結果，探索出有效的素食理念推廣的傳播模式。

O80

應用「合作式自律學習法」於大學英文閱讀課程之成效研究

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近年來心理學家及教育學者都相當肯定「自我調整學習策略」能有效協助學生提升其學習品質與成就。本研究意圖將此策略融入大學英文閱讀課程教學中，以協助學生提升其英文閱讀學習成效及增進其自我調整學習之能力。

本研究採不等組前後測準實驗設計，以修習大一英文閱讀課程的三個班學生作為研究對象，進行一學期（18 週），每班每週兩小時的實驗教學。三個班所使用之英文閱讀教材相同，上課時數相同，測驗亦同。

A 班採「合作式自我調整學習法」，教師在課堂上先以講述方式陸續介紹「自我調整學習策略」，並提供相關的文章供學生閱讀，且以小組合作方式進行英文閱讀學習及討論分享個人應用自我調整學習策略的心得，教師亦定時以檢核單來瞭解學生使用自我調整學習策略的情形。教師教授的自我調整學習策略包含：要求學生於學期初針對自己的英文閱讀學習概況進行瞭解，發現自己在英文閱讀學習上的問題，然後教師分次介紹有效的英文閱讀學習策略，並要求學生為自己每一次小考設定預期達成的目標，考後檢核自己的學習成果是否符合事前設定之目標，並省思如何改善及增進自己的英文閱讀學習成效。

B 班採「講授式自我調整學習法」，教師在課堂上以講授方式介紹教授與 A 班相同的「自我調整學習策略」，提供相同的文章供學生個別閱讀，也定時以檢核單來瞭解學生使用自我調整學習策略的情形。不同的是，學生採用個別獨立學習英文閱讀教材及運用自我調整學習策略，未進行小組合作討論分享個人應用自我調整學習策略的心得。

C 班採「合作式教學法」，教師未刻意教導學生自我調整學習策略，但是指導學生以合作學習方式進行英文閱讀學習。

結果發現：A、C 兩班的英文閱讀理解成績高於 B 班。由此結果可推論運用「合作學習」，有助於改善學生的英文閱讀學習成效。同時三組學生在期末施測的自我調整學習量表上的表現，並無顯著差異，且僅有三分之一左右的自我調整學習組（A、B 班）學生，認為自己有常應用自我調整學習策略於英文閱讀課程的學習上。此事實顯示自我調整學習能力的養成並不容易。但是本研究亦發現自我調整學習能力較高者，其英文閱讀學習成效較彰顯，這項發現肯定培養學生自我調整學習能力是有其價值與必要性。

關鍵字：自我調整學習、英文閱讀、合作學習

O81

(計畫名稱：The correlates of intra - and extra - cellular environmental alterations of facial motoneurons to functional recovery of reconnected facial nerve)

The role of nitric oxide in the regeneration of reconnected facial nerve

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Nitric oxide (NO), which can be released by neurons and glia, has been shown to play a critical role in regulating neuronal transmission, vasodilation, neuronal plasticity, and neuronal cytotoxicity. However, whether NO plays a role in the peripheral nerve regeneration remains unknown.

We transected and sutured left facial nerve of male SD rats. One group of animals received L-NAME, the NO blocker, treatment and another group did not. Functional recovery and axonal regeneration of injured facial motor neurons were measured by observation of vibrissae whisking and eye closure, nerve conduction study, axon and axon terminal immunolabeling, and retrograde tracing. We found that following nerve suture, the once transected nerve of both groups of animals conspicuously regenerate as their survival time prolonged. In comparison, the nerve regeneration was more rapid and thorough in the L-NAME-treated rats at 1-10 weeks after suture. The expression of nitrotyrosine was observed to increase in facial nerve of control group but was reduced in L-NAME-treated animals. Nevertheless, the expressions of neuronal nitric oxide synthase and inducible nitric oxide synthase in the facial motor neurons remained almost at the normal level in both groups.

These findings suggest that blockade of NO production will promote the earlier and better axonal regeneration and functional recovery of repaired facial motor neurons. This will help us to develop new therapeutic strategies for peripheral nerve injury.

O82

The trilogy of HCMV infection and autoimmunity

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The specific associations of antibody against lower matrix protein (pp65) of human cytomegalovirus (HCMV) and systemic lupus erythematosus (SLE) and the exacerbated autoimmunity by NZB/W and Balb/c mice following immunization of pp65 antigen have been reported. To reveal the auto-reactive/mimic epitopes within the pp65 antigen, we performed epitope mapping by ELISA and immunoblot with sera collected from SLE patients. A total of 48 SLE patient sera were analyzed against subcloned pp65 antigens, and 14 (29%) of them reactive against amino acids number 336-439 of the pp65 antigen ($pp65_{336-439}$). The reactivity reduced to less than 3 (6%) following deletion of amino acid before $pp65_{423}$. Computer and synthetic peptides analysis showed that the $pp65_{422-439}$ (GGGAMAGASTSAGRKRKS) not only share similarities to epitopes found on U1-70 kDa, but also recognize by 37.5 % of SLE patient sera. In animal tests, immunization of both pp65 N-terminus fusion protein ($pp65_{337-561}$) and synthetic peptide of $pp65_{422-430}$ (pp65-G) and $pp65_{430-439}$ (pp65-S) induce Balb/c mice to develop chronic autoreactivity to HeLa antigens of 60 kD and immune complex precipitation on glomeruli at older age. Thus, we speculate that through mimicry, HCMV pp65 antigen may induce tolerance break by genetically susceptible animals.

O83

Analysis for the role of *Saccharomyces cerevisiae* B-type cyclins in cytokinesis

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In *Saccharomyces cerevisiae*, Cdc14 is released from the nucleolus to the nucleoplasm and cytoplasm to initiate completion of mitosis and cytokinesis. The released Cdc14 can dephosphorylate Cdh1, a substrate-specific activator of APC (anaphase-promoting complex, an ubiquitin ligase) (Visintin et al., 1997). APC^{Cdh1} mediates ubiquitin-dependent proteolysis of Clb2 and Clb3 upon the exit from mitosis whereas Clb5 proteolysis is mediated by the association of the APC with Cdc20 (APC^{Cdc20}) (Visintin et al., 1997; Shirayama et al., 1999). Interestingly, recent studies has shown that Clb6 proteolysis occurs in late G1 and early S phase, and requires SCF^{Cdc4} (Skp1-Cullin-Cdc4) complex (Jackson et al., 2006).

In our studies, we found that overexpression of Clb6 could make *cdc14-1* mutant strain form cellular chains at the permissive temperature (25°C), and that much higher protein levels of Clb6 accumulated in *cdc14-1* cells than in *CDC14* cells when Clb6 was overexpressed. This observation raised the possibility that Clb6 was also the substrate of APC^{Cdh1} as Clb2 and Clb3. To clarify this, we examined Clb6 stability in G1 when the APC is known to be active. Cdc16 is an essential component of the APC and its defect will highly enhance the stability of the APC substrates (Irniger et al., 1995). Consistently, by the *pGAL1* shut-off experiment to measure the half-life of Clb6, we found that Clb6 was also more stable in *cdc16-123* G1 cells than in *CDC16* G1 cells at the non-permissive temperature (37°C). In addition, our results revealed that Clb6 proteolysis was also dependent upon Cdh1 in both early and late G1 cells. Finally, a KEN box and a destruction box in Clb6 were identified to be required for its proteolysis in G1. Taken together, we conclude that Clb6 proteolysis also requires the APC^{Cdh1}.

O84

(計畫名稱：平埔祖先的獵鹿文化變遷與臺中盆地古代鹿群 mtDNA 之親緣研究)

米、檳榔與卡瓦：從遺傳多樣性探討南島語族的遷徙與交換物的流動

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一個世紀以來，南島語族的起源和擴散始終是考古學、語言學、生物人類學和文化人類學嘗試解謎的重要課題。近年親源地理與分子生物科學的出現，提供了新的技術，得以檢驗並挑戰此一古老學術領域的各種假說。人類的遷徙從來不單只為餬口；人作為文化動物，常會在遷徙過程攜帶社會性的嗜好／作物，此類人擇植物具有非常重要的社會文化意涵。本計劃調查太平洋地區著名的主食作物與成癮性植物：稻米(Oryza sativa)、檳榔 (*Areca catechu*)與卡瓦 (*Piper methysticum*)，作為研究南島語族遷徙與擴散的切入點。稻又可分為水稻和旱稻(陸稻)二種適應生態型(ecotype)，由於花蓮的孤立地理區位，迄今仍有相當多原住民部落持續種植祖傳的野生型旱稻，是一個研究人群遷移與物種共同演化的極佳田野。旱稻具有很強的抗旱性，歷史上旱稻多種在降雨稀少的山區，也因而演化出許多特別的山地稻種。目前旱稻已成為人工雜交稻米的重要研究方向，在潛在經濟效益上能幫助農民節省大量灌溉用水。

檳榔種子中的植物鹼長期以來一直被東南亞及印度用作提神刺激的聖品。此一原產東南亞大陸的馴化物種早在南島語族到達之前便已出現在距今6400-6800年前的新幾內亞考古遺址中了。另一富含卡瓦素的卡瓦，則是廣泛分佈在波里尼西亞、美拉尼西亞、及麥克羅尼西亞南部的晚近馴化作物。台灣近年因為民俗醫療之故，卡瓦研究也引起民俗藥學方面的注意。

在今日許多南島社會（包括台灣）中，檳榔或卡瓦都是重要的嗜好／作物。一個是樹上的果，一個是地下的根，然而在文化上卻是「同類」的，作為休閒、鬆弛、社交的食品或藥物。檳榔與卡瓦的特殊性不在於作為主食或營養攝取，而在於深層社會文化的意涵，尤其是作為儀式交換的禮物和貿易的項目。檳榔和卡瓦在太平洋對應的地理區分佈呈現令人費解的斷裂甚至互斥，因此從近乎100年前英國人類學的鼻祖W. H. Rivers，即由此提出大洋洲兩波人群擴散的假說，而本研究在現有的語言學、植物型態學、植物化學、植物同功異構酶證據之外，使用最新的微衛星基因座技術來檢驗檳榔和卡瓦的親緣地理及遺傳結構，並利用分子時鐘估計人類馴化嗜好物之進程，所欲檢證的假說包括: 快車說、慢船說、俾斯麥群島原住民說、航海廊道說、和三源模型。檳榔和卡瓦在史前史中的基因流動與栽培數量，極可能關聯於南島語族的遷徙與其敵友間的互動。藉由檢證理論模式，現生檳榔和卡瓦的遺傳結構將輔助說明太平洋島嶼網絡間的人貨流動。

由於嗜好／作物主要是人攜帶且常是為了社會性目的而使用，此類研究一方面能讓我們瞭解人群流動，對嗜好物之生物多樣性所形成的影響，一方面也能一窺南島語族的遷徙歷史。由於嗜好物具有文化重要意義，特別需要借重民族誌的詮釋。本研究嘗試進行跨學科領域的整合，不僅將連結現今考古學和生物學研究探索人類移民到太平洋群島間的知識鴻溝，更補充了對亞洲大陸農業文化以迄大洋洲文化變遷與人群互動的理解。

O85

(計畫名稱：Effects of picture book group on the schizophrenia patients emotional intelligence)

The Effective of Emotional Picture Book Program on the mental disorder patient

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The picture books expand living scope, enrich learning experience, trigger the growth of personality and reinforce aesthetic perception and the capacities of cognition and language. Because of development of the illness, mental disorder patients tend to have unstable feelings which lead to the obstacles on social and interpersonal relationships. With the guidance of simple picture books, we can lead the groups. Lin (1985) designed artistic treatment program and had psychological guidance on the students in elementary schools through the groups of classes to increase the students' emotional expression and help them learning new living skills. This research aims to explore the effect of picture book group on emotional. The research design is based on "emotional experience scale" edited by the researcher Chiang. There are 36 questions and 3 scales. Cronbach α refers to 0.76, 0.78 and 0.80. The research targets are 7 hospitalized day-care patients and they are diagnosed as schizophrenia and biopalar disorder. Before and after the study with the groups, we treat "emotional experience scale" as the research tool. The study is practiced once a week and 45—50 minutes for each time. There are 4 picture book groups. The data analysis is based on SPSS 8.0. The statistical methods include the mean, standard deviation, pair t-test and correlation coefficient. The research result: the average age of the research targets is 41.2 years old and there are 4 females (57.1%) and 3 males (42.9%). Most of them believe in Buddhism (57.1%). Most of them have been ill for 4-6 years (42.9%). Before implementing picture book group program, the average score of emotional control in emotional wisdom scale is 15.57 points and after the implementation, it becomes 16 points which reaches significant change ($p<.001$). Research application: with the practice of picture book group and after collecting the mental disorder patients' emotional experience and learning of emotional share, we help the clinical nursing personnel to further be familiar with the patients' emotional change and dealing to allow the nursing personnel to have unique caring specialty.

Key word：emotional picture book、mental disorder patient

O86

Metabolic engineering of terpenoid synthases, ribosome-inactivating proteins and p-insulin from *Cucurbita* spp

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To understand the regulation in synthesis of bioactive compounds of *Momordica charantia* (bitter gourd), genes involved in controlling the biosynthetic fluxes becomes a main target for further investigation. Two cDNA clones involved in the pathway of terpene synthesis were cloned for further investigations insight into the metabolic engineering of terpenoid synthesis.

By using a PCR-based MARANTHON-like technique, we have attempted to obtain cDNA clones involved in the terpene synthesis from bitter gourd. Two fragments have been amplified through nested PCR reactions with two sets of degenerate primers designed following the highly conserved motifs of terpene synthases. These fragments of were used as template for further primers designs to specifically amplify the full-length genes of terpene synthase from bitter gourd through PCR walking method (5'-RACE and 3'-RACE). Expression of these genes will be further studied through sub-cloning of these terpene synthase genes in *E. coli* and yeast. The transformed cells will be further characterized for their ability of terpenoid synthesis. Functional genes isolated from bitter gourd will have academic importance and the new terpenoid will gain economic significance for future application.

O87

The study in the apoptosis control of human neutrophils by endogenous nitric oxide

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Neutrophils are the predominant leukocytes in the circulation and the essential components of the innate immune system in human. They form the first line of defense against microbial infections. The regulation of neutrophil apoptosis during an inflammatory response plays a critical role in its resolution. In many cells, NO participates in the regulation of the daily activities of cells as well as in growth and death. We investigated the effects of NO on neutrophil apoptosis through in vitro manipulating the cellular concentration and signaling of NO. Apoptosis was assessed by immunofluorescent staining and flowcytometry. Our results showed that the neutrophil apoptosis was accelerated by blocking either the endogenous NO generation or its downstream signals. The enhanced apoptosis was reversed by providing exogenous NO. The NO-mediated anti-apoptotic mechanisms are keeping studied.

O88

(計畫名稱：Characterization of NK1.1$^+$CD11c$^+$ cells in murine *Listeria monocytogenes* infection)

Characterization of Early IFN-γ Expression During Murine Listeriosis: Identification of NK1.1$^+$CD11c$^+$ cells as the Primary IFN-γ-expressing Cells

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Though it is well established that gamma interferon (IFN-γ) is crucial to the early innate defense of murine listeriosis, its sources remain controversial. In this study, intracellular cytokine staining of IFN-γ-expressing splenocytes early after *Listeria monocytogenes* (LM) infection revealed NK1.1$^+$, CD11c$^+$, CD8$^+$ T and CD4$^+$ T cells expressed IFN-γ 24 h after infection. Contrary to the previous report, most IFN-γ$^+$ dendritic cells (DC) were CD8α$^-$ DC. Unexpectedly, almost all CD11c$^+$ IFN-γ-expressing cells also expressed NK1.1. These NK1.1$^+$CD11c$^+$ cells represented primary IFN-γ-expressing cells after infection. *In situ* studies showed these NK1.1$^+$CD11c$^+$ cells were recruited to the border of infectious foci and expressed IFN-γ. A significant NK1.1$^+$CD11c$^+$ population was found in un-infected spleen, lymph node, blood and bone marrow cells. And its number increased significantly in spleen, lymph node, and bone marrow after LM infection. Using IL-12 p40$^{-/-}$ mice, IFN-γ expression was found to be largely IL-12 p40 dependent and the number of IFN-γ-expressing cells was only about one third of that of wild-type mice. Moreover, the IFN-γ expression was absolutely dependent on live LM infection as no IFN-γ was detected after inoculation of heat-killed LM (HKLM). Our findings not only provide an insight into IFN-γ expression after *in vivo* infection but may also change the current perceptions of DC, and natural killer (NK) cells.

