109 學年度

中山醫學大學生物醫學科學系

研究成果發表會

大會手冊

主辦單位:

中山醫學大學生物醫學科學學系

中華民國一百一十年六月二十五日

目錄

研究成果發表會議程

110 年 6 月 25 日(星期五)　線上視訊(Teams 名稱: 110 碩士班研究成果發表會)

9:00 主任致辭及負責老師講解規則		
時間	報告講題	學生/指導教授
口頭論文發表(12 分鐘講解 3 分鐘提問)		
9:10~9:25	洋蔥萃取物及槲皮素合併抗皰疹病毒藥物提升抗病毒效率及其機轉之探討 Effect of onion extracts and Quercetin combined with anti-herpes virus drugs to improve anti-viral efficiency and its mechanism.	陳偲茜/楊建洲 張元衍
9:25~9:40	多重轉譯體分析微角塵蟎在上皮細胞內誘發的發炎反應 Multiplex transcriptome profile analysis for the crude allergens from Der*matophagoides microceras* involved the inflammatory responses in the epithelium cell treatments	馮天浩/劉玉凡
9:40~9:55	探討α-Mangostin在小鼠視網膜病變模型中對碘酸鈉誘導細胞毒性和氧化傷害的保護作用 α-Mangostin protective effect of sodium iodate-induced cytotoxicity and oxidative stress in mice retinal degeneration model	葉睿軒/楊建洲 張元衍
9:55~10:10	透過 NAP 預處理使 BisGMA 誘發小鼠巨噬細胞的細胞毒性減緩，並且使小鼠巨噬細胞存活率上升 NAP pretreatment reduces the cytotoxicity of mouse macrophages RAW264.7 induced by BisGMA and increases the survival rate of RAW264.7 .	楊孟蓁/楊建洲 關宇翔
10:10~10:25	建立斑馬魚為模式動物來探討視網膜病變的機轉 Establish zebrafish as a model animal to explore the mechanism of retinopathy	周佩璇/楊建洲
10:25~10:40	探討台灣本土大蟬花 Wu-BFP-14 的菌絲體水萃物保護乙醯胺酚誘導急性肝損傷的分子機制 To explore the protective mechanisms of the water extract of mycelium of Taiwan native *Cordyceps cicadae* Wu-BFP-14 on propacetamol-induced acute liver injury	李季庭/王淑紅
10:40~10:55	高劑量 N-乙醯半胱氨酸治療舒疼消熱劑誘導急性肝損傷小鼠的副作用與毒性機制 The side-effects and toxic mechanisms of high-dose N-acetylcysteine therapy in mice with propacetamol-induced acute liver damage	謝承錡/王淑紅

10:55~11:05 休息		
11:05~11:20	芹菜素藉由影響 Pin 1 和 Wnt/β-catenin 信號路徑抑制三陰性乳癌的增生和侵襲 Apigenin suppresses cell proliferation and invasion via inhibiting Pin 1 and Wnt/β-catenin signaling pathways in triple-negative breast cancer.	廖泯嘉/陳威仁
11:20~11:35	SET7/9 在抗老化蛋白質表現，細胞衰老之角色探討 The role of SET7/9 in anti-aging protein expression and cell senescence in renal tubular cells	顏夷岑/林庭慧
11:35~11:50	MBNL3 參與卵巢癌的發展 MBNL3 Participates in ovarian cancer development	陳虹均/潘惠錦
11:50~12:05	精氨酸甲基化在 Klotho 蛋白質表現之機轉探討 Modulation of anti-aging Klotho expression by protein arginine methylation in NRK-52E cells	陳柏宇/林庭慧
休息		
海報論文發表(4 分鐘講解 1 分鐘提問)		
1:30~1:35	蛋白質精胺酸甲基化程度與核仁壓力對 FUS 蛋白質核仁分布的相關性研究 The Correlation Between Protein Arginine Methylation Degree and Nucleolar Stress on FUS Protein Nucleolus Distribution	劉玉環/李　娟
1:35~1:40	對稱性二甲基精氨酸多株抗體之製備及於酵素免疫分析法之開發 Production of Polyclonal antibodies and development of Enzyme-linked Immunosorbent Assay for Symmetrical dimethylarginine	林家君/余豐益
1:40~1:45	沙門氏菌 *Salmonella enterica* serovar Typhimurium LT2 的單股 DNA 結合蛋白質的純化、表現、結晶結構、DNA 結合特性與突變分析 Purification, expression, crystallization, crystal structure, DNA binding properties, and mutational analysis of single-stranded DNA binding protein from *Salmonella enterica* serovar Typhimurium LT2	羅仁宏/黃晟洋
1:45~1:50	早期乳癌患者的不良心血管事件主要風險因子探討-一項全國性人口研究 Risk of Major Adverse Cardiovascular Events in Early Stage Breast Cancer Patients – a Nationwide Population-based Study	楊蕙芳/張文瑋
1:50~1:55	探討柚皮素(Naringenin)和胡桃醌(Juglone)合併處理對乳癌細胞增生的影響 To explore the anti-cancer effects of combined treatment of Naringenin and Juglone on the proliferation of breast cancer cells	陳慶宇/陳威仁
1:55~2:00	山奈酚和槲皮素透過影響 PIN1 調控的訊號路徑抑制乳癌細胞的增殖和遷移	陳吟佩/陳威仁

	Kaempferol and quercetin inhibit breast cancer cell proliferation and migration through regulating PIN1-mediated signaling pathway	
2:00~2:05	探討台灣本土大蟬花 Wu-BFP-14 的生物安全性及保護乙醯胺酚誘導急性肝損傷效果 To decipher the acute toxicity and protective effect of Taiwan native *Cordyceps cicadae* Wu-BFP-14 on propacetamol-induced acute liver injury	李季庭/王淑紅

口頭論文摘要

1.

洋蔥萃取物及槲皮素合併抗皰疹病毒藥物提升抗病毒效率及其機轉之探討

Effect of onion extracts and Quercetin combined with anti-herpes virus drugs to improve anti-viral efficiency and its mechanism.

學生：陳偲茜　　指導教授：楊建洲、張元衍

摘要：

單純皰疹性腦炎 (Herpes simplex encephalitis，HSE) 為最常見的中樞神經系統病毒性疾病，具有高死亡率及高復發率，因此對全球公共衛生構成嚴重威脅，而此疾病產生的主要原因是單純皰疹病毒第一型 (herpes simplex virus type 1，HSV-1) 的感染，其臨床症狀包括唇皰疹、角膜炎、腦炎等。目前臨床上主要以核苷酸類似物進行治療，例如 Acyclovir (ACV)、Ganciclovir (GCV)、Foscarnet 和 Vidarabine 等。目前的研究指出病毒的感染會引起炎症反應，因此除了抗病毒藥物外，我們將以天然物來合併抗病毒藥來提升抗病毒及抗發炎的效果。槲皮素 (Quercetin) 為一種常見的類黃酮化合物，被認為天然抗氧化劑，已證實具有抗氧化、抗發炎及抗癌等功效。另外也有研究顯示洋蔥萃取物 (Onion extracts) 中槲皮素含量較其他蔬果多。因此本篇為探討洋蔥萃取物與槲皮素合併抗病毒藥來提升抗病毒及抗發炎的效果。

首先我們將 BV-2 細胞預先處理洋蔥萃取物與槲皮素 1.5 小時後，在感染 0.5 M.O.I. 的 HSV-1，並於不同時間點收集上清液及細胞萃取物，再利用酵素免疫分析 (Enzyme-Linked Immunosorbent Assay)、西方墨點法 (Western blot) 等方法來檢測 NO 與發炎相關細胞激素的作用機制。結果顯示，HSV-1 感染 BV-2 細胞後，會造成細胞死亡，同時會誘導出發炎相關因子 NO、IL-6、MCP-1 及 IP10，但經由槲皮素及洋蔥萃取物預處理的實驗組，可顯著地下降病毒誘導的 NO、IL-6、MCP-1 及 IP10 表現；同時也發現處理槲皮素及洋蔥萃取物會增加 HO-1 的表現量及抑制 iNOS 與 pNF-κB 的表現。

接著進一步探討洋蔥萃取物及槲皮素合併抗病毒藥來是否可提升抗病毒及抗發炎的效果。結果顯示單獨使用 ACV 與 GCV 的實驗組雖可降低病毒的複製，但無法抑制病毒誘發的炎症反應 (如 iNOS、IL-6、MCP-1 及 IP10 等)。而洋蔥萃取物及槲皮素合併抗病毒藥處理下，雖無法提升抑制病毒複製的能力，但能有效降低發炎反應。

因此，根據實驗初步的結果顯示，槲皮素及洋蔥萃取物合併抗病毒藥物能夠有效抑制 HSV-1 複製，且可通過抗炎反應來保護細胞。

Abstract：

Herpes simplex encephalitis (HSE) is the most common viral disease of the central nervous system, with high mortality and high recurrence rate. Therefore, HSE is considered to pose a serious threat to global public health. The main cause of this disease is infection with herpes simplex virus type 1 (HSV-1), whose clinical symptoms include fever, epilepsy, etc. HSV-1 is a two-strand DNA virus, and according to the statistics of WHO in 2020, about 67% of people in the world have been infected by HSV-1, which can cause many diseases, such as cold sores, keratitis, encephalitis, etc. And, the clinical treatment of HSV-1 is mainly based on nucleotide analogues, such as Acyclovir (ACV), Ganciclovir (GCV), Foscarnet, and Vidarabine. Quercetin is a natural flavonoid found abundantly in many plant foods and which is known for its antioxidant, anti-inflammatory and anti-viral activities. The onion extracts contain a lot of quercetin. The aim of this study, we will combine natural products (onion extracts or quercetin) with antiviral drugs to enhance antiviral and anti-inflammatory effects.