O89

Identification of DNA copy-number aberrations by array-comparative genomic hybridization in patients with Autism

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Autism is a childhood neurodevelopmental disorder with a strong genetic component in its etiology, yet the identification of autism susceptibility loci remains elusive. Based on recent reviews, cytogenetically detectable chromosome abnormalities are found in 7.4% of autism spectrum disorder (ASD) cases. The most frequent anomalies observed are 15q11-13 duplication, 2q37, 22q13.3 deletions and X chromosome aberrations. Therefore, chromosomal abnormalities are implicated as important markers for the pathogenesis in patients with autism.

In this study, we investigated 44 autism patients by a commercially available oligonucleotide based comparative genomic hybridization array(NimbleGen) to analyze DNA copy-number changes. We identified a male patient with 4q35 deletion and the other male patient with 8p23 deletion. These two variants were further confirmed by fluorescence in situ hybridization (FISH) and real-time quantitative PCR. The 8p23 deletion was not detected in 100 patients and 100 controls using real-time PCR as a screening method. In addition, we found several DNA copy number variants (CNVs) in this sample, including 1p36, 2p11, 3p25, 6p25, 7q11.2, 14q32.33, 16p11.2, 14q11, 15q11-13, 16p11-13, 17q21.3, 18p11.3, 22q11.2.

Although the sample size in this study is small, we identified two deletions in this sample, indicating array CGH is a useful tool to study the genetic defects of patients with autism. We also identified several other CNVs in this sample, whether these CNVs are associated with autism needs further study, especially comparison with normal patients.

O90

Ethanol Phosporylation of NMDA NR1 and NR2B Subunits in Rat Sympathetic Preganglionic Neurons : Involvement in Acute Tolerance to Ethanol Inhibition of NMDA Receptor Function

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Changes in phosphorylation states may have important affections for NMDA receptor function. Various kinases-mediated phosphorylation of NMDA receptor subunits has been shown to change NMDA receptor function. We hypothesized that kinase-mediated signaling involved in the development of acute ethanol tolerance. Immunohistochemistry studies demonstrated that the phosphorylated NR1 and NR2B subunit of NMDA receptor (pNR1-serine 896, pNR2B-tyrosine 1336) was found to be significantly increased at 40 min during acute ethanol tolerance in sympathetic preganglionic neuron (SPN). This effect was blocked by intrathecal applications of chelerythrine (100 pmol; a selective PKC inhibitor), PP2 (100 pmol; a selective Src family tyrosine kinase inhibitor) into the SPN. Moreover, the levels of pNR1-serine 897 expression was found to be significantly increased during prolonged ethanol exposure. Intrathecal applications of KT 5720 (200 pmol; a selective PKA inhibitor) significantly reduced the levels of pNR1-serine 897 expression. In vivo studies, reduction of NMDA-induced pressor effects was observed at 10 min but disappeared at 40 min after continuous ethanol infusion. This effect was dose-dependently blocked by intrathecal administration of chelerythrine (0.1 pmol -1 nmol) or PP2 (1 – 100 pmol) into the SPN 10 min post-injection of ethanol. Post-treatment with KT5720 (2 – 200 pmol), phorbol (20 – 200 pmol, a selective PKC activator) and PAO (0.01 – 1 nmol, a selective tyrosine phosphatase inhibitor) did not affect acute ethanol tolerance. We conclude that the phosphorylated status of NMDA NR1 and NR2B subunits was significantly increased during acute ethanol tolerance. PKC and Src tyrosine kinase-mediated signaling pathways may be involved in the development of acute tolerance to ethanol inhibition of NMDA receptor function.

O91

In vitro studies of isochaihulactone in human prostate cancer LNCaP cells

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Isochaihulactone is a novel lignan-like compound isolated from Bupleurum scorzonerifolium (BS-AE) and identified with various spectral techniques (^{1}H NMR, ^{13}C NMR, IR, and MS). Isochaihulactone has been shown to inhibit cell proliferation and as an effective inducer of apoptosis in a variety of carcinoma cell lines in previous studies. Moreover, isochaihulactone inhibited the growth of non-small cell lung carcinoma A549 xenografted in nude mice.

Recently, nonsteroidal anti-inflammatory drug-activated gene (NAG-1) expression was up-regulated by isochaihulactone through an ERK-dependent pathway involving the activation of EGR-1 in human lung carcinoma cell line A549. To determine the anticancer potential of isochaihulactone in prostate cancer, a human prostate cancer cell line, LNCaP, was tested in this study. By using a siRNA approach, we found that isochaihulactone-induced cell death in LNCaP could not be rescued by NAG-1 siRNA transfection. The results indicated that isochaihulactone-induced cell death in LNCaP was NAG-1 independent.

To determine the cytotoxic effects of isochaihulactone, we further analyzed the cell cycle profile of isochaihulactone-treated LNCaP. Our result showed that isochaihulactone caused cell cycle arrest (especially at G2/M phase) and apoptosis in a time- and concentration-dependent manner in LNCaP. The G2/M arrest was correlated with increased p21/WAF1 levels and a down-regulation of the checkpoint proteins cyclin B1/cdc2.

These findings indicated that isochaihulactone might induce cell death through different pathways in different cancer cell lines, and its anti-tumor activity should be further explored.

O92

Amiodarone Inhibits Epithelial to Mesenchymal Transformation and Causes Cardiac Valve Defect During Zebrafish Embryogenesis

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Amiodarone, a type III anti-arrhythmia drug, is commonly used to treat life-threatening arrhythmia. There are still some clinical possibilities in the early pregnancy with exposure to amiodarone. In order to develop an animal model for studying the effect of amiodarone on embryonic development, we used zebrafish due to its transparent embryo which makes dynamic spatiotemporal observation be possible.

When zebrafish embryos were treated with 15 *u* M amiodarone from 10 hours post-fertilization (hpf) to 72 hpf, blood regurgitation between ventricle and atrium was found. Whole mount *in situ* hybridization showed that *versican* and *has2*, molecular markers for cardiac valves were over-expressed ectopically at 72 hpf, suggesting that amiodarone causes embryos to have cardiac valve defect. In addition, the embryos treated with cyclosporine A, a chemical that inhibits valve development, also produced ectopic over-expression of *veriscan*. Moreover, α-smooth muscle actin (α-SMA), a marker of epithelial to mesenchymal transformation (EMT) which is an important process during cardiac valve formation was also absent at cardiac valves, indicating that EMT during cardiac valve development was inhibited by treating amiodarone. Using harmonic optical microscopy and two-photon fluorescence microscopy, we observed *in vivo* that valves were not formed in the amiodarone-treated embryos derived from zebrafish transgenic line *Tg(cmcl2:HcRFP)*, whose RFP reporter was driven by heart-specific promoter *cmcl2*. The endocushion was not observed at 87 hpf. TUNEL assay confirmed that apoptosis did not occur in endocushion forming region.

In conlusion, our findings reveal that amiodarone inhibits cardiac valve formation due to repression of EMT during embryonic cardiac valve development. We develop the zebrafish animal model to demonstrate *in vivo* the effect of amiodarone on the valve formation during cardiac development, which possesses significant pre-clinical implications.

O93

FGF10 signaling controls the intestinal cell differentiation in zebrafish

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FGF10 was required for epithelium cell proliferation and survival during mouse colon development. It was also reported to be required for cell proliferation and gland formation of stomach epithelium in chicken. In order to investigate detailed mechanisms of FGF10 during gut development, we studied the developing intestine using the FGF10 mutant zebrafish, *daedalus* (*dae*). In the *dae* fish, the swim bladder, which was the derivative of gut in teleost, was lost. HE staining indicated that the epithelia of esophagus and intestinal bulb had lower level of folding or unfolding. BrdU incorporation indicated that the intestinal epithelium cells were proliferated in both wild type and *dae* fish at 72 hpf. Remarkably, the proliferation cells were absent in the mid- and posterior intestine of *dae* at 120 hpf. In addition, we analyzed the expression of several differentiation markers of intestine. The IFABP, which was normally expressed in apical phase of enterocytes, was mis-expressed in the mutant fish. The zebrafish intestinal alkaline phosphatase (IAP) was localized to the intestinal lumen brush border in the wild type. However, it was not detected in the mutant fish. Therefore, we concluded that the FGF10 might be important for polarization of intestinal epithelium cells. The signal of Alcian blue-periodic acid Schiff staining, which detects the acidic mucin produced by goblet cells, was lost in *dae*. Furthermore, immunohistochemistry staining using 2F11 antibody could not detect the presence of goblet cells. We also found that the glucagon-expressing cells were reduced and Notch signaling was disturbed in the gut of *dae*. Taken together, these data indicated that FGF10 signaling is important for intestine development and it is involved in the epithelium proliferation and enterocyte/secretory cell differentiation.

O94

Molecular Mechanisms Underlying Urocortin-Induced Anti-proliferation In Neural Stem Cells

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Urocortin, a 40 amino acid neuropeptide, is a member of corticotrophin releasing hormone, which can mediate the hypothalamic-pituitary-adrenal (HPA) axis and coordinates the endocrine response to stress. Previous studies indicate that restraint stress decreases neurogenesis in hippocampus but stress-induced urocortin expression is observed in rat brain. However, there is no directing evident that urocortin can influent proliferation of neural stem cells (NSCs). We found that urocortin mRNA was expressed in rat neocortex in embryonic day 13.5. The corticotrophin releasing hormone receptor 1 and 2, receptors for urocortin, were found in NSCs in rat subventricular zone. We observed that urocortin decreased cell proliferation by using MTT proliferation assay and BrdU incorporation assay. This decrease was not caused by apoptosis but due to urocortin- induced phosphorylation of retinoblastoma protein and expression of $p21^{cip1/waf1}$, which are cell cycle progression inhibitors. Interestingly, we discovered that urocortin could direct inhibit the activity of histone deacetylase (HDAC) and induced histone acetylation, which is known to epigenetically regulate transcription of $p21^{cip1/waf1}$. These data demonstrates that urocortin has anti-proliferation effect, and might restrict self-renewal efficacy during development.

P01

The bubbling gut —a combined acoustic and direct imaging study

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Bowel sounds represent a physical sign that can be monitored easily, conveniently, painlessly and at low cost. However, the mechanisms involved in the genesis of bowel sounds are largely unknown. With the use of realtime ultrasonography and cineradiography, we were able to demonstrate a specific mechanism whereby a bubbling bowel sound was generated. Air bubbles were shown to produce *in situ* near the bottom of an upright intestinal loop. A bubble, once released from its submerged orifice, oscillated at its natural frequency. The acoustic signals thus emitted were studied through simultaneous recording and subject to subsequent analysis. We correlated the acoustic parameters derived from a computerized bowel sound analysis system (Enterotach®) e.g. time-expanded wave form analysis, power spectra and dominant frequency analysis, etc. with the image parameters e.g. the bubble size, shape and the spatial features of the loops. The results showed: (1) Bubbles are responsible for the bubbling bowel sounds and can be visualized adequately through current study design; (2) The oscillation frequency of a free rising bubble correlated well with its size; (3) Although not directly related with the bubble frequency, the spatial features of the bowel loops may further modify the acoustic signals in one or another ways; (4) Although difficult it might seem, the mechanisms underlying the genesis of bowel sounds can now be studied with the help of modern imaging modalities as used in this study.

P02

Interaction of ethanol with NMDA receptor antagonists on spinal NMDA-induced pressor responses in rats

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The present study was carried out to examine the interaction of ethanol with several selective NMDA receptor antagonists such as ketamine, memantine, and ifenprodil on spinal NMDA-induced pressor effects. Ketamine and memantine are uncompetitive, open channel blocker; ifenprodil has a high selectivity for the NR2B-containing subtype of NMDA receptor.. Repeated intrathecal injections of NMDA (2nmol) 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, ketamine or memantine or intrathecal injection of ifenprodil inhibited NMDA-induced pressor effects in a dose-dependent and reversible manner. Co-administration of ethanol with ketamine or ifenprodil, but not with memantine produced synergetic effects on the inhibition of NMDA-induced pressor effects. However, the above synergistic or addition effects were not observed while NMDA receptor antagonists were applied at 10 min after intravenous ethanol. The results indicated that acute ethanol exposure may interfere with the effects of NMDA receptor antagonists on spinal NMDA-mediated responses.

P03

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 tomography) imaging was able to applied for the evaluation of Parkinson's disease. Six rats were subjected to imaging analysis including three with 6-OHDA unilateral lesion and other as control group. The pharmacological challenge 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 approaching method could help scientists or clinicians to better understand the progression of PD and may potentially lead to realize the active cerebral area in PD patients.

P04

Enhancing permeability of blood-brain barrier as a therapeutic strategy in rabid rat

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Rabies infection is known to be lethal in human. However, the pathogenesis of this neurotrophic virus leading to acute death has yet been clear. Treatment with passive immunity for the rabid patients is effective only when the patients have not shown neural sign. This phenomenon leads us to investigate the permeability of blood-brain barrier (BBB) in rabies virus infection and purpose the enhancing BBB permeability combination with passive immunity could be a better therapeutic strategy in rabies. We inoculated the rabies virus to 8- to 10-week-old Lewis rats and measured the integrity of BBB by quantitative ELISA for either total IgG or albumin levels in the cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI). The results showed the permeability of BBB was slightly increased comparing with hypertonic arabinose we used to breakdown the BBB before. Our data indicated the limited BBB opening in rabies could be a reason of failure in passive immunotherapy when the patients showed neural sign. Basing on the above results, we administrated human anti-rabies immunoglobulin (HRIG, BayRab®) and neutralizing monoclonal antibody 8-10E and 22-4D before or after intracarotid hypertonic arabinose infusion. The immunoglobulin could be detected in CSF, but decreased with time. In the future, we will try to prolong the time of BBB opening and maintenance the titer of immunoglobulin in the brain. This strategy could be a therapeutic improvement in rabies patients.

P05

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. The hematopoietic system, one of adult stem cell systems, is a useful experimental model to study the coordination for cell differentiation because more than 8 distinct blood cell lineages (erythrocyte, megakaryocyte, neutrophil, macrophage, eosinophil, mast cell, B lymphocyte, T lymphocyte….) arise from single hematopoietic stem cell. However, due to the limit of cells source, the mechanism of how lineage specific genes controlled within individual hematopoetic cells still remains unknown. OP9 is a stromal cell line established from the calvaria of newborn macrophage colony-stimulating factor (M-CSF) deficient mouse. Embryonic stem cell-OP9 coculture system was shown to support hematopoietic differentiation of mouse embryonic stem (ES) cells into various lineage cells. Since OP9 is a primary cell line with only one-month duration, herein we used human papillomavirus E6 and E7 genes to immortalize OP9 stromal cells (I-OP9) which can successfully support megakaryocytic differentiation. 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. Six potential genes for megakaryocytic differentiation (RIS1, NFIX, ZFP36L1, BHLHB2, ZFP541, DACH1) were identified by microarray analyses. The potential roles of these genes in megakaryocytic differentiation will be characterized in HEL cell and embryonic stem cell-OP9 coculture system by shRNA knockdown analysis.

P06

Exercise Training Attenuates Severe Hemorrhagic Responses and Protects Organs from Damage

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Hemorrhagic shock is the most important cause of early death after major trauma. It has been well known that hemorrhage induces ischemia injury and an over inflammatory response performed. Some researchers report that regular exercise enhances the immune response and increases the cardiopulmonary function. However, the effects of exercise on hemorrhage-induced death and organ damage are less clear.The aim of this study was to evaluate the effects of regular exercise on the hemorrhage-induce physiopathological changes. Forty -eight Wistar-Kyoto rats were randomly assigned into two groups. The exercise-trained group (E group) received exercise training for 4 wk. The control group (Con group) was placed on the treadmill and remained sedentary for the same time period. According to different level of blood withdraw (20%, 40%, 60% of total blood volume), each group divided into 3 subgroups. The femoral artery was cannulated to monitor arterial pressure and HR. Blood samples were collected before and at various times after exsanguination. After 48 h, 3 rats of each subgroups were taken for animal activity examination. The liver, kidney and lung were taken for pathological examination and assessment after the experiment. After 60% blood withdraw, the E group of had lower aspartate aminotransferase, alanine aminotransferase, lactic acid dehydrogenase, creatine phosphokinase, glucose, lactate, and lactate dehydrogenase then Con group. Pathological examination revealed that hepatic, renal, and pulmonary injuries were more severe in the Con group than in the E group. In conclusion, exercise training attenuates severe hemorrhage shock responses and protects organs from damage.