In this study, the BV-2 cell line (mouse microglia) infected with HSV-1 was used to establish an *in vitro* HSE model. First, to elucidate the onion extracts and quercetin of potential immunomodulatory role of anti-inflammatory cytokines, we investigated the regulation effects of inflammatory factors (iNOS, IL-6, MCP-1 and IP-10, etc.) in response to HSV-1 infected BV-2 cells. These results showed that HSV-1 causes cell death and induce pro-inflammatory cytokine, such as TNF-α, IL-6, MCP-1 and IP-10 in BV-2 cells, but pretreated with quercetin or onion extracts can inhibit these cytokines. At the same time, it was also found that the treatment of quercetin or onion extract can induce the expression of HO-1 and inhibited iNOS and pNF-κB expression in HSV-1-infected BV-2 cells.

Then further explore whether onion extract and quercetin combined with antiviral drugs can enhance the antiviral and anti-inflammatory effects. The results show that ACV and GCV alone can reduce virus replication, but it cannot inhibit virus-induced inflammation (such as iNOS, IL-6, MCP-1, IP10, etc.). Although onion extract and quercetin combined with antiviral drugs cannot enhance the ability to inhibit virus replication, they can effectively reduce inflammation.

In the present study, we found that quercetin or onion extracts combined with antiviral agents can effectively inhibit HSV-1 replication and protect cells through anti-inflammation.

2.

轉譯體分析微角塵蟎在上皮細胞內誘發的發炎反應

Multiplex transcriptome profile analysis for the crude allergens from Der*matophagoides microceras* involved the inflammatory responses in the epithelium cell treatments

學生:馮天浩　指導教授:劉玉凡

摘要:

[Introduction]

家塵蟎 (House dust mites, HDMs) 是誘發氣喘的重要過敏原。台灣存在 3 種品種的 HDMs，包括微角塵蟎(*Dermatophagoides microceras*，*Der m*)，屋塵蟎(*Dermatophagoides pteronyssinus*，*Der p*)與粉塵蟎(*Dermatophagoides farinaea*，*Der f*)。前期的研究顯示台灣中部地區的 462 位特異反應性(atopy) 過敏幼童(2-16 歲)，有高達 80% 以上病患特別對 *Der m* 塵蟎，具有致敏作用(sensitization)，探討微角塵蟎致敏原刺激人類上皮細胞的致敏機制，從過敏症狀中呼吸道修補(Airway remodeling)的療效評估。

[Methods]

採用多重轉譯體分析(Multiplex transcriptional responses analysis)，進行 *Der p*、*Der f* 與 *Der m* 粗萃取液處理下，探討誘發人支氣管上皮細胞(BEAS-2B) 的致敏機制。採取不同的時間(4 和 24 小時)及不同處理條件，共十六組樣品的 RNA-seq 資料，包括: 對照組、纖維蛋白原組(fibrinogen, 重要的凝血因子)、*Der m 2* 組(主要致敏原)、藥物處理組(Dexamethason, 合成皮質類固醇)。其中分析三種粗萃過敏原蛋白粗萃物(*Der m*，*Der p* 和 *Der f*)、*Der m*+Dexamethasone 治療組，及 *Der m crude* 作用於凝血因子，不同時間點所得到產物(fibrinogen cleavage products，FCPs) 分別加入主要致敏原 *Der m 2*。採用次世代測序技術，使用 Hisat2，featureCounts 和 DESeq 等 RNA-seq 分析流程，並應用基因富集分析(GSEA)配合京都基因與基因組百科全書(Kyoto Encyclopedia of Genes and Genomes，KEGG) 與基因本體(Gene Ontology，GO)資料庫的註解。透用 Real-time qPCR 及 Western blot 分別針對目標基因表達與蛋白質層次的進行再驗證工作。

[Results]

使用三種粗萃過敏原蛋白提取物(*Der m*，*Der p* 和 *Der f*）處理的 4 小時組中的細胞因子（Il-6 和 IL-8）基因表現量高於 24 小時組的 5-8 倍，顯示致敏原誘發的發炎反應在 4 小時達到高點；首先應用 GSEA 於 KEGG 發現三種致敏原所誘發的共同富集路徑分別為 Toll-like 受體信號轉導通路(*P*=0.0001):，Nod-like 受體信號通路(*P*=0.0007)和 Jak-Stat 信號通路途徑(*P*=0.0004)，並且利用 Real-time qPCR 分別在驗證參與 Toll-like 受體信號轉導路徑的作用基因 Il-6, IL-8, NFKB1, PIK3R1, TBK1 與 RNA-Seq 分析的結果一致，並利用 Western blot 再驗證蛋白質機制，核轉錄因子 NF-κB 會由細胞質進入細胞核，藉此啟動下游基因的調控。其次分析 *Der m* 處理下的特有富集基因，是與第一型糖尿病的調控有關(*P*=0.0001)，其中上調基因有 IL-12A，IL-1 與 HLA-II，並透過 Real-time qPCR 再驗證了促 T 細胞增殖因子 IL-12A 為 *Der m* 誘發致敏作用的特有基因。接著在 *Der m*+Dexamethasone 治療組中，利用 GO 資料庫的 4D scatter plot，顯示藥物治療無效所影響的特有功能，經基因富集分析後有鈣離子結合(Calcium Ion Binding, P=1.65x10^{-7})、及微管蛋白結合(Tubulin Binding, P=2.33x10^{-7})等分子功能。最後在主要過敏原作用的分析中，顯示 1 分鐘 FCPs 加入 *Der m 2* 組別中所誘發的發炎反應具協同性的加乘作用(synergistically effect)，而利用基因富集分析顯示，顯示對於細胞連結(Adherens junction, (*P*=0.0003) 調控基因具有上調作用，說明 FCPs 透過細胞結合

素(Nectins)傳遞 Rac 訊號，來重組肌動蛋白及細胞骨架改變細胞通透性來調節 Adherens junction 的形成，改變細胞間黏附的作用。

[Conclusion]

結果顯示，三種家塵蟎的過敏原蛋白萃取物，誘發上皮細胞的致敏機制是共同經由 Toll-like 受體信號轉導通路，來引起核轉錄因子 NF-κB 進入細胞核使促發炎細胞因子的上調作用。另一方面暴露於微角塵蟎的環境下，相較其他兩種家塵蟎的致敏作用更容易導致β-cell 失去功能死亡而引發第一型糖尿病。在藥物治療組別中發現微角塵蟎有鈣離子結合、及微管蛋白結合等分子功能。最後，經微角塵蟎處理後的 FCPs 會導致 *Der m 2* 經由 Rac 傳遞訊號來調節 Adherens junction 的形成及改變細胞間的黏附作用，藉此增加主要過敏原 *Der m 2* 所誘發的發炎反應具有協同性的加乘作用。

Abstract:

[Introduction]

House dust mites (HDMs) are commonly known allergens to cause asthma in the subtropical area. There are three local breed HDMs in Taiwan including *Dermatophagoides microceras* (*Der m*), *Dermatophagoides pteronyssinus* (*Der p*) and *Dermatophagoides farinaea* (*Der f*). From the previous studies, more than 80% allergies sensitization rate exposure by the *Der m* allergens for children in the central Taiwan. Explore the sensitization mechanism of HDMs allergens to stimulate human epithelial cells, and evaluate the efficacy of airway remodeling from allergic symptoms

[Methods]

We proposed the multiplex transcriptional responses analysis approach to treatment of *Der p*, *Der f* and *Der m* crude extracts, explore the sensitization mechanism of human bronchial epithelial cells (BEAS-2B) we constructed different time (4 and 24 hours) and different processing conditions, a total of 16 sets of RNA-seq data including: a control group, fibrinogen (an important blood coagulation factor produced), *Der m 2* group(major allergens), drug treatment group(Dexamethasone, a synthetic corticosteroid to current treat allergies, asthma, rheumatic diseases, and skin diseases), three crude allergens protein extracts (such as *Der m*, *Der p* and *Der f*), in addition to *Der m crude* with fibrinogen, process different time points to obtain fibrinogen cleavage products(FCPs) and adding with *Der m 2*. we offered the next-generation sequencing (NGS) technology, and Hisat2, featureCounts and DESeq public packages for RNA-seq analysis. Gene enrichment analysis (GSEA) in Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (Gene Ontology, GO) database. Finally, Real-time qPCR and Western blot were used to verify the results of target gene expression and protein level.

[Results]

The gene expression of cytokines (Il-6 and IL-8) in the 4-hour group treated with three crude allergen protein extracts (*Der m*, *Der p* and *Der f*) was 5-8 fold change higher than that in the 24-hour group, showing that the allergen-induced inflammatory response reached a high point in 4 hours. First, GSEA was used in KEGG to find that the common enrichment pathways induced by the three allergens were Toll-like receptor signaling pathway (*P*=0.0001): Nod-like receptor signaling pathway (*P*=0.0007) and Jak-Stat receptor signaling pathway (*P*=0.0004). In addition, Real-time qPCR was used to verify that the genes Il-6, IL-8, NFKB1, PIK3R1, and TBK1 involved in the signal transduction pathway of Toll-like

receptors are consistent with the results of RNA-Seq analysis, and the results were verified by Western blot. Protein mechanism, nuclear transcription factor NF-κB enters the nucleus from the cytoplasm, thereby initiating the regulation of downstream genes. Secondly, analyze the unique enriched genes under *Der m* treatment, which are related to the regulation of type 1 diabetes (P=0.0001). Among them, the up-regulated genes are IL-12A, IL-1 and HLA-II, and they are reproduced by Real-time qPCR. It is verified that IL-12A (a T cell proliferation factor) is a unique gene for *Der m* to induce sensitization. Third, in the *Der m* + Dexamethasone treatment group, the 4D scatter plot of the GO database was used to show the unique functions affected by the ineffective drug treatment. After gene enrichment analysis, there was calcium ion binding (P=1.65x10^{-7}), and Tubulin Binding (P=2.33x10^{-7}) and other molecular functions. Finally, in the analysis of the effects of major allergens, it was shown that the inflammatory response induced by the addition of FCPs in the *Der m 2* group for 1 minute had a synergistically effect. The gene enrichment analysis showed that it has an up-regulation effect on cell connection regulatory genes (Adherens junction, P=0.0003). Its show that FCPs transmit Rac signals through Nectins to reorganize actin and cytoskeleton to change cell permeability, regulate the formation of Adherens junction, and change the role of cell adhesion.