P07

Immuno-modulation therapy with cytokines mediated by adeno-associated virus 2 in malignant brain tumor

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Background:
Immuno-modulation with cytokines is one of the most potential modality in cancer therapy. However, the adverse effect was miserable by systemic administration such as in the human clinical trail. Moreover, from the experience of clinical practice, malignant brain tumor always recurs or relapses within a short period from the completeness of initial treatment. So a superior method should be designed as long term expression of cytokines and local affection for the purpose of maximal therapeutic effectiveness and restricted systemic toxicity. In the environment of brain tumor, microglia and astrocyte are the dominant exiting cell types other than tumor cell itself. We postulate that AAV2 enable to transfect ether or all of those cells and the infected cells may constantly express the mediated cytokines for lasting a long period. From the stimulation of cytokines on resident microglia, astrocyte or other immigrated immune cells, a substantial anti-cancer activity proposes to be induced through the apoptosis of tumor cells or cytotoxic effect of activated immune cells.
Experimental design and proceeding:
We chose adeno-associated virus type 2 (AAV2) to be the vector for the reason of its safety and low pre-exited immune resistant. Four cell lines, including human glioblastoma cell line DBTRG, rat glioblastoma RG2, mouse microglia BV2 and mouse astrocyte, For achieving strong innate and adaptive immune responses, we chose two powerful cytokines interleukin-12 (IL-12) and interleukin-18 (IL-18). The transfection efficacy of four cell lines was carried out by using AAV2 encoded GFP. The anti-cancer activity was evaluated by secretion of tumor necrosis factor-alpha (TNF-α) from microglia stimulated by IL-12 or IL-18. The cytotoxic efficacy of microglia stimulated by IL-12 or IL-18 on RG2 was estimated with two different culture condition, co-culture and trans-well culture, and MTT assay. The translational experiment of animal models will be performed in subcutaneous cancer implantation of null mice and stereotatic implantation of cancer cell into rat brain.
Tentative results:
The transfection efficacy was quite well in RG2, DBTRG or astrocyte, near 100% cells expressed the GFP after co-culture with AAV2 encoded GFP. However, very few microglia could express GFP in the same condition. The RG2 and astrocyte secreted mouse IL-12 tremendously after co-culture with AAV2 encoded mouse IL-12. After stimulation by IL-12, IL-15 and IL-18, microglia secreted TNF-γobviously only in IL-18. In MTT assay, microglia exhibited no cytotoxic ability on RG2 cells in co-culture or transwell. But the cytotoxic effect could be induced by added in IL-12, IL-18 or two combined and persisted at least 72 hours. The microglia could be activated by RG2 infected by AAV2 encoded IL-12 and eliminated RG2 cells.

P08

The exploration of the illness behavior of patients with ESRD

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Taiwanese usually use western medical resources to deal with health problems. In acute disease situations, patient's primary responsibility is to seek for medical help, to comply with doctors' recommendation, and to have their recovery. Health care professionals are considered as experts to provide health care; patient is passively accepted caregiving. Suffering from multiple chronic diseases are the main features of health contemporary society. The principle of chronic care ought to strain on healing and based on patient's need and ought to be directed and controlled by patients (IOM, 2001). According to the published researches, end stage of renal disease has been one of the most important diseases in Taiwan. Patients with ESRD, usually with multiple-diseases, need life long dialysis to survive. There are many unexplored problems related to living with long-term hemodialysis. Patients must manage many problems by themselves in the socio-cultural situation. Most studies on ESRD patients with hemodialysis had focused on biomedical aspects and ignored the other dimensions of human beings.

This project intends to explore the illness behavior of ESRD patients with hemodialysis in a Northern Taiwan hospital. This project, based on medical anthropology, includs patients who have been on dialysis for more than 4 months, speak Chinese or Taiwanese, and agree to have several interviews and tape-recoding. The data for analysis will be collected with the methods of in-depth interviews with the patients, family and health professors. After individual interviews, the tapes were transcribed to texts. Content analysis was employed to analyze the texts.

6 patients have been included whose illness behaviors could be divided into medical seeking behavior, usage of complementary and alternative medicine, and self-management behavior. We'll study more to find how they use them integrally. The findings of this project will enhance our understanding of the holistic illness behavior related to the cultural background of the patients to provide further insight in developing a culturally relevant intervention for the ESRD in Taiwan.

P09

Endothelin-1 mediates TChi-2-induced constriction of LPS-pretreated mesenteric arteries of the rat

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Endotoxemia, a state of excessive endotoxin (lipopolysaccharide, LPS) in the circulation, is characterized by excessive proinflammatory cytokines and nitric oxide (NO) production, resulting in significant vasodilation and hypotensive shock followed by multiple organ dysfunction and failure. The specific drug treatment for this detrimental septic shock is not available yet. The aim of this study is to evaluate the beneficial effect of TChi-2 in managing LPS-induced vascular dysfunction and to investigate the possible mechanism of action of TChi-2 using *in vitro* tissue bath technique. The results indicated that in isolated rat tail arteries and mesenteric arteries with intact endothelial cells pretreated with LPS (200 ng/ml), TChi-2 (3 μM) induced moderate and persistent vasoconstriction. This TChi-2-induced constriction was not observed in endothelium-denuded arteries, suggesting that TChi-2-induced vasoconstriction is dependent on intact endothelium. We further examined if TChi-2-induced vasoconstriction was due to release of endothelin-1, a potent vasoconstrictor, from endothelial cells following LPS treatment. The contractile response induced by TChi-2 in LPS-treated rat mesenteric arteries was blocked by BQ 788 (an ET-1B receptor antagonist, 10 μM) and BQ 123 (an ET-1A receptor antagonist, 10 μM). BQ788 and BQ123 at similar concentrations, however, did not affect TChi-2-induced constriction in LPS-treated rat tail arteries. These results suggested that TChi-2 increased ET-1 release from mesenteric arteries but not from tail arteries. This is supported by preliminary results from our lab that mesenteric arteries released significantly higher amount of ET-1 than the tail arteries following LPS treatment. The exact mechanisms responsible for TChi-2 enhancement of ET-1 release from the endothelium remain to be investigated. TChi-2 appears to be a promising candidate to ameliorate the cardiovascular dysfunction in septic shock.

P10

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 is 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. It also revealed that, not only p-selectin, both PSGL1 and E-selectin play a role in IVIg amelioration effects. In this study, we performed antibody-induced thrombocytopenia in selectin knockout mice (P-sel-/-, PSGL1-/- and E-sel-/-), and we found that P-selectin, compare to PSGL1 and E-selectin, is more importantly involved in IVIg mediated amelioration. It also revealed that IVIg mediated amelioration is related to the regulation of FcγRⅡB and FcγRⅢ receptors on leukocytes when FcγRⅡB or FcγRⅢ knockout mice were used as a model system. In addition, in vitro analyses including leukocyte-endothelial cell adhesion system and flow cytometry based leukocyte-platelet phagocytosis system were used to study the action mechanisms of p-selectin in IVIg mediated amelioration.

P11

Mild hypothermia induced by slow fluid resuscitation ameliorates liver and gut damage in hemorrhagic shock

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Hemorrhagic shock (HS) is the major cause of death of trauma patients. In clinical, fluid resuscitation is an important treatment for traumatic HS. However, hypothermia frequently occurs during fluid resuscitation. Recent studies have suggested that hypothermia may protect the gut in intestinal ischemia reperfusion injury. This protection may be come from a pro-inflammatory cytokines effect. The aims of this study are to compare the status of hypothermia and pro-inflammatory cytokine under different resuscitation rates for HS in conscious rats. Thirty-two male Wistar–Kyoto rats were used. The volume of blood withdrawal was 40% of the total blood volume of rat and fluid resuscitation was given after blood withdrawal. Rats were randomly divided into control group, 10 minutes rapid group and 12 hours slow group. Levels of aspartate transferase (GOT), alanine transferase (GPT), blood urea nitrogen (BUN), creatinine, creatine phosphokinase (CPK), TNF-α and IL-10 were measured. The live and small intestine tissues were taken for pathological assessment at 48 hours after HS. Rapid resuscitation provided the BP and HR stability at early phase of HS. Slow resuscitation significantly decreased body temperature compare with rapid resuscitation, and decreased blood GOT, GPT, BUN, CPK, TNF-α and IL-10 levels after HS. The hypothermia with slow resuscitation suppressed the liver and gut damage after HS. In conclusion, mild hypothermia induced by slow fluid resuscitation ameliorates hemorrhage-induced liver and gut damage in conscious rats. These findings suggest that slow fluid resuscitation may be beneficial for prevention of organ damage in patients with HS.

P12

Analysis of overall gene expression in convergent pathway in mice frontal cortex after chronic treating of antipsychotics and psychositmulants

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Methamphetamine (MAP) and MK-801 are two psychotomimetic drugs that can induce psychotic symptoms in humans, while aripiprazole and amisulpride are two antipsychotic drugs that are used to treat psychotic symptoms. To identify the common pathway underlying the mechanisms of action of these drugs, we studied the gene expression profiles in the frontal cortex of ICR mice under long-term treatment with 2 mg/kg MAP, 0.5 mg/kg MK-801, 5 mg/kg aripiprazole, and 5 mg/kg amisulpride, respectively.

We were able to detect three genes, including activity regulated cytoskeletal-associated protein (Arc), nuclear receptor subfamily 4, group A, member 1 (Nr4a1), and early growth response 2 (Egr2), up-regulated in MK-801-treated mice, while these genes were down-regulated in amisulpride-treated mice. However, these genes were not found to have significant change in MAP- and aripiprazole-treated mice compared to control animals.

MAP and MK-801 act on dopamine and glutamate neurotransmission, respectively, while aripiprazole and amisulpride work on dopamine system. Aripiprazole is a partial antagonist of the dopamine D2 receptors, and amisulpride is a full antagonist of the dopamine D2 and D3 receptors. The complexity of the mechanism of action of these drugs makes the identification of common final pathway difficult. Nevertheless, the present study still offers a useful strategy in searching for susceptibility genes for psychosis or schizophrenia by selecting genes expressed in the opposite direction after treatment with psychotomimetic drugs and antipsychotic drugs.

P13

The Chinese herbs affect endothelial cells migration may be mediated through MMP-2

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Our laboratory have previously screen 288 Chinese herbs for their antiangiogenesis activity based on their effect on endothelial cell migration and proliferation. Now we are interested in evaluating the hypothesis that herbs inhibited endothelial cells migration may be mediated though MMP-2. From provide study, 28 herbs were selected based on their effects on migration. Among them, 16 had antimigration effect, 3 could enhanced migration and 9 had no effect on migration. CPAE(calf pulmonary artery endothelial cells) were seeded in $2X10^5$ cells/well in MEM supplemented with 20% FBS, in a total volume of 1.5 ml. Next day, cell were washed with PBS and then treated with herbs in various concentrations for 1 hr. The conditioned medium was collected and mixed with 2 fold sample buffer at room temp. for 10 min. Aliquet of 30 µl of the mixed conditioned medium was loading on gel, which was run at 90 v for 130 min. The gel was treated with renature buffer to remove SDS, with developing buffer to refold enzyme, incubated at 37℃ with gentle agitation overnight, and stained with Coomassie Blue. Our results showed that 28 herbs tested showed various potency to inhibite MMP-2 activity: one at 50 µg/ml, one at 100 µg/ml, 2 at 200 µg/ml, 4 at 400 µg/ml, and 1 at 800 µg/ml, 18 with no effect and 1 with promotion effect at 100 µg/ml. These results suggested that the inhibitory effect of herbs on migration was not well correlated with theie antiMMP-2 activity.

P14

The inhibitory effects of the aqueous extract of *Pluchea indica (L.) Less. and Saxifraga stolonifera Meerburg* on the migration of endothelial cell

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Chinese herbs have been used in China for hundreds of years as important remedies for maintaining health, prevention and treatment of chronic diseases. Recent studies have shown that extracts of Chinese herbs possess many biological activities including antibacterial, antiviral, antifungal, anti-tumor and immune-potentiation activities. Treatment with Chinese herbs for various diseases has become an important choice for alternative medicine.

Herbs with angiogenic activity can be used to rescue the myocardial ischemia and herbs with anti-angiogenic activity can be used to treat tumor. To understand the function of Chinese herbs in angiogenesis, in past years, our laboratory have screened 288 herbs for their anti-angiogenesis activity. Among them, we selected 17 Chinese herbs had anti-migration ability to investigate their effects on focal adhesion kinase (FAK), which is activated in migration. Our results showed that 17 Chinese herbs selected all can inhibit FAK activation. Then we further picked 2 Chinese herbs, *Pluchea indica (L.) Less.* and *Saxifraga stolonifera Meerburg*, to investigate what molecules are involved in the inhibition of endothelial cell migration. We first examined the effects of *P. indica* and *S. stolonifera* on the secretion of extracellular matrix (ECM)-degrading proteases in human umbilical vein endothelial cells (HUVECs). Analysis on gelatin zymography of serum-free conditioned medium revealed that HUVECs constitutively secrete MMP-2, and 2 Chinese herbs decreased the level of MMP-2. However, MMP-9 secretion was barely detectable in the same cells and not influenced by 2 Chinese herbs. We also observed that the membrane level of MT1-MMP, an activator of MMP-2, was decreased by *P. indica*, but not by *S. stolonifera*. In addition, we found that 2 Chinese herbs could induce apoptosis of HUVECs. In conculsion, 2 Chinese herbs inhibited endothelial cell migration by reducing MMP-2 level and induced endothelial cell apoptosis.

P15

Characterization of Interferon-inducible protein 10 expression during murine *Listeria monocytogenes* infection

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Interferon-gamma-inducible protein 10 (IP-10, CXCL10) is an important chemokine and is thought to be chemotactic to activated T cells. In this study, we investigated the expression of IP-10 during murine *Listeria monocytogenes* (LM) infection. Using *in situ* immunofluorescent staining, unexpectedly, naïve spleens from C57BL/6 mice were found to constitutively express high levels of IP-10. When the mice were infected with 1 x 10^4 CFU of LM, the expression levels of IP-10 decreased from day 4 to day 7 after infection and returned to the level as naïve spleen 10 days post-infection. Interestingly, when giving the mice a higher dose of LM (5 x 10^4 CFU), the expression of IP-10 diminished rapidly with obvious decrease at 24 h post-infection. These results indicate that the inflammatory signals induced by LM infection are responsible for the down-regulation of IP-10 expression and that intensity of signals is correlated with kinetics of IP-10 down-regulation. We also delineated the expression of IP-10 to white pulp region of spleen. Using F4/80 staining to outline red pulp, IP-10 staining and F4/80$^+$ staining were found to be mutual excluded. On the contrary, both T cell and B cell regions were co-localized with IP-10 staining. To further solidify our result, protein levels and mRNA levels of IP-10 were currently under investigation with Western blotting and quantitative RT-PCR respectively.

P16

Intermittent Hypoxia Induce Cell Death in cardiomyocytes of WKY and SHR rats

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Intermittent Hypoxia (IH) is a common symptom that can be found in many pathophysiological situations including nocturnal hypoventilation and obstructive sleep apnea syndrome (OSA). Past studies have shown that IH induces several cardiovascular features in OSA patients, including hypertension, sympathetic activation, ventricular hypertrophy, and left ventricular (LV) dysfunction, but the exact mechanism is still unclear. To better understand the mechanism, we exposed Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) to repetitive hypoxia-reoxygenation cycles (30 s of 5% O2 ; 45 s of 21% O2) or room air (RA) for 6 h/day during light phase (10 AM-4 PM) for 10, 20, 30 days and examined the effect of IH induce cell death in cardiomyocytes. We use the Lipid peroxidation and superoxide dismutase (SOD) activity assay to determine whether the free radicals increase in the IH. The propidium iodide (PI) staining was used for the plasma membrane integrity and distinction of necrotic cells. Apoptotic cells were also detected by TUNEL assay, DNA ladder, and Western Blot using the antibody against cytochrome C and caspase-3. The present study suggests that after the continuous process of 10, 20 and 30 days, respectively, IH activates necrotic machineries in cardiomyocytes of WKY and SHR, and the necrosis ratio of SHR was greater than that of WKY. However, there was no significant change in Poly (ADP-ribose)-polymerase (PARP) activation except WKY's cardiomyocytes lipid peroxidation and SOD activities were significantly lower than SHR. These findings conclude that the necrotic component is caused by a surge of free radicals, which results in ATP depletion and membrane permeabilization. In WKY, IH induce a smaller increase of free radicals and result in the release of cytochrome c and the condensation/ fragmentation of caspase 3-dependent chromatin. Therefore, the IH-induced cardiomyocytes apoptosis and necrosis can be related to the increase of free radicals. A smaller increase in free radicals will result in apoptosis in cardiomyocytes of WKY rats. In hypertensive rats, after giving the intermittent hypoxia, it is able to create a large oxidative stress and to cause the cardiomyocyte necrosis and heart dysfunction.