[Conclusion]

The results show that the allergen protein extracts of the three HDMs induce the sensitization mechanism of epithelial cells through the Toll-like receptor to cause the nuclear transcription factor NF-κB to enter the nucleus and promote the inflammation of cytokines upregulation. On the other hand, exposure to the environment of *Der* m is more likely to cause β-cell loss of function and death than *Der p* and *Der f*, leading to type 1 diabetes. In the drug treatment group, it was found that the *Der m* has calcium ion binding, tubulin binding and other molecular functions. Finally, the FCPs treated by *Der m* will cause *Der m 2* to transmit signals through Rac to regulate the formation of Adherens junction and change the adhesion between cells, thereby increasing the inflammation response induced by the major allergen *Der m 2*, it had a synergistically effect.

3.

探討α-Mangostin在小鼠視網膜病變模型中對碘酸鈉誘導細胞毒性和氧化傷害的保護作用

α-Mangostin protective effect of sodium iodate-induced cytotoxicity and oxidative stress in mice retinal degeneration model

學生：葉睿軒　指導教授：楊建州、張元衍

摘要:

老年性黃斑部病變 (age-related macular degeneration, AMD) 是一種會造成不可逆視覺損傷的多因素疾病，也是已開發國家中導致老年人失明的主因，到目前為止對於 AMD 的發病機制仍然不清楚，在臨床上也尚未有有效的治療方式。碘酸鈉 (sodium iodate, $NaIO_3$) 是一種天然強氧化劑，在先前的文獻已指出碘酸鈉具有誘導小鼠、大鼠等哺乳類動物視網膜色素上皮細胞 (retinal pigment epithelial cells, RPE) 產生氧化壓力 (oxidative stress) 以及與人類 AMD 相似的病理特徵，因此已被廣泛運用至建立 AMD 的動物模型。α-Mangostin 是一種屬於多酚類化合物 (polyphenol) 的山酮素 (xanthone)，目前已被證實在多種細胞中具有良好的抗氧化、抗癌及抗發炎能力以外，並且具有通過血液-視網膜屏障 (brain-retinal barrier, BRB) 的能力，可經由血液運送至眼球內，然而目前為止對於探討使用 α-Mangostin 這類山酮素對於治療 AMD 的文獻與相關資料並不多。因此本篇研究將使用碘酸鈉誘導之體內及體外 AMD 模型，探討 α-Mangostin 對於 AMD 可能的保護效果。

在 *in vitro* 實驗中，我們首先將 ARPE-19 細胞株 (人類視網膜色素上皮細胞) 預先處理不同濃度之 α-Mangostin (0, 3.75, 7.5, 15 μM) 1.5 小時後，再添加碘酸鈉 (6 mM) 反應 18 或 24 小時。通過 CCK-8 試劑測量經 α-Mangostin 與碘酸鈉共同處理下的細胞存活率，並藉由 JC-1 染劑、H_2DCF-DA 染劑、Annexin V 染劑、酵素活性檢測試劑及西方墨點法評估 α-Mangostin 對於碘酸鈉誘導之 ARPE-19 細胞氧化壓力及細胞凋亡的保護效果。在 *in vivo* 實驗中，首先我們在第一天利用腹腔注射 (intraperitoneal injection, IP) 給予 Balb/c 小鼠 α-Mangostin (20 mg/ kg) 2 小時後，再以尾靜脈注射 (intravenously injection, IV) 給予小鼠碘酸鈉 (40 mg/ kg)，之後每日補充一次相同劑量之 α-Mangostin，在第 7 天時經眼睛斷層掃描 (optical coherence tomography, OCT) 觀察小鼠視網膜結構出現扭曲、變薄後進行犧牲採血，並以 H&E 染色分析視網膜組織切片評估視網膜損傷程度及利用小鼠血清探討 α-Mangostin 在小鼠體內的抗氧化效果。

在 *in vitro* 實驗中的結果顯示，α-Mangostin 可以抑制碘酸鈉活化之 SOD 活性並增加 GSH 及 catalase 的表現量，減緩碘酸鈉誘導之 H_2O_2 堆積在細胞內降低了細胞內 ROS 的含量；此外 α-Mangostin 可以抑制碘酸鈉活化之 PI3K/Akt 訊息傳遞路徑及 Bax、cleaved-PARP 與 cleaved-caspase3 的表現量，並增加 Bcl-2 的表現量，以減緩碘酸鈉誘導之粒線體損傷及細胞凋亡的現象。在 *in vivo* 實驗結果中顯示，α-Mangostin 可以增加小鼠抗氧化的能力，並顯著地減緩碘酸鈉誘導的視網膜厚度變薄與結構扭曲的現象。

本篇結果顯示，α-Mangostin 可以通過增加視網膜色素上皮細胞的抗氧化能力以抵抗碘酸鈉誘導之氧化壓力，並且抑制細胞凋亡相關蛋白的表現，使視網膜色素上皮細胞免於氧化損傷進而保護視網膜結構。本篇研究顯示了 α-Mangostin 作為 AMD 保健食品的潛力，可達到延緩或預防 AMD 形成的功效。

Abstract:

Background:

Age-related macular degeneration (AMD), a multi-factorial disease with unclear mechanism, could

cause irreversible and severe vision impairment among the elderly in developed countries. Sodium iodate ($NaIO_3$), a strong oxidant with ability to induce AMD-like retinal pigment epithelial cells (RPE) oxidative stress in mammal (like mice, rat), was widely used in many studies to establish AMD models. α-Mangostin is a kind of the xanthone major from Mangosteen, which belongs to the polyphenol compound. It has been revealed with strong anti-oxidative capability in previous studies and also can through the blood-retinal barrier (BRB) into retina, but the influence of α-Mangostin on AMD remains indistinct. So the aim of this study was to explore the protective effect of α-Mangostin in $NaIO_3$-induced *in vivo* and *in vitro* AMD models.

Methods:

In vitro, ARPE-19 cells (human RPE cells) viability was measured by cell counting kit (CCK)-8 assay after α-Mangostin pre-treatment (0, 3.75, 7.5, 15 μM) for 1.5hr and $NaIO_3$ treatment (6mM) for 18hr or 24hr, and the anti-oxidant enzymes were measured by SOD, GSH, catalase, H_2O_2 assay kit and Western blotting. ROS production, mitochondria membrane potential, and cell apoptosis level were measured via H_2DCFDA, JC-1, and Annexin V staining by flow cytometry and fluorescence microscope. *In vivo*, Balb/c mice were intravenously injected with $NaIO_3$ (40 mg/ kg) to screen the protection of α-Mangostin (20 mg/ kg/ days) on $NaIO_3$-induced mice retinal degeneration with OCT (optical coherence tomography), H&E staining, and anti-oxidant enzyme activity by serum.

Result:

Our *in vitro* data showed that α-Mangostin significantly reduced $NaIO_3$-induced ARPE-19 cells oxidative stress through restoring the GSH, catalase and SOD activity, and relived $NaIO_3$-induced H_2O_2 and ROS generation. Besides, α-Mangostin decreased the cell apoptosis by up-regulation the expression of Bcl-2 and down-regulation the expression of PI3K/Akt signaling pathway, Bax, cleaved-PARP1, and cleaved-capsase3 in $NaIO_3$-induced ARPE-19 cells. In mice model, α-Mangostin not only increase the mice anti-oxidant ability, but also reduce the retinal thinning and protected the retinal structure from distortion induced by $NaIO_3$.

Conclusion:

The result of this article showed that α-Mangostin could protect RPE cells from oxidative stress to reduce $NaIO_3$-induced apoptosis. In addition, α-Mangostin can also protect the retinal degeneration of Balb/c mice induced by $NaIO_3$.Overall, the α-Mangostin may have the potential to prevent or delay the AMD formation.

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透過 NAP 預處理使 BisGMA 誘發小鼠巨噬細胞的細胞毒性減緩，並且使小鼠巨噬細胞存活率上升

NAP pretreatment reduces the cytotoxicity of mouse macrophages RAW264.7 induced by BisGMA and increases the survival rate of RAW264.7 .

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摘要:

BisGMA 是牙科常見的複合材料為樹酯的一種，經常使用在齲齒治療中修補的部分，BisGMA 在齲齒治療經過一段時間之後，可能會從修復後的牙齒中滲出，進一步造成周邊的組織產生發炎反應； NAP 是一種主要存在茄科植物中的有機化合物，通常由植物中提煉出來，NAP 經過實驗證明具有抗發炎、抗菌、抗病毒……等活性，衍生物則可被用來治療心血管疾病及治療傷口。在實驗中先透過 MTT Assay 與 LDH 檢測小鼠巨噬細胞存活率；再來利用 NO 檢測 BisGMA 所造成的毒性；使用 Annexin-V 觀察細胞凋亡之狀態； ROS 分析對核酸、蛋白質以及膜脂所造成破壞；JC-1 確認粒線體膜電位是否失衡；最後使用 Western blot 來對路徑進行更進一步的探討；經由上述實驗可以發現， BisGMA 對使用 NAP 預處理後的小鼠巨噬細胞傷害會減輕，並且在存活率有明顯的提高，結果證明 NAP 的預處理能夠有效減緩 BisGMA 對小鼠巨噬細胞所帶來的傷害。

關鍵字：小鼠巨噬細胞；抗發炎；樹脂基質

Abstract:

BisGMA is a common composite material in dentistry that is a kind of resin. It is often used in the repair process of caries treatment. After the caries treatment, BisGMA may leak out from the restored tooth, further causing inflammation in surrounding tissues. NAP is an organic compound usually extracted from plants. NAP posses anti-inflammatory, antibacterial, antiviral, and its derivatives can be used to treat cardiovascular diseases and wounds. In this research, the survival rate of mouse macrophages was detected by MTT analysis and LDH. NO were used to detect the toxicity caused by BisGMA. Annexin-V was used to detect whether the cell apoptosis has improved. ROS analysis the damage to nucleic acids, proteins and membrane lipids. JC-1 confirms whether the mitochondrial membrane potential is unbalanced. Western blot is used to further explore the pathway. Through the above experiments, it could be found that NAP pretreatment reduced the damage caused by BisGMA. The survival rate of RAW264.7 also improved.

Keywords: Raw264.7, Anti-inflammatory, BisGMA

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建立斑馬魚為模式動物來探討視網膜病變的機轉

Establish zebrafish as a model animal to explore the mechanism of retinopathy

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摘要：近年來，斑馬魚 (*Danio rerio*) 常被作為研究藥物和疾病的動物模式。視網膜病變 (Retinopathy) 是因疾病導致視網膜受損進而使視力喪失，而其中的機制被認為與氧化壓力有關。我們期望透過斑馬魚動物模式來探討視網膜病變的機制，並且了解其中視網膜損傷的分子機制，未來能夠對臨床上的應用有所幫助。本篇論文主要分成兩個部分進行探討，第一部分是因先前有許多研究證實可利用 $NaIO_3$ 建立視網膜病變的動物模式，但是在斑馬魚上做這類型的實驗較少，因此本研究想利用 $NaIO_3$ 建立視網膜病變的斑馬魚動物模式。第二部分是因有些報導指出，長期服用類固醇會造成眼睛疾病的副作用，所以本研究想利用類固醇 Betason-N 來瞭解造成眼睛疾病的致病成因和機轉。

首先我們建立了 3 個月至 24 個月野生型斑馬魚的眼壓值，發現眼壓並不會隨著年紀及性別而有所變化。在 $NaIO_3$ 的探討部分，我們針對 3 個月大的公魚，使用 2.0 mM 和 3.0 mM 的 $NaIO_3$ 誘導 7 天及 14 天，先進行眼壓的檢測，發現在利用 3.0mM 的 $NaIO_3$ 誘導 14 天的組別中，眼壓有顯著的上升。後續在 TUNEL 染色中看到視網膜色素上皮細胞 (Retinal pigment epithelium, RPE) 凋亡的訊號，證明 $NaIO_3$ 對於 RPE 具有專一性的傷害。接著是視網膜的組織切片，發現使用 2.0 mM 和 3.0 mM 的 $NaIO_3$ 誘導 7 天及 14 天的組別中，感光細胞外節都有變薄的情況。還有 3.0 mM 的 $NaIO_3$ 誘導 14 天的組別中，內叢狀層 (inner plexiform layer, IPL) 也有變薄的情形。再經由免疫螢光染色得知錐狀細胞 (cone cell) 和桿狀細胞 (rod cell) 的外節皆有損傷，最後是透過 q-PCR 的檢測針對光傳導的相關基因進行探討，發現 *opn1sw1*、*opn1sw2*、*opn1lw2*、*opn1mw1*、*rho*、*pde6b*、*otx2b* 皆有下降的趨勢，而在 *rpe65a* 的基因表現則是上升的。

在類固醇 Betason-N 的探討部分，針對 3 個月大的公魚，使用類固醇 Betason-N 和人工淚液 duratears 誘導 91 天，在眼壓檢測的結果中，發現 duratears 的眼壓有顯著下降。在類固醇 Betason-N 的組別中，RPE 一樣有凋亡的訊號、感光細胞的外節變薄和 cone cell 及 rod cell 的外節都有損傷的情形。

根據以上的結果得知，使用 2.0 mM 和 3.0 mM 的 $NaIO_3$ 誘導 7 天及 14 天的組別，還有類固醇 Betason-N 誘導 91 天的組別中有看到一些視網膜病變的症狀，包括感光細胞外節損傷、RPE 凋亡的情形以及光傳導基因的改變。因此我們認為可藉由 $NaIO_3$ 和 Betason-N 建立視網膜病變的斑馬魚動物模式，未來利用它們來探討臨床上視網膜病變的保護和治療藥物的可行性。

Abstract：

In the recent year, zebrafish (Danio rerio) was used as animal model for studying drugs and diseases. Retinopathy is a kind of retinal degeneration due to disease, which causes vision loss. And the mechanism is thought to be linked to oxidative stress. It is expected to explore the mechanism of retinopathy through the animal model of zebrafish and understand the molecular mechanism of retinal damage, which will be helpful for clinical application in the future. This study is mainly divided into two parts. The first part is the sodium iodate ($NaIO_3$), many previous studies have confirmed that $NaIO_3$ can be used to establish an animal model of retinopathy. However, there are few discussions about $NaIO_3$ acting on zebrafish. Therefore, we wanted to establish a zebrafish animal model for retinopathy by $NaIO_3$. The second part is

the steroid Betason-N. Long-term use of steroids will cause side effects of eye diseases, so we want to understand how steroids affect the eyes and cause damage to the retina.

First of all, we set up the intraocular pressure (IOP) value of wild-type zebrafish for 3 months post fertilization (mpf) to 24 mpf, we found that IOP doesn't change neither at different ages nor genders. In the $NaIO_3$, we took 3 mpf male fish and immersed them into 2.0 mM and 3.0 mM $NaIO_3$ for 7 days and 14 days. First, the IOPs were measured and suggested that the IOP was significantly higher than the control groups at the concentration of 3.0 mM of $NaIO_3$ for 14 days. The apoptosis signal of retinal pigment epithelium (RPE) can be seen by TUNEL staining in the $NaIO_3$ groups, it was proved that $NaIO_3$ had specific damage to RPE. And then the retinal tissue section was performed, it was found that the thickness of the photoreceptor cell outer segment (OS) was significantly thinner than the control groups at the concentration of 2.0 mM and 3.0 mM $NaIO_3$ for 7 days and 14 days. In addition to the thickness of inner plexiform layer (IPL) was also significantly thinner than the control groups at the concentration of 3.0 mM of $NaIO_3$ for 14 days. Through the immunofluorescence staining showed that the OS of cone cells and rod cells were damaged. Finally, q-PCR was used to investigate the photoconductive genes, and it was found that it was downregulation on *opn1sw1*, *opn1sw2*, *opn1lw2*, *opn1mw1*, *rho*, *pde6b* and *otx2b*. While *rpe65a* was upregulation.

In the steroid Betason-N, we took 3 mpf male fish and immersed them into Betason-N and duratears for 91 days. Among the results of IOP test, the IOP of duratears was significantly decreased. In the steroid Betason-N group, RPE also showed apoptotic signals, the thickness of the OS was significantly thinner and damage to the OS of cone-cell and rod cell.

Based on these results, 2.0 mM and 3.0 mM $NaIO_3$ for 7 days and 14 days and steroid Betason-N for 91 days showed some symptoms of retinopathy. Including damage to the OS, the appearance of RPE apoptosis and the change of photoconductive genes. Therefore, we believe that the zebrafish animal model of retinopathy can be established by using $NaIO_3$ and Betason-N. Looking forward to using them in the future to explore the feasibility of clinical protection and treatment of retinopathy.

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探討台灣本土大蟬花 Wu-BFP-14 菌絲體水萃物保護乙醯胺酚誘導急性肝損傷的分子機制

To explore the protective mechanisms of the water extract of mycelium of Taiwan native *Cordyceps cicadae* Wu-BFP-14 on propacetamol-induced acute liver injury

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摘要：

Backgroup:乙醯胺酚 (Acetaminophen; APAP)是一種被廣泛使用的消炎止痛用藥，而當 APAP 使用過量則會造成急性肝損傷(Acute liver injury)，且有可能惡化成致死率高的急性肝衰竭。舒疼消熱(Propacetamol)是 APAP 的前驅物，propacetamol 在肝臟中的代謝及誘導損傷方式與 APAP 一致，因此本次實驗使用 propacetamol 建立急性肝損傷動物模式分析大蟬花菌絲體的護肝作用。大蟬花(*Cordyceps cicadae*) 為一種寄生於蟬科幼蟲的真菌，含有核苷、多糖等被認為對肝臟保護具有功效的成分。因此，本篇研究主題探討人工培養大蟬花對舒疼消熱誘導的小鼠肝臟損傷的保護效果及作用機制。

Material and methods:我們連續餵食小鼠大蟬花菌絲體水萃物 3 天(62.5 mg/kg, 125 mg/kg, 250 mg/kg MW)之後，腹腔注射 propacetamol 600 mg/kg 誘導肝損傷誘，18 小時後分析血液中 ALT, AST、發炎因子(TNF-α, IL-1β)、肝臟 H&E stain、肝臟中的氧化壓力指標(MDA, GSH)、抗氧化酵素(SOD, GPx, Catalase)及 Propacetamol 代謝酵素(CYP2E1, UGT1A)，評估 MW 的保肝功效及機制。