P17

Neuroplasticity of Pulmonary C Fibers Induced by Intermittent Hypoxia in Rat: Role of Hydroxyl Radical and Cyclooxygenase Metabolites

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Long term exposure to intermittent hypoxia (IH), such as occurring in association with obstructive sleep apnea (OSA), may evoke reflex excitation of cardiopulmonary system and even generate systemic inflammation. Clinically, OSA may occur concomitantly with noturnal asthma and cough reflex, it is likely that involves a remarkable plasticity of pulmonary C fibers (PCFs) during airway inflammation. 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 mechanisms possibly underlying these effects. 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 activated 86.7% (13/15) of the PCFs tested and evoked a stimulatory effect. Indeed, stimulating PCFs with IH exhibited a long-term facilitation (LTF) on 93.3% (14/15) 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 dimethylthiourea (a hydroxyl radical scavenger) alone, indomethacin (a cyclooxygenase inhibitor) alone, or a combination of the two, attenuated the IH-induced stimulation, LTF, and hypersensitivity of PCFs. The suppressive effects of a combination of dimethylthiourea and indomethacin on the PCF responses were not superior to indomethacin alone. In addition, IH challenge produced a higher level of prostaglandin E2, a cyclooxygenase metabolites, in the bronchoalveolar lavage fluid. 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 hydroxyl radical and cyclooxygenase metabolites.

P18

Searching for cellular factors facilitating HCV replication

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Hepatitis C virus (HCV) infects more than 170 million people worldwide, leading to both acute and chronic liver diseases in patients. Hepatitis C virus (HCV) is an enveloped virus with a single stranded 9.6-kb RNA genome. Nowadays, the combined therapy with interferon-alpha (IFN-α) and ribavirin (RBV) remains the only available option for treatment of patients with chronic hepatitis C. There is no vaccine available. Moreover, the standard therapy [(pegylated) interferon alfa plus ribavirin] is only effective in 50–60% of patients and is associated with 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. Thus, cellular factors facilitating hepatitis C virus replication could be the anti-viral targets. To find out the cellular factors facilitating HCV replication, microarray assay and RNAi technology were used. Several candidate genes were identified.

P19

Identification of Major Impurities in Methamphetamine With Gas Chromatography -Mass Spectrometry

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Methamphetamine (MA) is one of the most important drugs of abuse in Taiwan. At the present time, individual methamphetamine stereoisomers (*S*)-(+)-MA ,(*R*)-(-)-MA and mixtures of the two stereoisomers are abused in Taiwan. For the synthesis of MA： (-)-Ephedrine (EP) or (+)-Pseudoephedrine (PEP) can both be used as the starting substances for (S)-(+)-MA manufacturing；(+)-EP or (-)-PEP can both be used as the starting substances for (R)-(-)-MA manufacturing. The aim of this work is to simultaneously identify the major impurities, concentration and optical isomers of EP and PEP in MA samples. Gas chromatography/mass spectrometry (GC/MS) with selected-ion monitoring (SIM) was employed after drugs were derivatized with (R)- (+)-α- methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA). A 50m HP-5MS column (0.25mm i.d., 0.25μm film thickness) can completely separate the stereoisomers of EP and PEP, after derivatization with MTPA. A typical GC-MS run time is about 30 min. Urine samples containing EP and PEP were alkalinized with 1N sodium hydroxide and extracted with dichloromathane . The recovery was 75％ to 83％. Linear range of the calibration curves of (+)EP and (+)PEP were observed from 50 to 10000 ng/mL (100 to 10000 ng/mL for (-)EP and (-)PEP), the R^2 values were all above 0.99. The limit of quantitation (LOQ) for (+)EP and (+)PEP was 50ng/mL, for (-)EP and (-)PEP was 100ng/mL. The limit of detection (LOD) for the 4 stereoisomers was 50ng/mL. Precision was determined with three different concentrations. The within-day precision and accuracy were below 9％ and ±11％. The between-day precision and accuracy were below 10％ and ±8％. We have analyzed MA samples to compares the prevalence of abuse with (S)-(+)-MA or (R)-(-)MA, and to determine the stereoisomers and concentration of EP and PEP in the MA samples. Urine samples positive of EPor PEP will be analyzed to identify their stereospecificity.

P20

Study of the mechanisms of hepatitis C virus core gene induced hepatocellular carcinoma

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Infection with hepatitis C virus (HCV) could result in the development of hepatocellular carcinoma (HCC). The ARFP/F protein is synthesized from the +1 reading frame of the hepatitis C virus (HCV) core protein gene. The function of this protein remains unknown. Our results indicate that the HCV ARFP/F protein can enhance the gene trans-activation activity of c-Myc, apparently by antagonizing the inhibitory effect of MM1. These results suggest that ARFP/F protein may play a role in hepatocellular transformation in HCV patients. To clarify the individual role of core and ARFP/F protein in the development of HCC, three different kinds of transgenic mice has been made (the protein expression is under the control of liver-specific albumin promoter): producing both core and ARFP/F protein, producing core protein alone, and producing ARFP/F protein alone (it is difficult to detect ARFP/F protein since it is very labile, therefore, we also generate the ARFP/F protein with myc tag) : The ratio of liver weight versus total body weight: transgenic mice with both core and ARFP/F protein ＞transgenic mice with ARFP/F protein alone ＞ wild type mice. At present, no HCC was observed in these transgenic mice (up to 51 weeks old). In our original plan, these three different kinds of transgenic mice will be inter-crossed with transgenic mice with H-ras to study the oncogenic properties of core and ARFP/F proteins (co-operation of Hras and myc will induce transformation). Transgenic mice with H-ras were also generated. However, most of them are embryonic lethal. Three mice were delivered but dead soon after they were born with liver two-fold bigger than that of wild type mice. Thus, the three different kinds of transgenic mice could not inter-cross with transgenic mice with H-ras to study the oncogenic properties of core and ARFP/F proteins as we planned. At present, these three different kinds of transgenic mice were treated with PB or DEN to study the development of HCC. After that, we can clarify the individual role of core and ARFP/F protein in the development of HCC. Furthermore, to study the effect of HCV core protein on the host cell gene expression, ddRT-PCR assay was performed in transgenic mice with core protein versus wild-type mice. Several genes were identified.

P21

The transcription mechanism for TPA-induced *p15* ^{INK4ß} gene expression in HepG2

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TPA (12-O-tetradecanoyl-phorbol 13-acetate) may inhibit cell growth of hepatoma cell HepG2 through activation of PKC-MEK-MAPK signal pathway and induction of gene expression of CDK (cyclin dependent kinase) inhibitor p15INK4b and p16INK4a。

Our research goal focus on investigation of the TPA-responsive regions on p15INK4b promoter for induction of this gene. Also, which transcriptional factors are required for activation of p15INK4b promoter will be identified. Of these, Snail will be highlighted.

In luciferase assay, The data show that TPA may induce luciferase activity of a specific p15INK4 promoter fragment, namely, p15pro230, covering -223/-75 bp upstream of the translational initiation site of this gene. On the other hand, by ChIP assay we found TPA may induce binding of Snail to the -223~1 (upstream of the translational initiation site) fragment on the p15INK4promoter in vivo. However, according to GENOMATRIX database there is no putative binding region for Snail. Thus we suspect Snail may play as a transcriptional co-activator of certain transcriptional factors capable of directly binding to p15INK4b promoter. Alternatively, there may be novel Snail binding sequence (to be identified) on this region for Snail to activate the p15INK4b promoter.

Therefore we search transcriptional factor binding site on -223/-75 promoter region of p15INK4b according to GENOMATRIX database. We design several DNA probes based on these regions were designed for EMSA. The data show mobility shift of the -192/-163 fragment was observed. According to the database, this promoter region contains binding sites for CTCF and EGR1 transcriptional factors.

Based on the above results, we suggest that TPA induce p15INK4b expression via activation of -223/+1 region on p15INK4b promoter. Moreover, transcriptional factors such as Snail, CTCF and EGR1 may be required for this activation the detailed mechanisms for which will be furtherly explored.

P22

Functional analysis of a colistin resistance associated gene in *Acinetobacter sp. ADP1*

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Multi-drug resistant *Acinetobacter baumannii* (MDRAB) can cause serious nosocomial infection. There were not suitable antibiotic to treat multi-drug resistant *Acinetobacter baumannii* infection before. Based on review papers, we found that colistin had been used to treat multi-drug resistant gram negative infection recently. According to our experiments, we found that after treated *Acinetobacter* spp. with colistin, the original *Acinetobacter* spp. becomes colistin resistant easily. However, the colistin resistant mechanisms in *Acinetobacter* spp. remain unknown. Therefore, the aim of our study is to examine the colistin resistance associated gene in *Acinetobacter sp. ADP1*. At the beginning, we treated the *Acinetobacter* sp. *ADP1* (ATCC33305) with gradually upgrading colistin concentration and got a colistin resistant strain from ATCC33305. We termed the induced colistin resistant strain 33305CR. In order to identify genes involved in colistin resistance, we performed two dimensional electrophoresis technology and data base search to find out several possible candidates. The first candidate gene we wanted to analyze in depth is *ACIAD0727* (corresponds to sensory histidine kinase in two-component regulatory system with *rstA*). RT-PCR analysis revealed that colistin resistant strain 33305CR had higher *ACAID0727* RNA expression levels compared with colistin susceptibility strain ATCC33305. We disrupted the ACAID0727 in ATCC33305 and ATCC33305CR by transposon mutagenesis simultaneously. We found that it is more difficult to get colistin resistance in *ACIAD0727* knockout mutant than the wild type ATCC33305. Besides, we found the MIC of colistin decrease apparently in *ACIAD0727* knockout mutant 33305CR. Thus, we conclude that *ACIAD0727* is a colistin resistance associated gene in *Acinetobacter sp. ADP1*.

P23

Morphological characterization of amyloid beta aggregates fibril in different environmental conditions

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Alzheimer'disease (AD) is a neurodegerative disease and the commonest form of dementia. One of the hallmarks of Alzheimer'disease is the self-aggregation of amyloid β peptide (Aβ) in extracellular amyloid plaques. Theses Aβ aggregates are toxic to neuron, and the toxicity induced by Aβ is highly dependent on environmental conditions. The examination of environmental factors accelerating Aβ formation and growth is an important key to unravel etiology of AD. Thus, in the present study, we investigated the process of Aβ aggregation and morphology of Aβ aggregates under different pH, concentration, incubation time and temperature. Several biophysical techniques, including Atomic force microscopy(AFM), fourier transform infrared(FTIR) spectroscopy and other spectroscopies, were used to visualize the morphology, secondary structure, and aggregation process. Results show that the morphology of Aβ aggregates form a rod-like shape under pH6.5-7.5, 4℃ and 48hrs. On the other hand, the morphology of Aβ aggregate shows a disk-like shape under disk-like under pH3.5-4.5, 37℃ and 4 weeks. The secondary structure of Aβ in nucleation state contains 56% β-sheet and 44% random coil at pH7.0 and 63% β-sheet and 37% random coil at pH4.0. Turbidity assay indicates that the aggregation of Aβ42 in rod-like fibril is more rapid than Aβ42 in disk-like fibril.

P24

The protective role of parkin against beta-amyloid(Aβ) induced neurotoxicity

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized pathologically by the deposition of beta-amyloid(A-beta) containing extracellular neuritic plaques and intracellular neurofibrillary tangles. The aggregation of Aβ (1-42) has been shown to induce neurotoxicity, protein oxidation, lipid peroxidation, and reactive oxygen species generation in neurons. A recent study have shown that overexpression of parkin protein can provide substantial protection stress through NF kappa B pathway. Furthermore, NF kappa B has also been shown to play an important role in Aβ-induced toxicity. Therefore, we constructed a parkin overexpressed PC12 cell and investigated the possible role of parkin in protection against Aβ-induced toxicity. Our result indicated that overexpression of parkin can protect PC12 cell against Aβ induced neurotoxicity,possibly through the I kappa B/NF kappa B pathway.

P25

Identification of unknown drugs with GC/MS and LC/MS

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The rapid increase of drug abuse lately has become a very serious problem that affects almost every community and family in some way.

In routine urinalysis of amphentamine and methamphentamine, urine samples were screened with immunoassays first. The positive urine samples were then confirmed with gas chromatography mass spectrometry (GC/MS). Sometimes unknown peak in the chromatogram were observed, it maybe a clinical medicine or a new abused drug. These unknown peaks can be investigated by searching the GC library of established mass spectra. Unfortunately, not all medicine was included in the GC library. We developed a method that employed gas chromatography mass spectrometry (GC/MS) and high-performance liquid chromatography mass spectrometry (LC/MS) to identify the unknown drug in urine sample. First, we collect urine sample that in routine test have shown unknown peak. The urine sample was extracted by liquid phase extraction (LLE). The extracts were analyzed with LC-MS. Compounds were separated on a ZORBAX SB-Aq Column (150mm× 2.1mm I.D, particle size 3.5μm). The HPLC method used gradient elution. The mobile phase constitutes of 90% of A (0.1% of formic acid in deionized water) and 10% of B (0.1% of formic acid in methanol). The initial mobile phase composition of 90% solvent A and 10% solvent B was maintained for 4 min. Between 4 and 22 min the percentage of mobile phase B was increased to100% and maintained for 7 min. Then back to the initial mobile phase composition within 0.6 min, and maintained for 0.4 min, with a total run time of 30 min. The mass spectra of compound was determined with electrospray ionization (ESI) and monitored with Auto MS2 mode. The LC/MS/MS spectral was compared with a library based on ESI spectra. After initial confirmation with the LC/MS/MS spectra, drug standard was purchased and reanalyzed with GC-MS for definitive confirmation and generation of GC-MS spectra library.

P26

The role of parkin in cell cycle

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Although the function of parkin, an E3 ubiquitin ligase, is well known as part of UPS machinery, a recent study showed that parkin can bind strongly to α/β tubulin hetereodimers and microtubule. Since chromosome segregation occurs on a bipolar spindle-shaped structure that is built from microtubule, it is proposed that microtubule-associated parkin may also involve in regulation of cell cycle, particularly in prometaphase and metaphase. In order to fully explore this hypothesis, we investigated the relationship between parkin and microtubule and its regulatory role in cell cycle. Our results showed that parkin overexpressed cell was resistant to the treatment of 400ng/ml nocodazole. The microtubule-associated parkin was localized at centromere from prophase to metaphase as visualized by confocal microscopy and could stabilize the microtubule-associated centrosome.

P27

Atomic Force Microscopy of pH Dependent Morphological Changs of *Escherichia coli* Flagella

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Flagella contribute to the virulence of pathogenic bacteria through chemotaxis, adhesion to and invasion of host surfaces. Previous investigations pointed out that a reduction in urethra pH level with a concomitant may reduce the adherent activity of the bacteria. It was also observed that the swarming ability of *Proteus* was inhibited by an alkaline condition of the medium. As flagella are the key factor for the adhesion and mobility of the bacteria, environmental pH may affect these bacterial abilities via affecting the bacterial flagella.

In this study, we firstly tested swarming ability of *E.coil* in pH 6, pH 7and pH 8. The results showed that swarming ability of *E.coil* was indeed affected by the environmental pH. The best swarming ability of *E.coil* was observed in pH 7, and the worst was in pH 8. Atomic force microscope (AFM) was then applied to characterise the morphology of bacteria flagella in different pH environments. Different diametres of flagella were measured in different pH by the AFM. The diametres of the flagella were 21.05±0.73 nm, 34.48±1.81 nm, and 5.93±0.36 nm in pH 6, pH 7and pH 8, respectively. These measurements were also confirmed by transmission electron microscopy (TEM). Our results indicated a strong correlation between flagella diametres and swarming ability of *E.coli* in different pH environments.

P28

Study on cellular factors interacting with influenza A virus NS1 protein

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Influenza A virus non-structural protein (NS1) is a multifunctional protein, and in virus infected cells NS1 modulates a number of host-cell processes by interacting with cellular factors. NS1 inhibits host gene expression through interacting with mRNA polyadenylation factors (CPSF and PABII) in the nucleus while it inhibits innate immune response through interacting with the pathogen sensor RIG-I(retinoic acid-inducible gene I) in the cytoplasm. To gain further insight into the role of NS1, yeast two-hybrid screening system was used to search for cellular factors interacting with NS1 protein. Homo sapiens proteasome (prosome, macropain) subunit, beta type, 4 (PSMB4) was found to interact with NS1 protein in this assay. Interacting domains of these two proteins were also determined using this assay. Interactions between these two proteins were further demonstrated using co-immunoprecipitation and confocal analysis. No significant effect of PSMB4 depleting on the replication of influenza A virus was found. We are studying on the effect of NS1 on cellular protease degradation pathway now.

P29

Effects of Magnolol on The 6-hydroxydopamine Model of Parkinson's disease

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Magnolol, a major bioactive constituent of the bark of *Magnolia officinalis*, has been reported to provide neuroprotection, which may be related to the anti-oxidative actions and antagonism of excitotoxicity induced by excitatory amino acids. It suggests that magnolol may modulate NMDA, non-NMDA and mGluR5 receptor functions. In this study, we examined whether magnolol is also effective against the neuronaltoxicity caused by 6-hydroxydopamine (6-OHDA), both in vivo and in vitro, which is relevant to Parkinson's disease (PD). In vitro study, co-treatment with magnolol reduced the cytotoxic effect of 6-OHDA on human dopaminergic SH-SY5Y neuroblastoma cells by measuring the cellular mitochondrial activity with methylthiazolyldiphenyl-tetrazolium bromide (MTT). Moreover, pre-treatment of magnolol reduced the neurodegeneration produced by a unilateral injection of 6-OHDA into the striatum in mice. Magnolol administration started 40 min prior to lesioning and continued daily for 14 days significantly ameliorated the numbers of apomorphine-induced contralateral rotations in 6-OHDA treated mice. In addition, magnolol administration started 7 days after 6-OHDA lesion and continued daily for 14 days significantly ameliorated the numbers of apomorphine-induced contralateral rotations. The findings raise the possibility that magnolol may be a novel candidate for neuroprotective treatment of PD.

P30

Cytoskeleton reorganization and migration inhibition by a brand-new compound PT-262

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The derivatives of 5, 8-quinolinediones contain anticancer activities. PT-262, a new synthetic compound derived from 6, 7-dichloroquinoline-5, 8-dione, was investigated the anticancer ability in this study. Treatment with 1-24 μM PT-262 for 6-72 h significantly induced the cytotoxicity via a concentration and time-dependent manner in A549 human lung cancer cells. Interestingly, PT-262 dramatically induced the cytoskeleton alteration and cell elongation. The average cell length was increased from 39.15 to 65.30 μm following treatment with 2 μM PT-262 in A549 cells. The Rho-ROCK signaling pathway has been shown to regulate stress fiber formation and cancer cell migration. Treatment with PT-262 or Y-27632 (a specific Rho-ROCK pathway inhibitor) inhibited the stress fiber formation and cancer cell migration. PT-262 was more effective on the inhibition of cancer cell migration than Y-27632. Together, our results suggest that Rho-ROCK pathway may participate in the regulation of cytoskeleton alteration and migration inhibition after treatment with PT-262 of the human lung cancer cells.

P31

Detection, identification and metabolic profiling of oxethazaine and its metabolites in relation to mephentermine and phentermine use in human urine using GC-MS

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During a sport competition event in Taiwan, several specimens were found positive for both mephentermine and phentermine. The donors claimed that they had taken Stoin for treating stomach pain and the medications were presented. Laboratory investigation found that Stoin, which contains oxethazaine, has been used as a topical anesthetic. Oxethazaine can be converted to mephentermine and phentermine.

Mephentermine and phentermine are synthetic sympathomimetic drugs prohibited in sport by the World Anti-Doping Agency. Following this report, we carried out several excretion study on six healthy volunteers who orally took 5 mg oxethazaine, 10 mg mephentermine or 10 mg pentermine. Urine samples collected at various times were analyzed using Gas chromatography-mass spectrometry. The results demonstrated that (a) an efficient and reliable analytic method were developed oxethazaine, phentermine and mephentermine and their metabolites in the urine; (b) the mass spectra of phentermine and mephentermine were identified with characteristic ions of m/z 168, 110, 91 and 154, 132, 91, respectively; (c) in comparison to the concentrations of mephentermine and phentermine in the urine when mephentermine was administered, the results were generally reversed when oxethazaine was ingested with phentermine greater than mephentermine.

P32

Role of Akt and Securin on the Baicalein-induced Cancer Cell Death

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Baicalein is a major bioactive flavonoid component of *Scutellaria baicalensis* exerting anticancer activity, although the mechanisms are not fully understood. The phosphatidylinositide 3'-OH kinase (PI3K)/AKT signaling pathway plays an important role in the regulation of cancer cell proliferation and survival. Securin, also known as pituitary tumour transforming gene (PTTG), is highly expressed in many cancer cells and regulates cell cycle progression. However, the role and regulation of AKT and securin on the baicalein-induced apoptosis in human cancer cells remain unclear. Baicalein inhibited cell viability in a variety of human cancer cell lines including bladder, breast, cervical, colon, and lung cancers. The protein phosphorylation of AKT was increased by baicalein in human cancer cells. Blockade of PI3K/AKT pathway by wortmannin increased the cytotoxicity in the baicalein-treated cells. Interestingly, the protein expression of securin was reduced by baicalein in human cancer cells. The securin-wild type cancer cells were more susceptible on the cytotoxicity than the securin-null cancer cells after treatment with baicalein. Furthermore, the protein phosphorylation of AKT was increased by baicalein in both the securin-wild type and -null cancer cells. Together, our results suggest that the existence of securin promotes the baicalein-induced cytotoxicity, and the phosphorylation of AKT by baicalein resists cancer cell death in a securin-independent pathway.

P33

活化轉錄因子保護心臟免於壓力過負荷下造成的心室肥大

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過去研究顯示活化轉錄因子 3(activating factor 3, ATF3)是一種壓力(stress)誘發的轉錄因子 (transcription factor) ，屬於鹼性區域白胺酸夾鏈(bZIP)家族中 ATF/cAMP 反應元素結合蛋白(CREB)次家族的一員。然而，ATF3 在心臟壓力過負荷(pressure overloading)下，如高血壓或主動脈瓣膜狹窄等疾病中，所扮演的角色仍有待釐清。因此，本研究利用 ATF3 基因剔除小鼠(knockout mice)進行主動脈縮窄術(aortic banding)造成心臟壓力過負荷，觀察其後續之病理生理變化，其目的是探討在壓力過負荷下 ATF3 是否具有心肌保護作用。

我們的研究顯示：(a) 利用心臟超音波檢查，ATF3 基因剔除小鼠，在主動脈狹窄四星期後，其心室腔比野生型(wild type)小鼠更寬 (LVIDs: 0.285 vs 0.197cm, n=5, $p<0.05$)，而且心肌短縮分率(fraction shortening)也比野生型小鼠差(FS: 33.3 vs 20.5%, n=5, $p<0.005$)，表示心室收縮功能變差．(b) 心臟組織在膠原染色(Masson stain)下，ATF3 基因剔除小鼠顯現更廣泛的纖維化現象；利用 TUNEL 染色發現 ATF3 基因剔除小鼠比野生型有更嚴重的細胞凋亡(apoptosis)，而且更早進入細胞壞死的階段．

為探討 ATF3 之心肌保護機制，(a) 我們在培養的心肌細胞，利用腺病毒感染轉殖 ATF3 基因，應用重氫白胺酸攝入實驗([H^3] Leucine incorporation)，在表現 ATF3 的心肌細胞顯示會抑制 Phenylephrine(PE)誘發之心肌細胞肥大(cardiomyocyte hypertrophy)，而且利用西方墨點方法也發現，ATF3 會降低 CREB 的磷酸化(phosphorylation)，因而抑制心肌細胞的肥大; (b) 為進一步探討 ATF3 與細胞凋亡的關係，我們以 ATF3 促進因子 Thapsigargin (TGG)處理心臟細胞，藉由西方墨點法我們發現 HAX-1 蛋白有增加的現象，HAX-1 為一抗凋亡的因子，因此 ATF3 可能與 HAX-1 的調控有關．

結論：在壓力過負荷下，ATF3 會抑制心肌細胞肥大及凋亡，因而具有心肌保護作用。未來在臨床上將可作為開發心臟衰竭治療藥物的標的。

P34

Cholinesterase Inhibitor Blockade of Sympathetic nAChR-mediated Neurogenic Dilation in Porcine Basilar Arteries

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Cholinesterase inhibitors (ChEls) have been used for the treatment of Alzheimer's disease (AD). The clinical efficacy of these drugs, however, is less than satisfactory. The possibility that ChEIs may have effects other than ChE inhibition, such as negative modulation of neuronal nicotinic acetylcholine receptors (nAChRs), was explored in the present study. We examined if donepezil and huperzine A inhibited nAChR-mediated nitrergic vasodilation in isolated porcine endothelium-denuded basilar arterial rings using in vitro tissue bath technique. Consistent with our previous findings, activation by nicotine of nAChRs located on the perivascular sympathetic nerve terminals facilitated nitric oxide (NO) release resulting in vasodilation. Donepezil (1-30μM) and huperzine A (10-300 μM) in a concentration-dependent manner inhibited nicotine (100μM) and transmural nerve stimulation (8 Hz)-induced vasodilation with the former more potent than the latter. Both ChEIs did not affect relaxation induced by sodium nitroprusside (0.3-100 μM) or isoproterenol (0.01-10 μM). Furthermore, donepezil and huperzine A inhibited nicotine-elicited inward currents in α7-nAChR-expressing *Xenopus* oocyte. These results suggest that donepezil and huperzine A directly inhibit nAChRs located on cerebral perivascular sympathetic nerve terminals, leading to blockade of neuronal NO release and cerebral neurogenic vasodilation. Inhibition of nAChRs by ChEIs may in part explain the limitation of the effectiveness of these drugs in AD therapy. Huperzine A appears to be safer than donepezil.

P35

Modulatory mechanisms of granulocyte-colony stimulating factor in suppressing NO overproduction and iNOS expression of BV-2 microglia caused by lipopolysaccharide

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Microglia activation enhances expression of the inducible NO synthase (iNOS) to over produce nitric oxide (NO), leading to neuronal damage. Granulocyte-colony stimulating factor (G-CSF) has neuroprotective effect. It is not known whether G-CSF modulates microglia function to protect neurons. We demonstrated G-CSF receptor on BV-2 microglia cell. The microglia cell subjected to stimulation of lipopolysaccharide (LPS) enhanced iNOS expression and NO production. These enhancements were suppressed by G-CSF. In addition, G-CSF caused an increase in phosphorylated-Akt, associated with an increase in phosphorylated-GSK3β, a decrease in GSK3β, and an increase in cytosolic nuclear factor-kappa B (NF-κB). In conclusion, G-CSF-induced reductions in GSK3β may attenuate translation of iNOS and translocation of NF-κB. These reductions may result in suppression of NO over production from the activated microglia. This novel mechanism for G-CSF suppression of the activated microglias may be important in neuronal protection in neurological diseases.

P36

G-CSF augments iron-overload cardiac dysfunction and induces cardiac thrombosis whereas can be attenuated via simvastatin in mice

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Objective: We aimed to investigate the effect of G-CSF on chronic cardiomyopathy induced by iron overload. Although recent reports have suggested that G-CSF may exert a beneficial effect on left-ventricular remodeling after acute myocardial infarction, its effect in chronic cardiac disease is unclear. Methods and Results: Male C57B6 mice iron overloaded (n=11) and iron-overloaded mice treated with G-CSF (I+G mice, n=11) both showed decreased cardiac diastolic function and conduction defect. Unexpectedly, 7 of the I+G mice showed mural thrombi in the left ventricle. RT-PCR studies showed increased level of inflammatory coagulants, including ICAM-1, MCP-1, tissue factor, and TNF-α, in the affected myocardium of I+G mice, as well as recruitment of monocytes and neutrophils. Simvastatin treatment to the I+G mice can markedly attenuate the thrombus formation with decrement of inflammatory markers IL-6 and TNF-α. Conclusion: G-CSF augments iron-overloaded cardiac dysfunction and induces cardiac thrombosis in mice, in which can be abrogated by simvastatin treatment. We propose that iron overload increases oxidative stress and damages the cardiac endothelium, and further administration of G-CSF promotes the pro-inflammatory pathway and result in thrombus formation. We proposed here a novel animal model to study inflammatory-dependent cardiac thrombosis.

P37

Glycine transporter 1 inhibitor (Sarcosine) alleviates toluene-induced behavitoral neurotoxicity

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Toluene is a commonly abused organic solvent and produces significant behavioral dysfunction. Electrophysiological studies demonstrate that toluene can inhibit NMDA receptor-mediated currents. In the present study, we examined whether sarcosine, a glycine transporter 1 inhibitor, could reverse toluene-induced behavioral and neurochemical responses. Male NMRI mice were pretreated with sarcosine (100 or 300 mg/kg, i.p.) 15 minutes prior to administration of toluene (750 mg/kg, i.p.). Thirty minutes later, the rectal temperature, rotarod, social interaction and novel object recognition task (NORT) were examined. The mortality rate was measured at toluene (2000 mg/kg i.p.) with pre-treatment and post-treatment of sarcosine (800-1600 mg/kg i.p.). Sarcosine reduced acute toluene-induced hypothermia, motor incoordination, social interaction deficits, cognitive impairments and mortality. In subchronic test, mice received injection per day of either toluene (600 mg/kg) or oil at P35-P37, toluene (750 mg/kg) at P38-P39 and P42-P46. Sarcosine (300 mg/kg) was either co-treated with toluene or given 10 days after toluene withdrawal for 14 days. Then, the social interaction and NORT were tested. After behavioral test, the mice were scarified. DA, 5-HT, and their metabolites were measured in mPFC, striatum and nucleus accumbens. In subchronic toluene-exposed mice, co-treatment of sarcosine could reverse the cognition deficits induced by toluene, but not social interaction, whereas post-treatment of sarcosine alleviated social interaction deficits and cognition impairment. However, the effects of sarcosine on the levels of DA and 5-HT were not in parallel with the behavioral responses. These findings suggest that sarcosine has potential to act as an antidote for acute toluene intoxication and beneficial effect on subchronic toluene-induced behavioral dysfunction.

P38

T cell responses after treated with dengue NS1 elicited anti-DR autoantibodies

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Dengue virus infection is an emerging tropical threat . It includs mild dengue fever and severe dengue hemorrhagic fever . The mechanism of how dengue virus triggers abnormal immune response is not understood . Many groups involved the researches of dengue infection and the ability of NS1 protein to induce autoantibody , which was first found by Falconar at 1997 .

According to our preliminary data , it showed that immunoglobulin from dengue hemorrhagic fever patient's sera could cross-react with one of the TNF receptor / death receptor (DR) superfamily members . In addition , we have found dengue NS1 protein elicited anti-DR autoantibodies might be involved in the pathogenesis of dengue virus infection .

Here we would like to investigate weather dengue NS1 protein elicited anti-DR autoantibodies could inhibit the function and the survival of T cells .

P39

Molecular Cytogenetic Study on Familial Epilepsy

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Epilepsy is a common but genetically complex neurological disorder that affects 1-2% of world population. Previous studies showed the chromosomal or genetic abnormalities may confer susceptibility to epilepsy, but the genes causing epilepsy have not been well known or characterized to date. In the present study, we reported 6 individuals with epilepsy in the same family, and the clinical work up did not reveal any environmental or infectious factors to cause the disease in this family. We hypothesize genetic factors are major contributed to this familial epilepsy. To investigate the chromosomal aberrations for the familiar epilepsy, chromosome karyotyping and molecular cytogenetic techniques were used for this study. The karyotyping results showed that no numerical or structural abnormalities were found in these epileptic individuals. Furthermore, genomic DNA was isolated from whole blood cells for array-based comparative genomic hybridization (array-CGH) analysis. The array-CGH data showed that duplication was identified in chromosome 14q11.1-11.2 from epileptic individuals of this family. The identified genomic gain regions were further verified in affected individuals by real-time quantitative PCR and the results were consistent with those from array-CGH. These results indicated that chromosome 14q11.1-11.2 may be a candidate region for epilepsy and used for further investigation to determine the genetic factors of the familial epilepsy.