Results: Propacetamol 經由 CYP2E1 代謝成有毒 NAPQI, GSH 可以中和 NAPQI,過多的 NAPQI 造成氧化壓力上升，並增加發炎反應，促使肝臟細胞壞死，ALT, AST 也會顯著提高。我們的結果顯示餵食 3 天 62.5 mg 及 125 mg MW 能顯著降低 APAP 誘導肝臟細胞壞死面積及血液中 ALT, AST 數值，顯示 MW 具有保護 APAP 肝損傷的能力，我們進一步分析 MW 的保護機制，MW 組降低 APAP 誘導的 CYP2E1 表現，但不影響 UGT1A1 的表現，顯示 MW 前處理減少 APAP 代謝毒物 NAPQI 在肝臟中產生，但不影響 APAP 的無毒代謝途徑。另外氧化壓力方面，62.5MW、125MW 組可以恢復 APAP 降低的 GSH 量，同時 125MW 組降低 APAP 誘導的脂質的過氧化物 MDA，顯示 MW 能降低 APAP 誘導的肝臟組織氧化壓力，不過抗氧化酵素 SOD, GPx, Catalase 的活性並沒有因為餵食了 MW 而提高。但 MW 組明顯的降低 APAP 誘導的 TNF-a 及 IL-1B 發炎因子。

Conclusions: 大蟬花菌絲體水萃物(MW)具有保護 propacetamol 誘導肝損傷的能力，主要的分子機制是透過降低 propacetamol 誘導的 CYP2E1 表現，降低氧化壓力，及降低發炎因子，最終降低肝細胞壞死，更詳細分子機制有待將來進一步研究。

Abstract:

Background: Acetaminophen (APAP) is a widely used analgesic and antipyretic drug. Excessive APAP can cause acute liver injury (Acute liver injury), and may worsen into acute liver failure with a high fatality rate. Propacetamol is the prodrug of APAP. The metabolism of propacetamol in the liver and the way in which it induces damage is the same as APAP. *Cordyceps cicadae* (C. cicadae) is a fungus that parasitizes the larvae of the cicada family. It contains nucleosides, polysaccharides and other ingredients that are believed to be effective in protecting the liver. Therefore, this study explored the protective mechanisms of water extract of cicada on propacetamol-induced liver damage.

Material and methods: The mice was continuously pretreated with water extract of the mycelium (MW) of the C. cicadae for 3 days (62.5 mg/kg, 125 mg/kg, 250 mg/kg) and then intraperitoneal injection with

propacetamol to induce liver damage. The serum ALT/AST value, H&E staining of pathological sections, inflammatory factors (TNF-α, IL-1β), liver oxidative stress marker (MDA, GSH), antioxidant enzymes (SOD, GPx, Catalase) and metabolic enzymes (CYP2E1, UGT1A) was detected to evaluate the hepatoprotective effect and mechanism of MW 18 hours post propacetamol injection.

Results: The results show that the 62.5mg and 125mg MW pretreatment can significantly reduce the propacetamol-induced liver necrosis ratio and serum ALT and AST values, showing that MW has the ability to protect APAP induced liver damage. MW pretreatment reduced APAP-induced CYP2E1 expression, but UGT1A1 expression was not significantly different from propacetamol group.

Obvious MW pretreatment reduces the production of APAP metabolic toxicant NAPQI in the liver, and does not affect the non-toxic metabolic pathway of APAP. In addition, in terms of oxidative stress, 62.5mg and 125mg MW pretreatment can restore the amount of GSH reduced by APAP, while 125mg pretreatment reduces APAP-induced lipid peroxide MDA, indicating that MW can reduce APAP-induced oxidative stress in liver tissue. However, the activity of antioxidant enzymes, SOD, GPx, and Catalase is not improved by MW pretreatment. However, MW pretreatment significantly reduced APAP-induced TNF-α and IL-1β inflammatory factors.

Conclusions: The water extract of mycelium (MW) has the ability to protect propacetamol-induced liver injury. MW pretreatment reduce the expression of CYP2E1 induced by propacetamol, reduce oxidative stress, and reduce inflammatory factors, and ultimately reduce liver cell necrosis. More detailed molecular mechanisms need to be further studied in the future.

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高劑量 N-乙醯半胱氨酸治療舒疼消熱劑誘導急性肝損傷小鼠的副作用與毒性機制

The side-effects and toxic mechanisms of high-dose N-acetylcysteine therapy in mice with propacetamol-induced acute liver damage.

學生:謝承錡　　指導教授:王淑紅

摘要：

乙醯胺酚 (acetaminophen; APAP)是一種常見的鎮痛解熱藥，但在過量的使用下，會導致急性肝衰竭，且有極高的死亡率；N-乙醯半胱氨酸 (NAC)在臨床上可用於治療 APAP 中毒，但臨床上 NAC 標準治療劑量並無法適用於每個 APAP 中毒病人，尤其是高劑量 APAP 中毒者，沒有證據顯示是否提高 NAC 治療劑量可以提高治療效果。舒疼消熱劑 (propacetamol)是 APAP 的前驅物，常用於術後減輕疼痛，舒疼消熱劑引起的急性肝衰竭的作用機制與 APAP 一樣，APAP 誘導小鼠的急性肝損傷的病理機制與人類相似，因此，我們以小鼠研究高劑量 N-乙醯半胱氨酸治療舒疼消熱劑誘導急性肝損傷小鼠的副作用與毒性機制。

我們首先測試不同品系小鼠在不同劑量舒疼處理後 168 小時存活率，以舒疼處理 BALB/c 與 C57BL6/J 小鼠 48 小時的致死劑量分別為 1400 mg/kg 與 1600 mg/kg；以不同劑量 NAC 治療舒疼 1200 mg/kg 中毒並分析 168 小時存活率的實驗中，我們發現以 275 mg/kg 的 NAC 治療 APAP 中毒，兩種品系小鼠存活率皆為 100%，但以 400、800 mg/kg 的 NAC 治療皆會造成兩種品系小鼠的存活率下降；因此給予 275 mg/kg 的 NAC 是對兩種品系小鼠是一個較好的治療劑量。

我們選用了對於舒疼較為敏感的 BALB/c 小鼠作為我們後續 NAC 過量治療急性肝損傷分子機制實驗的實驗動物，我們分析給予小鼠 1200 mg/kg 舒疼在 1.5 小時後以 125、275、400、800 mg/kg 的 NAC 治療急性肝損傷後的 12、24、48 小時的小鼠肝臟外觀與病理切片、血清中的 ALT 與 AST 後，我們發現使用 125、275 mg/kg 的 NAC 治療急性肝損傷有劑量效應，雖然 NAC 400 與 NAC 800 治療組 ALT 與 AST 數值與細胞死亡比例相當，NAC 400 與 NAC 800 處理會造成 APAP 中毒小鼠與正常小鼠的死亡，N800 處理顯著降低了 APAP 中毒小鼠與正常小鼠的肝臟 GSH 水平，並誘導發炎因子 IL-6 的上升和肝臟微泡脂肪變性。此外，N275 和 N400 處理降低了正常小鼠血清三酸甘油酯 (TG) 但增加了肝臟三酸甘油酯，而 N800 處理明顯的增加了肝臟中的游離脂肪酸與三酸甘油脂及血清總膽固醇量。總結以上結果，過量 NAC 抑制肝臟 GSH 量，干擾肝臟代謝促進脂肪肝形成，誘發系統性發炎，進而造成 N800 組與 N800 治療組的小鼠死亡，高劑量 NAC 的詳細毒性分子機制有待未來進一步研究。

Abstract:

Acetaminophen (APAP) overdose induces acute liver damage and even death. The standard therapeutic dose of N-acetyl cysteine (NAC) cannot be applied to every patient, espe-cially those with high-dose APAP poisoning. There is insufficient evidence to prove that increas-ing NAC dose can treat patients who failed in standard treatment. Proacetamol (propacetamol) is the precursor of APAP. It is often used to relieve pain after surgery. The mechanism of acute liver failure caused by the propacetamol is the same as that of APAP. The pathological mechanism of APAP-induced acute liver injury in mice is similar to that of humans. Therefore, we used mice to study the side effects and toxicity mechanisms of high-dose N-acetylcysteine in the treatment of propacetamol induced acute liver injury mice and healthy (normal)

mice. We first tested the 168-hour survival rate of different strains of mice at different doses of propacetamol. The lethal doses of BALB/c and C57BL6/J mice treated with propacetamol for 48 hours were 1400 mg/kg and 1600 mg/kg, respectively. Two inbred mouse strains with different sensitivities to propacetamol-induced hepatotoxicity were treated with different NAC doses. The results show that 275 mg/kg NAC can completely rescue propacetamol poisoning (1200 mg/kg) in two strains of mice. However 400 and 800 mg/kg (NAC 400 and NAC800) treatment decreased the survival rate in two strains of mice. Therefore, 275 mg/kg of NAC is a better therapeutic dose for propacetamol poisoning (1200 mg/kg) of the two strains of mice. We selected BALB/c mice that are more sensitive to propacetamol to explore the molecular mechanism of different NAC dose in the treatment of acute liver injury. The result show that the therapeutic effects of NAC on propacetamol induced hepatotoxicity were dose-dependent from 125 (N125) to 275 mg/kg (N275) according to pathological section of liver and serum ALT/AST level from 12 to 48 hours treatment. In the NAC400 and NAC800 therapy groups, the ALT and AST values and the percentage of cell death were equivalent. Elevated doses of NAC (N400 and N800) caused additional deaths in both propacetamol-treated and normal mice. N800 treatments significantly decreased hepatic GSH levels and induced inflammatory cytokines (IL-6) and hepatic microvesicular steatosis in both propacetamol-treated and normal mice. Furthermore, both N275 and N400 treatments decreased serum triglyceride (TG) and induced hepatic TG, whereas N800 treatment significantly increased hepatic TG, hepatic free fatty acid and serum total cholesterol levels. In conclusion, NAC overdose decreased hepatic GSH level, induces hepatic and systemic inflammations and interferes with fatty acid metabolism, which in turn causes the death of mice in normal and proacetamol poisoning mice. The detailed molecular mechanism of high-dose NAC toxicity needs to be further studied in the future.