P40

Mechanism of how *Bacillus anthracis* Lethal Toxin affects the expression of Aurora B in megakaryocytes

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Bacillus anthracis can cause many clinical symptoms, include abnormal bleeding and thrombocytopenia, but the mechanisms were still remained unknown. Anthrax lethal toxin (LT) is the major virulence factor produced by *Bacillus anthracis* and our previous studies demonstrated that LT interferes megakaryocytic polyploidy in TPA treated HEL (erythro/megakaryocytic) cell line. Megakaryocytes (MKs) are large and polyploidy cells that can produce platelets. As they mature, MKs through a process called endomitosis that defined by abortion of mitosis in late anaphase and incomplete of cytokinesis, but remain DNA synthesis result in polyploidy. Aurora B is a subunit of the chromosomal passenger protein complex that plays an essential role in mitosis. Previous studies have been reported that continuous repressions of Aurora B during MKs maturation are necessary processes. Here we have identified the protein level of Aurora B was decreased in TPA treated HEL cell line and increased when pretreated with LT by Western blot. Aurora B is recognized by APC/C-cdh1 and degraded by ubiquitin-proteosome degradation pathway. Taking together, in this study we will exam whether the increased of Aurora B in LT pretreated HEL cell is mediated by inhibition of the ubiquitin-proteosome degradation pathway.

P41

Potential roles of cofilin in megakaryocytic polyploidy

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Anthrax lethal toxin (LT), a critical virulence factor of *Bacillus anthracis*, is a complex of protective antigen (PA) and lethal factor (LF). PA acts to deliver LF to the cytosol of target cell and LF is a Zn^{2+}-dependent metalloprotease, cleaves mitogen activated protein kinase kinases (MAPKKs) family through proteolysis of their NH_2-terminal. Megakaryocyte maturation involves the development of polyploidy cells via endomitosis. Our previous studies have demonstrated that LT can block megakaryocytic polyploidy in TPA (12-*O*-tetradecanoylphorbol-13-acetate) treated HEL (Human erythroleukemia) cell line. Cofilin is a key regulator of actin filament dynamics and plays important roles in cell-cell adhesion, cell migration and cytokinesis. The activity of cofilin is regulated by phosphorylation and dephosphorylation at Ser-3, with the phosphorylated form being inactive. The major aim of this study is to characterize whether cofilin play an important role in megakaryocytic polyploidy. We have found that phosphorylated cofilin was increased when TPA treated and reduced when LT pretreated. We will use immunoprecipitation Western blotting analysis to demonstrate phospho-cofilin increased maybe affect cell division to result in megakaryocytic polyploidy.

P42

Pathogenic effects of dengue virus nonstructural-1 protein elicited antibodies on B lymphocytes

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Infection of dengue virus, one of the Flavivirus, can cause dengue hemorrhage fever and dengue shock syndrome. The antibodies against dengue virus nonstructural protein 1 (NS1) could cross-reactive to self-antigens. Previously we showed that dengue patients sera and rabbit anti-NS1 antibodies could cross-reactive to the tumor necrosis factor receptor / death receptor families. These are also B cell antigens. We would like to investigate the pathogenic role of anti-NS1 antibodies on B lymphocyte. C57BL/6J mice animal model were immunized with GST、NS1 recombinant protein to address the question. After immunization with GST、NS1 recombinant protein, we founed subsequently immunized of T-independent antigen showed different titers between different experiment groups. Our data suggest that anti-NS1 antibodies could directly affect on B lymphocyte.

P43

Analysis for the role of LMO7 in mitosis progression

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The spindle assembly checkpoint（SAC）can ensure precise separation of paired sister-chromatids into two daughter cells during mitosis. The SAC components include MPS1, CENP-E, BUBR1, BUB3, MAD1, MAD2, ZW10, and ROD. By yeast two-hybrid screening, we have identified that MAD1 interacted with LIM domain only-7 (LMO7). Fluorescence microscopic analysis revealed that LMO7 localized to nucleus, actin filament, and seemingly the centrosome. The LIM domain of LMO7 was sufficient for its localization to both the actin filament and centrosome. LMO7 was not involved in the formation of cleavage furrow. We further made a series of LMO7 truncation construct to define its function in the SAC. We found that overexpression of the full-length LMO7 could significantly resulted in a defect in the SAC, and that deletion of the LIM domain could relieve the negative effect on the SAC caused by overexpression of LMO7. Besides, deletion of LIM domain made LMO7 very diffusively distributed with the cytosol in both interphase and mitotic cells. These observations indicated that localization of LMO7 could determine its function in the SAC. However, more analyses are still required to confirm the cellular functions of LMO7 in mitosis.

P44

Role of the cleaved signal peptide of HCV core protein in autoimmunity

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Infection with hepatitis C virus (HCV) could cause severe liver diseases, e.g. autoimmune hepatitis, liver cirrhosis, and hepatocellular carcinoma. Mechanisms of HCV pathogenesis are largely unknown. HCV core protein is a 191-amino-acid product released from the polyprotein by cleavage with cellular signal peptidase. This 191-amino-acid core protein will be further processed by signal peptide peptidase (SPP) into the mature form of core protein with about 179 amino acids in size. The function of the cleaved signal peptide of HCV core protein (a.a. 180-191) is unknown. This cleaved signal peptide was found to have high binding affinity with HLA-0201 molecule and have high homologies with peptides of cytochrome c proteins: CYP2A6 and CYP 2A7. Thus, we hypothesize that the cleaved signal peptide of HCV core protein will induce autoimmunity in HLA-0201 patients. To address this issue, we have raised the transgenic mice with HLA-0201 molecules. These transgenic mice will be immunized with the cleaved signal peptide of HCV core protein or peptides of CYP2A6 and CYP 2A7 later. Using this mouse model, we will be able to study the role of the cleaved signal peptide of HCV core protein (a.a. 180-191) in autoimmunity.

P45

Modulation of metastatic functions of hSecurin through its interaction with COPS5

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Pttg1 (Pituitary Tumor-Transforming Gene 1) is an oncogene and was found to be over-expressed in many tumor types. The PTTG1 protein regulates cell cycle at metaphase/anaphase transition and is identified as the human securin (hSecurin). In a microarray study, hSecurin had been found to be over-expressed in most metastatic carcinomas. hSecurin was also found to be a transcription factor. It can also interact with other proteins. Thus hSecurin can exert its metastatic functions through regulating its target gene or through interacting with its associated proteins thus modulating their functions. To find out the function of hSecurin in metastasis, we used yeast-two hybrid screen to identify proteins that interact with securin. We identified several proteins. One of these is Jun activation domain-binding protein 1 (Jab1).

Jab1 is also called COPS5. COPS5 is a multifunctional protein which is involved in various cellular mechanisms including development in Drosophila and mouse, cell cycle control and signal transduction pathways. Several studies showed that COPS5 functions as a nuclear exporter and inducer of cytoplasmic degradation for several proteins including p53.

Using pull-down and co-immunoprecipitation assays we demonstrated that COPS5 interacts with hSecurin in vitro and in vivo. hSecurin has already been proven to interact with p53. This interaction blocks the specific binding of p53 to DNA and inhibits its transcriptional activity. But the mechanism still remains unclear. Our results indicated that COPS5 could not induce the cytoplasmic localization and p53 degradation in HCT116 cells if the cells are devoid of PTTG1/securin ($sec^{-/-}$ HCT116). Therefore, we suggested that COPS5 induced the cytoplasmic localization and degradation of p53 may be through the interaction with hSecurin.

P46

A novel regulatory pathway confers elevated expression and anti-apoptosis ability of claudin-1 in nasopharyngeal carcinoma cells

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Claudins are the main tight junctional proteins and play a paracellular transport barrier to maintain tissue homeostasis as well as cellular polarity. Recently, aberrant expression of claudins are illustrated in various tumor cells, however, the biological functions are still largely unknown. Here, we reported an elevated expression of claudin-1 in nasopharyngeal carcinoma cells (NPC) under apoptotic insults including serum deprivation and Fluorouracil treatment. Meanwhile, in the beginning, cell apoptosis was increased but lately decreased whereas claudin-1 consistently increasing expression. By using RNA interference and ectopic overexpression of claudin-1 in tumor cells, respectively, the anti-apoptotic activity of claudin-1 was clearly demonstrated. However, cell proliferation was not induced by claudin-1 overexpression. Conversely, the aforementioned feature of claudin-1 was potentially driven by cell proliferation which was enforced underwent apoptotic insult. Moreover, epithelial-mesenchymal transition (EMT) process apparently involved cell proliferation followed claudin-1 expression. Taken together, our data suggested a certain role of claudin-1 in tumor cell growth, and provided a novel regulatory pathway of expression as well as anti-apoptosis ability of claudin-1.

P47

Effects of Bacillus anthracis Lethal Toxin on Erythropoiesis

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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. Lethal toxin is the major virulence factor of B. anthracis, which was found to be a mitogen-activated protein kinase kinase (MAPKKs) inhibitor. Treatments of lethal toxin to experimental mice or cell culture could reproduce certain anthrax-like pathogenic responses in vivo; this made it an idea molecular tool to study anthrax-mediated pathogenesis. Since anemia and hypoxic tissue damages are involved in anthrax-mediated mortality, these observations prompt us to further analyze whether lethal toxin could influence erythropoiesis. According to our experiments, we found that treatments of lethal toxin indeed inhibit proliferation of human erythro/megakaryocytic cell line (HEL and K562), decrease the number and size of erythroid colonies in mouse colony-forming cell (CFC) assay, and change the percentage of erythroblasts in mice. In addition, pretreatments of erythropoietin (EPO) significantly ameliorate the morality of lethal toxin-treated mice. Our data might suggest a new model that lethal toxin is primarily block erythropoiesis and then results anemia, hypoxic tissue damages and death.

P48

Visible-light photocatalyst-mediated cytotoxicity on tumor cells

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Photo-excited titanium dioxide (TiO_2) substrates are primarily induced by UV-light irradiation to exert their biocidial activities through oxidation and reduction. Recently, some modified TiO_2 substrates were proved to have equally potent antimicrobial activities against bacteria under visible-light illumination. Whether or not visible-light photocatalysts (VLPs) contain any anti-tumor activities remains to be further studied. In this study, we found that tumor cells can be killed by visible-light illuminated VLPs in both *in vitro* and *in vivo* experiments. These findings suggest that VLP may be a novel approach to develop anti-tumor strategies.

P49

Coagulopathy and vasculopathy induced by dengue envelope protein domain III

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Dengue virus , a member of the flavivirus family , causes an emerging global health threat. Infection of dengue virus can result a range of clinical symptoms, the more severe forms is DHF/DSS, are characterized by thrombocytopenia, hemorrhagic manifestations and evidence of increased vascular permeability; however, the mechanisms of DHF or DSS development are not completely understood. In this study, we found purified recombinant soluble dengue envelope protein domain III (rsEIII) bound to human platelet directly, and sequently induced platelet activation as measured by P-selectin expression on flow cytometry. Treatment of rsEIII can induce thrombocytopenia and leukopenia in B6 mice, wich were similar to clinical sign observed at early stage of dengue virus infection patients. Activated partial thrombin time(aPPT) assay show that rsEIII could also neutralize heparin function and prolong plasma clotting time similar to the usage of protamine, a heparin antidote for clinical use. At the same time, we also found rsEIII has antiangiogenic effect as observed it's can inhibit endothelial survival and impaired VEGF165 mediate endothelial cell proliferation in vitro and chorioallantoic membrane assay in vivo. These data suggest that dengue EIII protein region might be an important role in dengue-induced coagulopathy and vasculopathy.

P50

Structural Characterization of HCV E1 and E2 Proteins

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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.

We would like to know which glycoprotein, E1, E2, or both, is responsible for the fusion event? Also which region of the E1 or E2 protein is the fusion peptide? We have prepared several constructs containing truncated E1 or E2. The truncated E1 or E2 protein can be expressed and purified from *E. coli*. Circular dichroism analysis of these expressed proteins showed little difference between pH 7 and 4. Chemical crosslinking is used to identify if there is any protein-protein interaction between these proteins.

P51

Biological Effects of hHB-EGF on the Mammary Gland of Transgenic Mice Carrying WAP T/t

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Hepairin-binding epidermal growth factor-like growth factor (HB-EGF) has been show to stimulate the growth of a variety of cells in an autocrine or paracrine manner and to be involved in stromal proliferation. Although HB-EGF is widely expressed in tumors compared with normal tissue it's contribution to metastasis is unknown. HB-EGF can be produced as a membrane-anchored form (pro-HB-EGF)and later processed to soluble form (s-HB-EGF).A recent study demonstrated that HB-EGF can binding to N-arginine dibasic convertase is highly specific for HB-EGF among EGF family members. Its specific binding modulates HB-EGF-induced cell migration via EGF receptor. we observe to carry WAP T/t and hHB-EGF transgenic mice tumor proliferation and metastasis. In this study , we found that HB-EGF induced tumor proliferation and metastasis.

P52

Central Chronic Effects of 3-Nitropropionic Acid in Mice

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Introduction : Mitochondria play a critical role in cell death by releasing apoptogenic factors from the intermembrane space into the cytoplasm. Because mitochondrial dysfunction has been shown to be involved in several neurodegenerative diseases, mitochondrial toxins are largely used to model these disorders. For example, 3-nitropropionic acid (3-NP) is a neurotoxin that inhibits mitochondrial complex II, which causes prolonged energy impairment and replicates most of the clinical and pathophysiological features of Huntington's disease, including spontaneous choreiform and dystonic movements, as well as selective degeneration of striatum. In this in vivo study, we investigated and compared the neurotoxic and neuroprotective properties. Objective : To observed whether body weight, food consumption and behavior of mouse were influenced by chronic treatment with the 3-NP. Furthermore, we also studied the brain damage by histology.

Meterials and Methods : The guide cannule were implanted into the third cerebroventricle in adult male C57BL/6 mice. Animals were randomly divided into different groups and received the intracerebroventricle treatments of 3-NP. The various dosages of 3-NP (0.05, 0.5, 5 or 50 ng/day) were microinjection from day 0. Using the body weight, food consumption, lifespan and behavioral tests were as the damage indices. Conclusion : In this study, the toxic effects of 3-NP have the dose-dependent treatments. Animals treated with 3-NP showed limp paralysis, dystonia, or other abnormal behaviors after 4-6 days microinjection. Body weight and food consumption were significantly decreased in the 50 ng treated group of 3-NP than other dosages of 3-NP. Similarly, the survival rate was significantly lowest in the 50 ng treated group of 3-NP. According to the indices of body weight, food consumption, and lifespan. We will test whether the neuroprotective agents block or delay the toxic effects of 3-NP in the future studies.

P53

Circadian Rhythm-related Genes Expression Influenced by Insulin Resistance

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Population of type 2 diabetes mellitus (T2DM) is increased with high rate in well-developed and developing countries. Except some related to heredity, most of T2DM are related to the imbalance in the ratio of energy income and outcome which regulate by liver. Recently International Diabetes Federation defined that central obesity was involved in the pathology index of T2DM. Another important sign to develop T2DM is insulin resistance (IR). Both obesity and IR increase free fatty acids (FFAs). When glycolysis proceeding, FFAs from adipose tissue or intestine chylomicrons hydrolysis enter to mitochondria in liver and are implicated in the necessary reoxidation of NADH + H^+ to NAD^+, thereby enabling maintenance of glycolytic flux. Interesting, The DNA-binding activity of the CLOCK:BMAL1 heterodimers (master the circadian rhythms) is also regulated by the redox state of NAD cofactors in a purified system. The neonatal male Sprague-Dawley rats subcutaneously injected STZ on postnatal day 2 (PND2) or intraperitoneally injected nicotinamide (NA) and STZ on PND21 were used in this study. For monitoring the physiological responses of treatments, we measured the body weight and plasma glucose level of fasting or glucose treatment by intraperitoneal glucose tolerance test (IPGTT). We found that the body weight of the experimental groups were lower than the control groups in the first 12 weeks. They displayed gradually increase of fasting glucose level, and exert the trend of insulin resistance even DM after treatment. Using the reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) to measure the gene expression, the circadian rhythm-related genes, such as *Per1*, *Per2*, *Per3*, exhibited the diurnal rhythmicity in all groups. Interestingly, the *Clock* gene expression did not have this rhythmic pattern. Moreover, the levels of *Per1*, *Per2*, and *Per3* gene expression were changed and showed the increasing following the animals developed the IR. In conclusion, the circadian rhythm-related genes have the potentials as the indices for the IR, but the further studies are needed to make this clearer.

P54

Rapid enhancement of endothelium-derived nitric oxide contributes to neuroprotection of granulocyte-colony stimulating factor against ischemia-reperfusion in the brain

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Granulocyte colony-stimulating factor (G-CSF) has beneficial effect on ischemic and functional recovery, but its effect on ischemic cerebral blood flow is unclear. Therefore, we intend to investigate the effect of G-CSF on ischemic cerebral blood flow. Transient cerebral ischemia /reperfusion (TCI/R) model induced by microinjection of endothelin-1 (ET-1, 400 pmol/10 μl) were used in Sprague-Dawley rats. Real-time monitoring of blood flow and NO release were measured with a laser Doppler flowmeter and an NO-selective electrode, respectively. A single dose of G-CSF (200 μg/kg, subcutaneously) or saline was administered immediately after ET-1 injection. Total infarction volume were measured by 2,3,5-Triphenyltetrazolium chloride staining. In our TCI model, the reduction of striatal blood flow (<17.5 % of basal) was recovered to 90 % by G-CSF administrated. The peak of NO production during ischemia preceded the onset of blood flow increase. G-CSF significantly promoted an earlier and faster restoration of reduced blood flow during ischemia while exaggerated NO production, which proceeded the striatal blood flow restoration period. G-CSF markedly reduced the infarct size and neurological deficit at 24 hr after TCI/R, which were abolished by pretreatment of L-N^G-*nitro-arginine methyl ester* (L-NAME, 30 mg/kg intraperitoneally) at 30 min prior to G-CSF administration. G-CSF enhanced phospho-eNOS protein expressions at 15 min after TCI suggest that G-CSF-induced increased in NO production is mediated via activation of eNOS pathway. Pretreatment of L-NAME blunted G-CSF protective effect on the infarction volume and neurological deficit. G-CSF shorten the cerebral ischemic period, accelerate the blood flow restoration in ischemic area, reduced neurological deficit, which can be a valuable therapeutic option for the acute cerebral ischemia in clinical implication.

P55

Comparative Proteomic Analysis of Biofiom and Planktonic cells of *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus is one of the major pathogenic agents of bacterial gastroenteritis that exists in multiple cell types for adapting appropriately in natural environments and hosts. *V. parahaemolyticus* No.93 (VP93), which carriers neither *tdh* nor *trh* gene, was isolated from a case of collective seafood poisoning We observed VP93 produces strong biofilms under conventional growth conditions in our laboratory. Therefore, we hypothesize that biofilm formation of VP93 might play an important role in adherence to abiotic and biotic surfaces. For this reason, we want to compare proteins between planktonic and bofilm cells and find out the protein involved in biofilm formation. The planktonic and bofilm cells were collected, proteins were extracted and then separated by two-dimensional polyacrylamide electrophoresis (2DE). Proteins exhibiting significant spots were then identified by mass spectrometry and basic local alignment and search tool (BLAST). In this study, we showed that the development of biofilm in VP93 depends on temperatures and nutrients. Vp93's adherent ability is better on hydrophilic substrate, specifically borosilicate glasses. Based on SDS-PAGE analysis, protein profiles are definitely different between exponential phase and staionary phase. Analysis 2DE gels by PDQuest, we also found 489 proteins that are from biofilm cells and 13 proteins are enhanced in biofilms cells compared to planktonic cells at stationary phase. Diminished protein expression was observed that those proteins with the pI range 4.5 – 6 and with molecular masses of 10-70 kDa. The results show that biofilm cells possess novel proteins, of as yet unknown function, that are not present in planktonic cells.

P56

TTC1138 from *Thermus thermophilus* HB27 Involved in Biofilm Formation

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Bacteria exposed to transient 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 the *T. thermophilus* HB27. In genetic approach, a *TTC1138* (response regulator) deletion mutant was still on constructing. Attempt to construct the mutant, the PCR product of *TTC1137*、*TTC1139* and kanamycin resistant gene were cloned into plasmid pGEM-3Zf(-) that to occur homologous recombination .The *TTC1138* gene will be mutated by kanamycin resistant gene. In biochemical approach, the gene encoding TTC1138 protein in *E.coli* was overexpressed and purified by the Ni^{2+} affinity chromatography analysis that to prepared the antibody. It will be crystallizing structure studies and identified the period and location of protein expression via the western blot analysis. The relationship of *TTC1138* and biofilm development will be investigated in the future. The differences of protein expressions profiles between sessile cells and planktonic cells of wildtype and TTC1138 mutant will be own specific interest in the future.

P57

Construct a Mutant Library of *Thermus Thermophilus* HB27

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Constructing a mutant library could do researches on thousands or ten thousands of mutant strains of their phenotypes. By understanding their phenotype expressing, we could further get into genetic fuctions of these mutant ones. In Taiwan, there are many researchers constructing mutant libraries of many species, and sharing the genetic libraries informations with the world. Seeing that lacking of mutant libraries of *Thermus thermophilus* spp., we will construct a mutant library of *T.thermophilus* HB27. Except for exporing its unique properties, higher optimal growth temperature and producing thermostable enzyme and protein in *T.thermophilus* spp. treasure-house, we put our focus on its biofilm formation and connecting the related factors. By way of ligasing newly cloning plasmid *kan*/pGEM3zf(-) and radom digestion of chromosome DNA fragments of *T.thermophilus* HB27. Then, utilizing nature transformation of *T.thermophilus* HB27 let the previous ligased product to double crossing over the original chromosome DNA of *T.thermophilus* HB27. As the result, we could collect large amount of different mutants and by screening the activity of biofilm formation. We might find out important genes causing the biofilm formation.

P58

Diet influence the composition of gut microbiota in wild rodents

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The gut ecosystem has great impacts on host fitness, and myriad of microbial species makes it one of the most complicated ecosystem on earth. Environmental changes, especially food contents and its nutritional value, may directly affect the composition of gut microbiota. Most studies were carried out on mice maintained in simplified laboratory environments, therefore may not reflect the gut ecosystem diversity.

We have obtained 135, 127, and 158 isolates from the guts of wild rodents *Mus musculus*, *Apodemus semotus*, and *Rattus exulans*; and 37, 42, and 75 of them were anaerobes, respectively. The cultured bacteria were identified as members of *Clostridium*, *Enterococcu*, *Lactobucillus* and *Veillonella*, according to their 16S rRNA gene sequences. Cellulase activity, which represents the ability to use the most abundant plant-derived carbohydrates, was found in many isolates with apparent heterogeneity. *In vitro* culture experiments with fresh fecal pellets as inocula and *in vivo* feeding experiments demonstrated that mucin or different plant polysaccharide supplements can change microbial community composition when mucin and different plant polysaccharides were used as major carbon sources. The change in community structure was evident even within hours, which highlights the dynamic nature of the gut ecosystem.

P59

Functional analysis of inner envelope membrane components of the chloroplast protein import apparatus *in vivo*

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Most chloroplast proteins are post-translationally imported from the cytosol through a translocon complex located in the chloroplast envelope. Translocon components in the outer membrane are named as Toc (translocon at the outer- envelope-membrane of chloroplasts) proteins and those in the inner membrane are named as Tic (translocon at the inner-envelope-membrane of chloroplasts) proteins. We have investigated the function of Tic40 through analyzed an Arabidopsis T-DNA-tagged *tic40* mutant. Protein import and cross-linking experiments demonstrated that Tic40 was part of the Toc/Tic translcon complex. The C-terminal half of Tic40's hydrophilic domain is composed of a TPR domain followed by domain shared by co-chaperones Sti1p/Hop and Hop. Previous data support that Tic40 may functions as a co-chaperone in the stromal chaperone complex that facilitates protein translocation across the inner membrane. In this study, we investigate the interacting domains among Tic40 and proteins that are known to associate with Tic40 are being mapped by yeast two-hybrid assay, and use biochemical approach and proteomic tools to identity new components associated with Tic40 by co-immunoprecipitation. Due to the accuracy of *Arabidopsis* genome sequence and annotation, many individual proteins can be unambiguously identified from a protein mixture using mass spectrometry. Functional significance of these interactions between with Tic40 and other novel components will also be analyzed. Knowledge obtained from this study will help us understand the composition and functional mechanism of chloroplast protein import machinery.

P60

Characterization of Chaperonin from *Thermus aquaticus* YT-1 Involved in Biofilm Formation

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Thermus aquaticus YT-1 is a gram-negative bacterium that did not have motility. It was suitable for growing at 55~60℃ and was found in Yellowstone of U.S.A. at 1969. It was not only isolated from hot spring but also have the ability to form biofilm. *Thermus aquaticus* YT-1 reached log phase at the 6th hour and it enter stationary phase after 12 hours cultivation. The planktonic cell and sessile cell were collected in 4 periods of time at the 6th、12th、18th、24th hour. Preliminary analysis was performed by one dimension gel electrophoresis. There were different proteins expression profile in the 6th hour and the 24th hour. Two-dimensional electrophoresis was applied to analyze that the protein of the planktonic cell and sessile cell expression. Utilize commassie blue G250 to be dyed, it could find out that the majority different protein focuses at pI 5~6. Then extracted the protein and analyzed the protein by ESI-QUAD-TOF and MALDI-TOF. In addition, compared data with Matrixscience database. Result shows those proteins was involved in translation(8%)、transcription (3.2%)、replication, recombination and repair(6.2%) 、 posttranslational modification, protein turnover, chaperones(5.5%)、energy production and conversion(11%)、
carbohydrate transport and metabolism (4%)、amino acid transport and metabolism (3.2%)、coenzyme transport and metabolism (3.2%)、lipid transport and metabolism (4.8%)、inorganic ion transport and metabolism(4.8%)、secondary metabolites biosynthesis, transport and catabolism(0.8%)、general function prediction only(8%)
、cell wall/membrane biogenesis(2.4%)、cell cycle control, mitosis and meiosis (0.8%)、intracellular trafficking and secretion(0.8%)、signal transduction mechanisms(2.4%)and some protein with unknown function(30.9%). According to previous results, I have selected chaperonin (GroE) for futher study. The GroE can be divided into GroES and GroEL, mainly for proteins folding and protection. Then we cloned the *groES* to BL-21 by transformation, and induction by IPTG. We had purified 11 mg of GroES by nicol chelate affinity chromatography. In addition, I have test nature transformation frequency that transfrred pUC19-PYK189 plasmid to *Thermus* spp. for transformation test. In the future I will construct the GroES mutation plasmid that containd truncate *TTHA0271* and *TTHA0273* to pGEM-3Zf(-) beside kanamycine resistant gene. Nature transformation will be performed to transfer GroES mutation plasmid to *Thermus* spp.. Disrupting the *groES* via the double crossing over, and then mutants were generated. I will study the relationship of GroES and biofilm formation in the future.

P61

Complementation of different tRNA Synthetases between yeast and Arabidopsis

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tRNA synthetase is the basic and the key enzyme of translation. It can direct the amino acid to the corresponding tRNA. In our experiment, we try to look the complementation of different tRNA Synthetases（Valyl-tRNA synthetase, Glycyl-tRNA synthetase）between yeast and Arabidopsis. Because mutations of the tRNA synthetase are embryonic lethal, we use heterozygous mutants (TWN2/twn2, Gly/gly) for our purpose. If yeast tRNA synthetase can complement Arabidopsis, we should be able to identify homozygous mutants from our transgenic plants.

P62

同源近親品系小鼠的建立：得自一自發性突變鼠的經驗

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建立一同源近親品系 (congenic inbred strain) 意將具特定目標基因型的原品系 (donor strain)，回交 (backcross) 至另一近親品系 (recipient strain) 數代，用以得到一帶有此基因型的新品系。換言之，此一新品系的特徵除帶有該基因的特定性狀，其餘基因背景與回交的近親品系相同。故可 1) 用於了解一基因在不同遺傳背景下所造成的影響，2) 排除其他基因對目標基因的影響，3) 確認目標基因是否為 QTL (Quantitative trait loci)。本探討將藉由一在 BALB/cByJNarl 所發生的自發性突變小鼠，回交至 C57BL/6NTac 育種的經驗，來說明與討論如何有效率地建立同源近親品系。

P63

Tumor Necrosis Factor-α mediates Pseudorabies Virus-induced Apoptosis via the Activation of p38 Mitogen-activated Protein Kinase and c-Jun N-terminal Kinase /Stress-activated Protein Kinase Signaling

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Pesudorabies virus (PRV) is a member of Alphaherpesviruses family, and contains double strand DNA genome about 143 kbp with casid surrounded. The capsid is surrounded by a layer named the tegument and virus-encoded proteins called envelope. The mortality of PRV infection in piglets is up to 100 percent, and cause abortion and stillbirth in sow. What's more, PRV not only infect pig but also cattle and then spread with high speed, hence the pecuniary loss is serious. Apoptosis assay, DNA fragmentation and acridine orange stain expressed that PRV infection induce apoptosis. Further experiments using p38 and JNK inhibitors show PRV-induced apoptosis via p38 and JNK Mitogen-activated protein kinase pathways. We confirm this result by p38 and JNK western blot. Except above mentioned, according to real-time PCR, we know the level of TNF-α mRNA increase with virus does-dependent. The intracellular mature form of TNF-α and the secretion of TNF-α are see the same phenomenon too in Western blot and ELISA. Therefore, we suggest that TNF-α mediates PRV-induced apoptosis via p38 and JNK Mitogen-activated protein kinase pathways.

P64

Caffeine Overrides Radiation-Induced Prolonged G_2/M Arrest and Results in increased apoptosis in DNA-Dependent Protein Kinase Deficient Malignant Glioma Cells

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Glioblastoma multiforme (GBM) is the most common and aggressive type of primary brain tumor. A malignant glioma is radioresistant and always recurs, even after a high dose of radiation. DNA-dependent protein kinase (DNA-PK) plays an important role in the repair of DNA double-strand breaks (DSB) induced by ionizing radiation (IR). Lack of DNA-PK causes prolonged G_2/M arrest and radiosensitization. Caffeine has been shown to disrupt the G_2/M checkpoint and sensitizes tumor cells to ionizing radiation. However, the mechanism of caffeine by radiation-induced prolong G_2/M arrest in DNA-PK deficient malignant glioma cells remain unclear. Two type of human malignant glioma cell lines, M059K (DNA-PK proficient) and M059J (DNA-PK deficient) cells, were investigated on the action of caffeine by irradiation. Treatment with caffeine markedly decreased radiation-induced prolong G2/M arrest and increased apoptosis in M059J cells. Furthermore, caffeine decreased clonogenic survival in M059J cells. As a whole, our result indicate that caffeine override prolong G_2/M arrest by undergo apoptosis after radiation in DNA-PK deficient malignant glioma cells. This may point to an effective approach toward improving radiotherapy outcomes of DNA-PK deficient malignant glioma.

P65

Hydrogen-consuming *Hydrogenobacter* streamer from hydrothermal environment in Hualien, Taiwan

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Many members of *Hydrogenobacter* form macroscopic filamentous aggregates in hydrothermal environment. White 4 to 6 cm long filaments were found in local hot spring in the spring outflow, with one end firmly attached to the substrate. Microscopic examination revealed microbial cells within aggregates are a diverse community in which motile Gram-negative rods dominate. These bacterial grow well, and grew well aerobically in heterotrophic media. Electron microscopic observation shown that the filamentous aggregates collected on site were composed of threads of long inter-connected rod cells, with a diameter approximately 0.5 μm. Cells appeared to be shorter when grown in broth culture. The dominant sequence amplified with universal bacterial 16S rRNA primers was found to be unique and closely related to *Hydrogenobacter hydrogenophilum*. Denaturing gradient gel electrophoresis analysis indicated diverse bacterial composition in this community, and the *Hydrogenobacter* fragment was found in both the filament and in the spring water. Cocci, rods, and filamentous cells were found in enrichment cultures in microscopic examination. Addition of either organic compounds or H_2/CO_2 can support bacterial growth, suggests the presence of fermentors and hydrogen consumers in this community.

P66

Momordica charantia reduces viability of gastric cancer cells via distinct mechanisms

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Gastric cancer is considered to be a major health problem worldwide. Although chemotherapy significantly improves survival in patients with gastric cancer, additional agents effective against gastric cancer are still needed to increase the available regimens. Diets rich in bioactive phytochemicals are known to be associated with reduced risk of certain cancer and several diet-based strategies hold promise for both prevention and treatment of colon cancer. *Momordica charantia,* commonly called bitter gourd , is recently reported to have some biomedical activities such as anti-inflammation and anti-glycemia effects. Previous researches indicated that *Momordica charantia* has the anti-tumor activity on some tumor cells, however, the effect of this diet on gastric cancer cells has not been investigated. This study is designed to use gastric cancer SCM-1 cells to evaluate the anti-proliferation activity of *Momordica charantia.* Ethanol extract of whole fruits was found to be significantly effective on the growth inhibition of SCM-1 cells at a treated dose higher than 0.5mg/ml. *Momordica charantia* induced apoptosis was evaluated by using DNA fragmentation, DAPI/PI staining and flow cytometry assay. The increase of stress-induced gene expressions, P53 and cyclin-dependent kinase inhibitors such as p15, p18 and p19, indicated that ethanol extract of *Momordica charantia* would probably induce both apoptosis and cell cycle arrest in SCM-1 cells. Results from the protein analysis of caspase 3, substrates of caspase 3 and some proapoptotic Bcl-2 family proteins indicated that the ethanol extract of *Momordica charantia* has a potential to be developed as an anti-proliferation diet with more detailed investigations.