8

芹菜素藉由影響 Pin 1 和 Wnt/β-catenin 信號路徑抑制三陰性乳癌的增生和侵襲

Apigenin suppresses cell proliferation and invasion via inhibiting Pin 1 and Wnt/β-catenin signaling pathways in triple-negative breast cancer.

學生:廖泯嘉　指導教授:陳威仁

摘要:

背景：WNT /β-catenin 信號通路的異常在人類腫瘤發生中起重要作用。 β-catenin 的功能異常會導致下游基因（如細胞週期蛋白 D1）的過度表達，並加速人類癌症的細胞週期進程。芹菜素是一種富含於蔬菜和水果的類黃酮。據報導，芹菜素具有抗癌活性，具有低的細胞毒性和誘變活性，但芹菜素抑制三陰性乳腺癌細胞增殖和侵襲的機制仍不清楚。為此，我們進一步探討芹菜素是否通過調節 Wnt /β-catenin 信號通路和 Pin 1 蛋白來抑制三陰性乳腺癌的增殖和 EMT。

方法：在這項研究中，我們進行了 MTT，傷口癒合和 transwell 入侵試驗，以分別評估細胞毒性，增殖，細胞遷移和侵襲。進行西方墨點法以監測參與 WNT /β-catenin 途徑的活性分子的蛋白質水平。

結果：傷口癒合和 transwell 入侵試驗的結果表明，芹菜素以濃度依賴性方式抑制細胞遷移和侵襲。Western 印跡分析顯示芹菜素降低了 Pin 1 和 β-catenin 蛋白的水平，並同時抑制了 EMT 相關蛋白的表達。

結論：芹菜素可以通過抑制 Pin 1 蛋白來調節 β-catenin 在核內的表現量，進而抑制 EMT。因此，我們推薦芹菜素可以作為三陰性乳腺癌患者輔佐治療藥劑。

Abstract:

Background: Abnormal WNT/ β-catenin signaling pathway plays an important role in human tumorigenesis. Aberrant β-catenin function leads to overexpression of its effector gene such as cyclin D1 and accelerates cell cycle progression in human cancers. Apigenin , a kind of flavonoids abundant in vegetables and fruits, has been reported to possess anti-cancer activity with low cytotoxicity and mutagenic activity, but the mechanism by which apigenin inhibits triple-negative breast cancer cell proliferation and invasion still remains unclear. To this end, we investigate whether apigenin inhibits proliferation and EMT of triple-negative breast cancer via inhibition of Pin 1 and the Wnt/β-catenin signaling pathways.

Methods: In this study, we conducted MTT, wound healing and, transwell invasion assays, to evaluate cytotoxicity, proliferation, cell migration, and invasion, respectively. Western blotting were performed to monitor the protein levels of active molecules involved in WNT/ β-catenin pathway.

Results: The results of wound healing and transwell invasion assays demonstrated that apigenin inhibits cell migration and invasion in a dose-dependent manner. Western blot analysis revealed that apigenin decreases the level of Pin 1 and β-catenin protein level, and concomitantly suppresses the expression of EMT-associated protein.

Conclusion: Apigenin can regulate β-catenin level to inhibit EMT through inhibiting Pin 1. We thus suggest that apigenin may act as a potential adjunctive therapeutic agent for triple-negative breast cancer patients.

9.

SET7/9 在抗老化蛋白質表現，細胞衰老之角色探討

The role of SET7/9 in anti-aging protein expression and cell senescence in renal tubular cells

學生:顏夷岑　指導教授:林庭慧

摘要:

硫酸吲哚酚（IS）是一種典型的尿毒症毒素，由於其腎毒性，在慢性腎臟疾病（CKD）的發展中非常重要。 SET7 / 9 是一種蛋白質離氨酸甲基轉移酶，可催化組蛋白 H3 的離氨酸甲基化，影響染色質結構並參與基因調控。我們的研究目標，旨在闡明 SET7/9 在 IS 調節的抗老化蛋白表達和細胞衰老過程中是否扮演調控角色。我們使用 1200 μM IS 處理 NRK-52E 細胞 24 小時，並藉由 Western Blot 分析檢測抗老化蛋白質（包括 Klotho、sirtuin1）的蛋白質表達。同時，也檢視了細胞衰老標記，包括衰老相關的 β-半乳糖苷酶 (SA-β-gal) 染色、Cdk 抑製劑蛋白 p21 和 p16 的表達增加以及 p53 轉錄因子的活化，也利用免疫熒光顯微鏡檢視了 IS 對 P16 蛋白螢光信號和 NPM1 蛋白在核中位置的影響。我們的結果表明，IS 減弱了抗老化蛋白（Klotho 和 Sirtuin1）的表達，並通過在 NRE-52 E 細胞中表達 β-半乳糖苷酶、p16、p21 和 p53 來誘導細胞衰老。 NRK-53E 細胞使用 SET7/9 抑製劑或通過 siRNA 沉默 SET7/9 ，受 IS 抑制的 Sirtuin1 蛋白表現量恢復。此外，當 SET 7/9 被抑制時，IS 處理的 NRK-2E 細胞中細胞衰老標誌物 β-半乳糖苷酶和 p21 的上調減弱。這些實驗結果說明，SET7/9 蛋白質在調控抗老化蛋白 Sirtuin1 表達及細胞衰老上扮演重要角色。

Abstract:

Indoxyl sulfate (IS), a typical uremic toxin, is involved in the development of chronic kidney disease (CKD) with its nephrotoxicity. SET7/9 is a protein lysine methyltransferase which catalyze lysine methylation of histone H3, affects chromatin structure and participates in gene regulation. Our study aimed to clarify the regulatory role of SET7/9 in IS-modulated anti-aging protein expression and cell senescence. Kidney epithelial cells (NRK-52E cells) were treated with 1200 μM IS for 24 hours, and protein expression of anti-aging proteins, including Klotho, sirtuin1 was detected by Western Blot analysis. Biomarker of senescent cells including senescence-associated β-galactosidase (SA-β-gal) staining, increased expression of the Cdk inhibitor proteins p21 and p16, and activation of p53 transcription factor were examined in IS-treated NRK-52E cells. The effect of IS on P16 protein fluorescent signal and NPM1 protein subnuclear localization were explored by immunofluorescence microscopy. Our results demonstrated that IS attenuated anti-aging proteins (Klotho and sirtuin1) expression and induced cell senescence with expression of β-galactosidase, p16, p21 and p53 in NRE-52 E cells. Treatment of NRK-53E cells with either SET7/9 inhibitor or silencing of SET7/9 by siRNA restored IS- suppressed sirtuin1 protein expression. Moreover, upregulation of cell senescence marker β-galactosidase and p21 in IS-treated NRK-2E cells was attenuated when SET 7/9 was inhibited. These results indicated that SET7/9 protein plays an important role in modulating IS-induced cell senescence and anti-aging sirtuin1 proteins expression.

10

MBNL3 參與卵巢癌的發展

MBNL3 Participates in ovarian cancer development

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摘要:

MBNL3 是一種調節選擇性剪接的 RNA 結合蛋白，主要在增殖組織中表達。據報導，在許多癌症中 MBNL3 扮演促進的角色，例如乳腺癌、肝癌、非小細胞肺癌等。先前，我們實驗室發現卵巢癌細胞株中的 MBNL3 表現量高於正常卵巢細胞，並且 MBNL3 弱化會降低卵巢癌細胞的生長速度。我們想確定 MBNL3 的表達如何影響細胞。我培養卵巢癌 HeyC2 細胞，用 si-MBNL3 轉染細胞弱化 MBNL3 表現量，並以 si-NC 作為對照組。使用 RT-PCR 和西方點墨法檢查弱化 MBNL3 後，確認 MBNL3 表達量降低了大約 50%。我們通過傷口癒合、transwell 和聚落形成試驗觀察了細胞行為。另一方面，我也使用 Hela 細胞轉染 DNA，過量表達 MBNL3，再用相同的方式觀察細胞行為。我們的結果表明，弱化 HeyC2 中的 MBNL3 降低了細胞的遷移能力，以及細胞聚落的形成；而過表達 MBNL3 可促進 Hela 細胞遷移的能力。

關鍵字：MBNL3、HeyC2、Hela、卵巢癌、細胞遷移、聚落形成

Abstract

MBNL3 is an RNA-binding protein that regulates alternative splicing and is mainly expressed in proliferative tissues. It has been reported that MBNL3 plays a promotive role in many cancers, such as breast cancer, liver cancer, non-small cell lung cancer and other cancers. Previously, our laboratory discovered higher MBNL3 expression in ovarian cancer cell lines than in normal ovarian cells, and MBNL3 knockdown decreased the growth rate of ovarian cancer cells. To determine how MBNL3 expression impacts on the cells, we cultured ovarian cancer HeyC2 cells and transfected the cells with si-MBNL3 to knockdown MBNL3, as well as with si-NC as a control. We confirmed decreased MBNL3 expression after MBNL3 knockdown using RT-PCR and Western blotting. And we examined cell behaviors by wound healing, transwell, and colony formation assays. On the other hand, we transfected DNA to overexpress MBNL3 in Hela cells, and examined the cell behaviors in the same way. Our results revealed that knockdown MBNL3 in HeyC2 decreased the migration ability of the cells, as well as the colony formation ability. Overexpression of MBNL3 in Hela cell also promoted cell migration.

Key word: MBNL3, HeyC2, Hela, ovarian cancer, cell migration, colony formation

11.

精氨酸甲基化在 Klotho 蛋白質表現之機轉探討

Modulation of anti-aging Klotho expression by protein arginine methylation in NRK-52E cells

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摘要:

Indoxyl Sulfate (IS)是一種尿毒素，是造成慢性腎臟病之致病因子。具抗老化功能的 Klotho 蛋白質在慢性腎臟病的實驗動物，和患有末期腎臟疾病患者的腎臟組織中表現量下降。表觀遺傳修飾，包括 DNA 甲基化和蛋白質甲基化，可調節 Klotho 蛋白質基因的表達。在我們之前的研究中，我們證明了 PRMT6 和 NF-κB 的相互作用與 NF-κB 上精氨酸甲基化有關，且影響 NF-κB 進入細胞核以抑制 Klotho 蛋白質表達。為了進一步確認 Klotho 受到蛋白質甲基化的調控，我們研究了離氨酸和精氨酸甲基化是否會影響 Klotho 蛋白質的表現。我們使用 IS 來處理大鼠腎臟近端小管細胞(NRK-52E cells)，觀察到 IS 的處理抑制了 Klotho 蛋白質的表達，並且增加了精氨酸甲基轉移酶 4（PRMT4）、精氨酸甲基轉移酶 6（PRMT6）與第 7/9 型離胺酸甲基轉移酶 (SET7/9)的表現。經過 DNA 甲基轉移酶抑製劑（5-Aza-2'-dc）、專一性的精氨酸甲基轉移酶抑制劑(AMI-1)、離胺酸甲基轉移酶抑制劑(SET7/9 inhibitor) 處理過後，可使被 IS 抑制的 Klotho 蛋白質表現量回升。NF-κB 被認為是精氨酸甲基化的受質，使用免疫沉澱實驗，確認 NF-κB 與 PRMT4、PRMT6 的相互作用。並利用免疫螢光染色實驗確認 AMI-1、 SET7/9 inhibitor 阻斷 IS 誘導的 NF-κB 核移位。另外使用放線菌酮(CHX)阻斷實驗，證明了透過專一性的精氨酸甲基酶抑制劑作用，增加 Klotho 蛋白質的穩定度。這些發現提供了調控 Klotho 蛋白質表現之分子機轉，或可提供一些因缺乏 Klotho 蛋白質而導致之腎臟疾病的治療策略。

Abstract：

Indoxyl Sulfate (IS) is a pathogenic factor that cause chronic kidney disease (CKD). In the renal tissues of CKD animal models and patients with end-stage renal disease, expression of Klotho, a protein with anti-aging function, is down-regulated. Epigenetic modifications, including DNA methylation and protein methylation modulate gene expression. In our previous study, we demonstrated that lipopolysaccharide (LPS) up-regulated the expression of protein arginine methyltransferase 6 (PRMT6) which affect the activation of NF-κB, consequently regulating the expression of Klotho protein. To further confirm the role of protein methylation in modulating Klotho protein expression, we treated rat kidney epithelial cells (NRK-52E cells) with IS and investigated whether lysine and arginine methylation affect Klotho expression. IS downregulated Klotho expression and upregulated PRMT4, PRMT6, SET domain containing lysine methyltransferase SETD7 (SET7/9) expression in NRK-52E cells. Expression of the Klotho was diminished by IS-treatment and was restored by DNA methyltransferase inhibitor (5-Aza-2'-dc), protein arginine methyltransferase inhibitor (AMI-1) or protein lysine methyltransferase inhibitor (SET7/9 inhibitor). These results showed that both DNA methylation and protein methylation could regulate the expression of Klotho protein. Immunoprecipitation experiments showed that NF-κB was identified as a substrate for arginine methylation and interact with PRMT6 in NRK-52E cells. In addition, inhibition of PRMT activity by AMI-1 blocked IS-induced NF-κB nuclear translocation in NRK-52E cells as revealed by immunofluorescence staining experiments. Moreover, cycloheximide (CHX) chase assay was used to analyze the protein stability of Klotho in the treatment of IS and AMI-1. Our data indicate AMI-1 maintain Klotho protein in a more stable level and restored protein expression of Klotho suppressed by IS treatment in NRK-52E cells. Our findings suggest protein methylation play an important role in modulating anti-aging Klotho protein expression in NRK-52E cells.

海報論文摘要

1.

蛋白質精胺酸甲基化程度與核仁壓力對 FUS 蛋白質核仁分布的相關性研究

The Correlation Between Protein Arginine Methylation Degree and Nucleolar Stress on FUS Protein Nucleolus Distribution

學生:劉玉環　指導教授:李娟

Abstract:

Protein arginine methyltransferases (PRMTs) lead to protein arginine methylation. An RNA-binding protein FUS can be arginine methylated by PRMT to modulate the subcellular distribution and form granules in nucleus in a hypomethylated environment. I want to know the effect of protein arginine methylation on the distribution of FUS in cells and knocked down PRMT1 by lentivirus infection or methyltransferase Inhibitor, such as K313 and AdOx to prepare hypomethylated environment. I also treated cells with a nucleolar stress drug CX-5461. Only a few FUS granules which were not co-localized with the nucleolar marker fibrillarin appeared in hypomethylated cells. Nutlin can upregulate the level of a paraspeckle scaffold lncRNA NEAT1 level. As predicted, there were more FUS granules co-localized with a paraspeckle marker NONO forming in HSC-3-PRMT1-KD cell when treated with Nutlin. I also used qPCR to detect NEAT1 level in PRMT1-KD cells. The NEAT1 level and the number of paraspeckles were higher in PRMT1-KD cell than in control cell. Therefore, our study suggests that protein arginine methylation might modulate the distribution of FUS in cancer cells and make PRMT1-KD cells form paraspeckles.

Key word: protein arginine methylation, FUS, Nucleolar distribution, paraspeckles

2.

對稱性二甲基精氨酸多株抗體之製備及於酵素免疫分析法之開發

Production of Polyclonal antibodies and development of Enzyme-linked Immunosorbent Assay for Symmetrical dimethylarginine

學生：林家君　　指導教授：余豐益

摘要：

對稱二甲基精氨酸 (symmetrical dimethylarginine, SDMA) 是非對稱性二甲基精氨酸 (asymmetrical dimethylarginine, ADMA) 的結構異構物，當體內的細胞於蛋白質更新時都會產生 SDMA，此物質可自由且大量的被腎臟過濾到尿液中，因此是早期偵測腎臟過濾功能很好的一個生物標記物。國際腎臟權益組織 (International Renal Interest Society, IRIS) 在慢性腎病 (Chronic kidney disease, CKD) 指引中更指出 SDMA 對於診斷和治療的重要性。因此開發一快速且便利的免疫檢測系統，對於社會的健康問題是很重要的。由於 SDMA 為小分子標記物，且擁有一個常用之活性基團-羥基 (-OH)，因此我們使用了 succinic anhydride 在 SDMA 的羥基 (-OH) 衍生出羧基 (-COOH)，再以 1-ethyl-3-[3-dimethylaminopropyl]Carbodimide (EDC)/N-hydroxysuccinimide (NHS) 活化羧基，使其與載體蛋白質上的胺基 ($-NH_2$) 做結合成具有免疫原性之抗原 SDMA-SH-KLH，並將此抗原免疫入 Balb/c 小鼠體內以製備抗體，再藉由 competitive indirect ELISA (ciELISA) 來觀察實驗動物血清中是否具有 SDMA 專一性抗體產生。在 ciELISA 測試的結果當中可以發現，免疫過後的小鼠血清效價有持續上升的現象，不過卻無法產生對 SDMA 具有專一性之抗體，因此在未來實驗中，本研究想要克服目前無法有良好的抗體效價以及專一性的問題，將運用其他的載體蛋白，如 BTG 或 γ-globulin 來與 SDMA 進行接合，期望免疫小鼠可以產生更好的專一性及效價的抗體。

3.