Key Words: *Momordica charantia,* gastric cancer cells, apoptosis, cell cycle arrest.

P67

Genetic characterization and molecular cloning of genes affected in chloroplast biogenesis in *Arabidopsis thaliana*

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The majority of chloroplast proteins are encoded within the nuclei synthesized on cytoplasic ribosomes. These proteins must be imported into the organelle post-translationally by a translocon complex at the chloroplast envelope. The translocon at the outer membrane of chloroplasts (Toc complex) and the translocon at the inner membrane of chloroplasts (Tic complex) act cooperatively during this import process. In order to further our understanding the import progress, we analyzed a collection of the T-DNA-tagged mutants affected in chloroplast biogenesis in *Arabidopsis thaliana*.

These mutants were obtained from the *Arabidopsis* Biological Resource Center (ABRC) in the Feldmann Collection and display the pale-green or albino phenotype. The locus of the T-DNA insertion will be identified by TAIL-PCR (thermal asymmetric interlaced PCR) or plasmid rescue. With the completion of the *Arabidopsis* genome sequencing project, we can clone these genes and predicate the function of gene easily. In this study, we provide the data indicate a gene that deduced protein sequence shows high homology to a know Toc component, and the other mutant may affect acetyl-CoA C-acyltransferase activity. Progress on characterization the functions of these novel genes will be studied by biochemical methods and genetic tools. Knowledge obtained from this study will help us advance our understanding of chloroplast biogenesis in general.

P68

Involvement of DNA-damage Pathway in Pseudorabies Virus-induced Apoptosis

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Although induction of apoptosis by Pseudorabies virus (PRV) has been demonstrated in vivo and in cultured cells, to date, the involved signaling pathways are still largely unknown. The current research will investigate whether PRV utilize DNA damage response in mediating viral replication and apoptosis induction, may like other members of hepesviridae family, including herpes simplex virus 1 and Epstein-Barr virus. In the presence of DNA damage signaling inhibitor caffeine, the viral titer and PRV-induced apoptosis were suppressed significantly. Cytopathetic effect observation and trypan blue dye exclusion revealed the caffeine treatment increased the cell survival rate upon PRV infection. Expression and phosphorylation of DNA damage sensor protein Ataxia telangiectasia mutated-Rad3-related kinase（ATR）, DNA-dependent protein kinas (DNA-PK) and their downstream transducer checkpoint homologue 1 (chk1), chk2, as well as DNA damage effector p53 were increased in a dose dependent pattern following PRV infection. Two p53 downstream apoptotic effects bax and bak expression were upregulated, whereas, expression of another proapoptotic protein bcl-Xs did not altered. bcl-Xs without p53 binding sites within its promoter region. In addition, caffeine reduced the expression of chk1 and chk2 induced by PRV. Taken together, our data support a model in which PRV-induced apoptosis is mediated via DNA damage signaling.

P69

Intermittent Hypoxia-Induced Astrocytes Proliferation Inhibition

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Intermittent Hypoxia (IH) is accompanied with several pathophysiological conditions, including obstructive sleep apnea syndrome (OSA), asthma, and apneas in premature infants. Recent studies indicate that Chronic intermittent hypoxia (CIH) increases oxidative damage to the brain cortex and decreases neurocognitive function in rodent models resembling human OSA. However, the mechanism of neuronal cells death and astrocytes dysfunction caused by IH is unclear. Astrocytes play a key role in central nervous system functions and have diverse functions in many aspects of ischemic brain damage. IH is a more potent inducer of cell death than continuous hypoxia, probably because of a substantial increase in free radicals generation. In this investigation, we exposed cultured cerebellar astrocytes to hypoxia-reoxygenation (5% O_2 30 min, 21% O_2 30 min) for 0~4 days and examined the effect of IH on astrocyte cells proliferation. The present study suggests that overproduction of reactive oxygen species in IH. For example, in astrocyte cells, hydroxyl radical ($OH^{\bullet}$) is one of the major causes of proliferation inhibition and activation PARP activities, which may result in intracellular ATP depletion and cell cycle arrest corresponding to an increase in p53 and p21(waf1) protein expression.

P70

Effect of *Vibrio vulnificus* infection on the physiological function of zebrafish

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In mammals, many studies revealed that heart rate variability (HRV) decrease when individual was infected. In contrast to the large number of HRV studies in mammals, there is little known about fish. The aim of this study is to exam the change of cardiovascular function of zebrafish during *Vibrio vulnificus* infection. The zebrafishes were divided into experimental and control groups. In the beginning, electrocardiograms (ECG) were recorded for 5 minutes as baseline cardiac activity. The experimental group then were raised in the infection solution containing *Vibrio vulnificus* (C.F.U.=10^8 /ml). The control group were raised in germ-free distil water for the same time. After 2 hour infection, ECG was recorded for 5 minutes each hour and last for 6 hours. On the next day, the ECG of zebrafishes was recorded for the other 6 hours. The heart rate of control group during the recording period is relatively more stable compared than the experimental group. The heart rate of experimental group increase gradually during infection period of first and second days. The result suggests that the physiological function of zebrafishes would be affected by the infection of *Vibrio vulnificus* and the ECG recording may be useful for monitoring the condition of fishes.

P71

Isolation and characterization of a novel *Paenibacillus* species from hydrothermal environment in Hualien, Taiwan.

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Paenibacillus has been isolated from diverse environment including hydrothermal environment. A novel bacterial strain, RS01-w-092606-The7ana01, was isolated from a local hydrothermal environment in Hualien. The temperature and pH readings in the natural habitat were 50°C and slightly alkaline when the samples were collected. The isolated microorganism is a Gram-negative rod, able to grow on various heterotrophic media, and form large white colonies. This strain grew well when cultured in either aerobic or anaerobic conditions, but the colonies were morphologically different. This strain was motile in medium containing up to 0.5 % agar. Growth occurred at temperatures between 30 and 50°C (optimum at 40°C), pH between 5 and 12 (optimum at pH 8.0), and in media supplemented with 0-2.5 % NaCl. This strain produced acid from cellobiose and maltose, but not from galactose or sucrose. Additional substrate utilization profile was assayed with BIOLOG GN system. The G+C content of the genomic DNA is estimated 43.6 mol%. Analysis of the 1457 bases of 16S rRNA gene sequence shows that this bacterial species is genetically distinct but closely related to *Paenibacillus barengoltzii* strain SAFN-125 (97 %) and *P. macerans* (95 %). We propose that this isolate should be classified within *Paenibacillus* and is likely to be a new species.

P72

Depletion of Securin Protein Expression Increases the Radiosensitivity and Apoptosis-Independent Growth Arrest in Human Colorectal Cancer Cells

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Securin, a proposed proto-oncogene, has been shown to regulate cell proliferation and tumorigenesis. Moreover, the over-expression of securin induced aneuploidy, genetic instability, and apoptosis. It has been shown that securin participates in DNA repair after radiation. However, the expression and role of securin on radiation-induced cell cycle arrest and cell death remain unknown. Two types of cell lines, the securin wild-type and –null human HCT116 colorectal caner cells, were investigated on the expression of securin by irradiation. The securin-null cells exhibited greater susceptibility on the cell death to X-ray radiation than the securin wild-type cells. Moreover, the securin-null cells enter prolonged G2/M arrest than securin wild-type cells following X-ray irradiation. Interestingly, the levels of phopho-p53 (Ser-15), p53 (DO-1) and p21 were elevated in both cells by irradiation. Furthermore, the existence of securin in HCT116 cells more susceptible to the induction of apoptosis than the securin-null cells after radiation. Meanwhile, radiation increased the levels of phopho-ERK1/2 proteins in securin-null cells; conversely, phospho-p38 was elevated in securin wild-type cells by irradiation. Together, it is the first time to prove that the blockage of securin protein expression increases the radiosensitivity and ERK mediates cell death via a securin-dependent pathway following radiation exposure.

P73

Tryptophan fluorescence studies of HCV E1 protein

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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.

We have successfully expressed and purified different E1 ectodomains. Tryptophan fluorescence was measured under different pH. Both E_{260} and E_{328} showed pH sensitive spectral shift.

P74

Acid Treatment Response of *Vibrio* spp.

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Vibrio parahaemolyticus was first discovered in sea food from Japan. The strain in this study was *Vibrio parahaemolyticus*93 and 14A *Vibrio parahaemolyticus*11 which were clinical strain and environment strain. Many bacteria must endure or survive transient encounters with extremely low or high pH outside the range optimum for growth. The aim with this research was that why *Vibrio* spp. can resistant stomach acid and reach to small intestines. *Vibrio* spp. reached to midde-log phase and stationary-phase after 2 and 4 hours, respectively. In this research, we use two methods to treat cells, acid tolerance response and acid adaptation response. At acid tolerance experience, we found *Vibrio* spp. can resistant acid to pH 4. On the other hand, acid adaptation experiment, we did not proof *Vibrio* spp. have adapted to acid environment, but we found that *Vibrio* spp. showed higher acid resistant ability at log-phase.

P75

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

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So far these has been studied in Thermophilic bacteria for a long time. The sciences study in the strain in the molecular and physiological levels. We are interested in biofilm formation in *Thermus thermophilus* HB27, and the goal of this study is the relationship between *luxS* and biofilm formation. Biofilm formation which could helps bacteria to defend the toxins in environment is produced by bacterial secreting polysaccharide. And there are many effects on the ability of biofilm formation in previous study. For example, quorum sensing is a effective one. Quorum sensing is a bacterial intercommunication system that controls the expression of multiple genes in response to population density. The Lux QS system regulates the expression of several virulence factors in a widw variety of pathogenic. Pili are defined by their shared structural, biochemical, and morphological features. The type IV pili are proteinceous surface structures found ubiquitously in gram-negative species of medical, environmental and ecological importance. The method we use is exchanging the site specific gene. After *luxS* junction genes were amplified by polymerase chain reaction, the products will be clone in kanamycin resistant gene /pGEM3zf(-) to obtain the mutant. Previous study indicated that the loss of *pilD* gene significantly reduces the ability of *Vibrio vulnificus* to persist in *Crassostrea virginica*, and strongly suggesting that pili expressed by this bacterium play a role in biofilm formation. Therefore, we would like to know the function of *pilD* gene in *Thermus thermophilus* HB27. We could ensure the relationship between *luxS* and biofilm formation, furthermore, the relationship between *luxS* and *pilD* in biofilm formation.

P76

Temperature Tolerance Response and Cross Protection of *Vibrio* spp.

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In nature, organisms encounter a lot of stresses including temperature variation. The major part of this study was to observe that *Vibrio* spp. change themselves when the temperature changed. I observed the growth curve to establish the log phase and stationary phase of different *Vibrio* spp. The growth curve test also showed the growth rate of *Vibrio* spp in optimum temperature. Temperature tolerance response was that changing the temperature become 4°C and 60°C. I observed the changes of growth rate. The result showed that growth rates of *V. parahaemolyticus*93 and 14A *V. parahaemolyticus*11 had not significant changes at 4°C in 24 hours. The survival condition of both bacteria in stationary phase were batter than in log phase. 14A *V. parahaemolyticus*11 had superior survival than *V. parahaemolyticus*93 in both phase. The survival condition of *V. vulnificus* YJ016 had significant changes in different mediums and different phases. *V. vulnificus* YJ016 had stable survival rate in TSB supplement with 1.5 % NaCl and Marine broth in stationary phase but don't in log phase. In Artificial seawater, the survival condition of *V. vulnificus* YJ016 in log phase and stationary phase had no changes. There are some problems in treating at high temperature. It is too high to *Vibrio* spp survive at 60°C. Except 14A *V. parahaemolyticus*11 in log phase had few colony, all of them had no colony. Next, I do the study of cross protection. The method is that doing the cold adaption on *Vibrio* spp., and tying the different stresses like acid, osmosis, oxidation. To observed the changes of growth rate. The result showed that cross protection not happen on *Vibrio* spp.. In the future, I want to find the suitable temperature of the high temperature test, to obtain the genes can against cold tolerance by mutation, and to observe the protein about cold shock from two-dimensional electrophoresis.

P77

Differential physiological responses and morphology of *Vibrio vulnificus* under aerobic and anaerobic conditions

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Vibrio vulnificus is a motile gram-negative rod with polar flagella, and it can cause fatal infection in human, eel and tilapia. Proper response to anaerobic or microaerobic conditions can be critical in viability in natural environment or inside animal intestinal tract, and the reduced oxygen concentration may be used as a environmental cue for switch in metabolism or virulence. In this study we compare the physiological response of *V. vulnificus* in aerobic and anaerobic condition. *V. vulnificus* demonstrate reduced motility on soft agar, reduced hemolytic activity, and loss of protease activity in anaerobic environment, compared with their aerobic counterpart. Moreover, the ability of biofilm formation was greatly reduced in anaerobic condition. Anaerobically-cultured *V. vulnificus* were less virulent to zebrafish. Electron microscopic examination revealed differences in cell morphology and flagellation. This study demonstrates that *V. vulnificus* cultured in anaerobic condition may have different physiological and morphological characteristics, and these differences may be associated with virulence towards vertebrate hosts.

P78

Isolation and characterization of potential pathogen-inhibiting probiotics from the environments for aquaculture application

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Isolates obtained from marine environments or cobia intestines were tested for their potential as probiotics. Among the 938 isolates, 11 were found to inhibit growth of *Photobacterium damselae* subsp. *piscicida* (Pdp, cobia isolate), *Vibrio anguillarum* (eel isolate), or *V. vulnificus* (Vv, tilapia isolate), and form wide inhibition zone in co-culture experiments. These isolates were tested for virulence toward zebrafish through intraperitoneal infection, and 4 of them found to be of no or very low virulence. These isolates therefore have potential as being used as probiotics.

Among the 4 potential probiotics, strain L.PP02-F-041807-TSAS19 is selected for further study. This isolate was found to be associated with *Enterobacter* using 16S rDNA-based characterization. Culture supernatant of this isolate can inhibit growth of pathogens Pdp and Vv. This isolate can inhibit bacterial growth in broth co-culture experiment. The addition of this isolate seems to induce aggregate formation in Pdp, which may be an additional mechanism to combat pathogen besides direct growth inhibition.

P79

TChi-2 Enhances Endothelin Release in LPS-treated Endothelial Cells

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Severe sepsis and septic shock are among the most common causes of morbidity and mortality in the intensive care unit. Septic shock in adults refers to a state of acute circulatory failure characterized by persistent arterial hypotension. Although routine treatments for sepsis including antibiotics, intravenous fluids and vasopressors are beneficial, ideal drug treatment for more serious septic shock is still lacking. Our goal is to find drugs that will improve cardiovascular functions with ultimately positive outcomes and minimum side effects in septic shock. Our preliminary results indicated that TChi-2, a natural compound isolated from Scutellaria baicalensis, attenuated the lipopolysaccharides (LPS)-induced fall in heart rate, mean arterial pressure, and TNF-a production. These results suggest a possible role of TChi-2 in protecting against and/or treating detrimental cardiovascular effects in septic shock. The exact mechanism of action of TChi-2 remains unknown. Our preliminary studies further demonstrated in isolated rat mesenteric arteries treated with LPS that TChi-2-induced vasoconstriction was blocked by endothelin-1 (ET-1) receptor antagonist. This result suggests that ET-1 released from the endothelium of the mesenteric arteries is involved in TChi-2-induced vasoconstriction. Therefore, in the proposed study, we examined if TChi-2 enhanced the release of ET-1 in cultured mesenteric artery pretreated with LPS. The results indicated that TChi-2 appeared to increase the release of ET-1 in cultured mesenteric arteries following LPS-treatment. This effect of TChi-2 was not seen in cultured aorta or tail arteries. Similar results were found in mesenteric arteries obtained from septic rats induced by LPS pretreatment. In conclusion, TChi-2 increases ET-1 release from the mesenteric arteries but not the aorta or tail arteries in LPS-treated rats. Release of ET-1 which causes vasoconstriction may contribute to the TChi-2 enhancement of blood pressure in sepsis.

Memo