沙門氏菌 *Salmonella enterica* serovar Typhimurium LT2 的單股 DNA 結合蛋白質的純化、表現、結晶結構、DNA 結合特性與突變分析

Purification, expression, crystallization, crystal structure, DNA binding properties, and mutational analysis of single-stranded DNA binding protein from *Salmonella enterica* serovar Typhimurium LT2

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摘要:

沙門氏菌(*Salmonella enterica*)是革蘭氏陰性菌，屬於腸內菌科，根據其基因體的相似程度及生化特性，共可細分為 6 個亞種與近 3000 種不同的血清型。 *Salmonella enterica* serovar Typhimurium LT2 隸屬於第一型亞種鼠傷寒血清型(*S.* typhimurium)的沙門氏菌，是哺乳動物中最常見的沙門氏菌，也是人類胃腸炎的主要病原菌，並被用作人類傷寒的小鼠模型。沙門氏菌的生存力很強，但感染後可以抗生素治療。近年抗抗生素的沙門氏菌陸續被發現，感染後常面臨無藥可醫之窘境，因此異於針對細胞壁或核醣體之新型抗生素標靶亟待開發與研究。革蘭氏陰性菌的單股 DNA 結合蛋白質(single-stranded DNA binding protein; SSB)是一種必要蛋白質，於細菌 DNA 複製、修復與重組時扮演重要角色，也許可當成新型抗生素的研發標的。由於人類複製 DNA 所使用的單股 DNA 蛋白質是 RPA，其 DNA 結合特性與結構都與 SSB 不同，因此預期所據之而研究出的抗生素副作用應較小; 然而，目前沙門氏菌的 SSB (SetSSB)結構並未解出，使得無法以 SSB 作為抑制標靶來理性設計藥物。在此研究，我們大量表現並純化出 SetSSB，來研究其結構-功能關聯性。為了得到結晶結構，我們利用懸吊法進行蛋白質晶體成長，在數千次篩選過程與修飾條件後，找到一極佳的晶體成長條件為 14% PEG 4000、100 mM HEPES、pH 7.5、125 mM sodium acetate 與 50% glycerol。目前此晶體已經由國家同步輻射 X 射線獲得繞射點並據之解出結晶結構，此結果已通過蛋白質資料庫的物理與化學因子的審查，並等待主論文發表後公開，本人為第一作者 [Ren-Hong Luo, Yen-Hua Huang, and Cheng-Yang Huang (2021) Crystal structure of SSB from *Salmonella enterica* serovar Typhimurium LT2, PDB ID 7F25]。結構顯示其 N 端含有典型的寡核苷酸結合位功能區(OB-fold domain)，然而在不同的結構中，我們同時也觀察到在之前不甚了解的自身蛋白質-蛋白質交互協同作用，可能與其他未知的功能有關。我們目前利用電泳遲滯法與結構為基礎的定點突變驗證其功能與其他 SSB 異同之處。此研究後續的結構與功能之結果希望能具體結論出 SSB 重要點位與 SetSSB 本身獨特點位，作為抑制劑甚或抗生素藥物開發之參考依據。

4

早期乳癌患者的不良心血管事件主要風險因子探討-一項全國性人口研究

Risk of Major Adverse Cardiovascular Events in Early Stage Breast Cancer Patients – a Nationwide Population-based Study

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Abstract:

Background: Breast cancer is the most common cancer in women worldwide. Adjuvant treatments such as chemotherapy and radiotherapy after breast cancer surgery cause long-term cardiac toxicity. We conducted a nationwide population-based study to evaluate the risk factors for major cardiovascular events (MACE) after breast cancer treatments.

Methods: Patients diagnosed with breast cancer between January 1, 2007 and December 31, 2014 were identified. Accordingly, three national databases were used, namely the National Health Insurance Research Database, the National Mortality Database, and the Taiwan Cancer Registry. The incidence risk of fatal and nonfatal MACE was calculated, and the adjusted cumulative hazard ratio (aHR) was estimated.

Results: Overall, 26,874 patients were included for analysis. Age at diagnosis and pathological stage significantly influenced the risk of non-fatal MACE. No difference in the risk of nonfatal MACE was noted between different adjuvant treatment groups. The age at diagnosis older than 60 increased the risk of fatal MACE (aHR 2.05, 95% CI: 1.60-2.63). Patients who received adjuvant chemotherapy and radiotherapy had an increased risk of fatal MACE (aHR 2.65, 95% CI: 1.76–4.01) compared with those who received no adjuvant treatment. Patients with stage II disease also had an increased risk of fatal MACE compared with patients with stage I disease (aHR1.62, 95% CI: 1.26-2.09).

Conclusion: In general, age at diagnosis, pathological stage, adjuvant treatment is correlated with the risk of fatal MACE.

Key Words: breast cancer; adjuvant treatment; major adverse cardiovascular event

5.

柚皮素(Naringenin)和胡桃醌(Juglone)合併處理對乳癌細胞增生的影響

To explore the anti-cancer effects of combined treatment of Naringenin and Juglone on the proliferation of breast cancer cells

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摘要:

乳腺癌在台灣女性癌症排名一直都是位於前三，為人類主要癌症之一。在藥物治療方面除了用化學合成藥物外，合併使用天然化合物作為治療佐劑也是一直在研究的方針。在天然植物萃取物中柚皮素 (Naringenin) 和胡桃醌 (Juglone) 已經有研究分別證實可以抑制乳腺癌細胞的生長並使其凋亡。

柚皮素可以將乳癌細胞周期停止在 G0/G1 時期並活化細胞凋亡；而胡桃醌可以通過抑制 Pin1 蛋白活性來抑制乳癌細胞的增生並誘導細胞產生自由基(reactive oxygen species)後發生細胞凋亡。本研究想探討合併這兩種藥物處理乳癌細胞,是否可增強彼此抑制乳癌細胞增生與誘發細胞凋亡的能力，並探索其可能機制。初步結果中，比起單獨使用胡桃醌或柚皮素，在細胞存活率實驗(MTT assay)中共同處理可以使細胞存活率下降更多。使用 western blot 觀察柚皮素對 Pin1 蛋白表現時發現 Pin1 蛋白表現量和對照組相比沒有顯著性變化，推測柚皮素可能是透過其他途徑影響細胞週期而胡桃醌在其他研究表明不影響 Pin1 蛋白的表達主要作用為使 Pin1 蛋白失去活性，且兩者都可以抑制 Wnt/β-catenin signaling 從而抑制細胞增生，但是否調節路徑為一致還有待實驗佐證。

後續實驗主要想觀察兩種藥物合並處理乳癌細胞對 ROS 的產生以及其誘導細胞凋亡的能力，還有對細胞週期的調節是否會有更大的影響,以及能否對不同基因型的乳癌如三陰性乳癌產生抑制增生作用。

6.

山奈酚和槲皮素透過影響 PIN1 調控的訊號路徑抑制乳癌細胞的增殖和遷移

Kaempferol and quercetin inhibit breast cancer cell proliferation and migration through regulating PIN1-mediated signaling pathway

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Abstract:

Both kaempferol and quercetin are flavonoids found in plants. The evidence in literatures proved that they have a wide range of physiological activities, including anti-inflammation, anti-oxidation and anti-angiogenesis. PIN1 (Peptidylprolyl cis/trans isomerase 1) is a well-known PPIase that alter protein structure and function by controlling the isomerization of the phosphor Ser/Thr–Pro motif. PIN1 is highly expressed in cancers and regulates many signaling pathways associated with tumorigenesis, suggesting that inhibition of PIN1 expression may be a useful therapy for breast cancer. Therefore, we will investigate whether kaempferol or quercetin can inhibit cell proliferation and migration by modulating PIN1-mediated signaling in breast cancer cells. Firstly, we used MTT assay to detect the effect of kaempferol or quercetin on human breast cancer MCF-7 cell survival, and used wound-healing assay to examine cell migration in vitro. We also transfected PIN1-expressing plasmid (NM_006221 human tagged ORF clone) to MCF-7 cells to establish an overexpression condition in the cells. Finally, we will investigate whether kaempferol or quercetin can reduce the expression of PIN1 to affect PIN1-mediated signaling pathways required for cell proliferation and migration. Our research hopes to prove that Kaempferol or quercetin has the potential to inhibit breast cancer caused by aberrant PIN1 function and to develop kaempferol or quercetin as a leading drug for the treatment of breast cancer.

7.

探討台灣本土大蟬花 Wu-BFP-14 的生物安全性及保護乙醯胺酚誘導急性肝損傷效果

To decipher the acute toxicity and protective effect of Taiwan native *Cordyceps cicadae* Wu-BFP-14 on propacetamol-induced acute liver injury

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摘要：

Background:大蟬花(Cordyceps cicadae)是一種寄生於蟬科幼蟲的真菌，被認為與冬蟲夏草有相似的成份及功效，最大的好處即是可人工培養，進而改善品質及降低成本。大蟬花含有多糖、腺苷、蟲草素等有效成份，在數千年前就被作為中藥所使用，具有保肝、保腎、提升免疫等功效。

Methods:本次實驗使用固態培養子實體 (F)及液態發酵菌絲體 (M)的乾燥粉末進行急毒性測試，餵食小鼠 1g/kg 或 5g/kg M 及 F，並在 14 天後進行犧牲並探討此劑量對小鼠是否有毒性。之後的肝臟保護實驗，我們使用了酒精或純水萃取 F 及 M 粉末，並將萃取液凍乾成粉末。首先管餵小鼠酒萃子實體(FA)、酒萃菌絲體(MA)、水萃子實體(FW)、水萃菌絲體(MW)四種不同粉末，並使用舒疼消熱(Propacetamol)進行腹腔注射 600 mg/kg 誘導肝損傷誘，18 小時後分析血清中與肝損傷有關的 ALT, AST 及腎損傷有關的 BUN, CREA 並針對有效組別進行組織 H/E 染色，探討不同萃取法 F 與 M 的肝臟保護效果。

Results: 當 ALT, AST, BUN CREA 上升時代表肝腎受到傷害，而餵食 1g/kg, 5g/kg 劑量的 F 及 M 粉末不會造成 ALT, AST, BUN CREA 上升，因此認為餵食 1g/kg 的 F 及 M 不會有肝腎毒性。不過在 5g/kg 的劑量兩組別都造成小鼠體重降低。Propacetamol 在肝臟代謝成有毒物質，促使肝臟細胞壞死，而肝細胞的死亡會使 ALT、AST 顯著提高。在保肝的結果可以看到，在 FA, MA, FW, MW 四個組別都能看到顯著降低 ALT 的效果，不過在 AST 的部分，則僅 MW 有顯著降低。在肝臟 H/E 染色的結果可以看出使用 MW 處裡的小鼠，肝臟細胞的壞死面積明顯小於單純誘導肝損傷小鼠。

Conclusion:在 1 g/kg 以下的 F、M 劑量是對小鼠不具有毒性的，而 5 g/kg F、 M 會使小鼠體重下降。MW 具有保護肝臟降低 Propacetamol 誘導的損傷效果，未來將繼續對其最佳劑量及作用機制進行研究。