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研究成果發表會

會議手冊

2020.6.10-6.12

議程表

日期與時間	活動主題	地點
6 月 10-11 日(星期三、四) 12:00-13:00	海報論文發表	正心樓 1 樓走廊 (圖書館前) 碩一學生 12:00~1:00 站於 海報旁穿戴整齊(避拖鞋) 教師詢問海報內容並評分
6 月 12 日(星期五) 09:50-10:00	報到(全體碩士班學生)	正心樓 0733 教室
10:00-10:10	開幕(系主任致詞) 全體教師出席	
10:10-11:30	口頭論文報告	
11:30-11:40	發餐盒 等候成績統計	
12:00-12:15	頒獎 大合照	

摘要目錄

1.

探討β-Mangostin 對碘酸鈉(Sodium iodate)誘發視網膜色素上皮細胞氧化傷害之保護機轉

Protecting effect of β-Mangostin on sodium iodate induced oxidative damage in retinal pigment epithelium cells

學生:葉睿軒　指導教授:楊建洲、張元衍

摘要:

老年性黃斑部病變(Age-related macular degeneration, AMD)是一種會造成老年人不可逆視覺損傷的視網膜疾病其發病機制仍不明瞭，而目前關於 AMD 的主要發病機制被認為和視網膜色素上皮細胞(Retinal pigment epithelium, RPE)的氧化損傷有關。從先前研究已知碘酸鈉(Sodium iodate, NaIO3)具有誘導人類和小鼠視網膜色素上皮細胞產生氧化壓力及類似於 AMD 的病理特徵，因此被廣泛運用在許多研究中建立 AMD 的模型。天然化合物β-Mangostin 是一種萃取自山竹果果皮的山酮素(Xanthone)，在許多研究中指出它具有很強的抗氧化能力，並且已經廣泛運用在腫瘤、糖尿病等疾病治療中。所以在本篇研究中我們想要探討β-Mangostin 對 NaIO3 誘導人類視網膜色素上皮細胞株(ARPE-19 cells)產生氧化傷害及自噬作用的影響。

我們先藉由 CCK-8 (Cell counting kit-8)及 LDH (Lactate dehydrogenase)測量細胞存活率及細胞毒性，並以 H2DCF-DA 染劑使用流式細胞儀(Flow cytometry) 偵測細胞內的活性氧(ROS)，接著我們以抗氧化酵素(GSH, SOD, Catalase)及 H2O2 的檢測試劑評估細胞的抗氧化能力，最後以 western blot 探討β-Mangostin 對抗氧化及細胞自噬的相關分子機制。

我們的結果顯示，NaIO3 會誘導 ARPE-19 細胞內 H2O2 增加，而導致細胞 ROS 產生，增加細胞自噬相關蛋白 Beclin-1、LC3 II 的表達，進而促使細胞存活率下降；而預先處理β-Mangostin 可以回復細胞的存活率及減緩細胞的毒性，且發現β-Mangostin 會藉由增加 GSH 的活性，並降低 SOD、H2O2、ROS 的表現量，進而減少細胞的氧化壓力，並且抑制 NaIO3 誘導的 Beclin-1、LC3 II 的表現。

根據我們初步的結果顯示,推測β-Mangostin 可藉由抑制 NaIO3 誘導細胞的 ROS 來降低細胞自噬作用，因此我們認為β-Mangostin 可能具有預防或延緩 AMD 形成的潛力。

2

洋蔥萃取物和槲皮素抑制單純疱疹病毒-1 型誘發 BV-2 細胞炎症反應之探討

Protective effects of Onion extract and Quercetin on HSV-1 induced inflammatory respones model in BV-2 cells

學生:陳偲茜　指導教授:楊建洲 張元衍

摘要:

HSE(單純疱疹腦炎，Herpes simplex encephalitis)是最常見的中樞神經系統病毒性疾病，又稱單純疱疹病毒性腦炎(HSVE)，是由單純疱疹病毒 (Herpes simplex virus, HSV)感染所引起的急性腦炎。目前臨床上針對 HSE 的治療，主要是通過 Acyclovir 來阻止 HSV 在體內的複製，但患者的死亡率仍高達 20%至 30%，因此仍需一種有效且具有治療病毒誘導急性腦炎的藥物。HSV 可分為 HSV-1(Herpes simplex virus type-1) 和 HSV-2(Herpes simplex virus type-2)。百分之九十的單純疱疹腦炎病例均由 HSV-1 引致，因此本研究將使用 HSV-1 感染 BV2 細胞株(鼠微膠質細胞)建立體外 HSE 模型。槲皮素是蔬果中主要的類黃酮化合物之一，為一種天然抗氧化劑，目前已被許多研究證實具有抗氧化及抗發炎的功效；此外，有研究指出：槲皮素在 Raw264.7 細胞株(小鼠巨噬細胞)中，可藉由抑制 TLR-3 的表現，減緩 HSV 所引起的發炎及氧化壓力(Seulki Lee, et al., 2017)。因此本研究將通過 HSV-1 誘導的體外 HSE 模型，探討洋蔥萃取物(Onion)與槲皮素(Quercetin)對 HSE 可能的保護效果。

首先，我們探討不同時間點及病毒劑量下，HSV-1 感染 BV2 cells 後誘發的炎症相關的細胞激素及死亡率。結果發現在 0.5 (M.O.I.)的 HSV 感染 15 小時後細胞顯著死亡，並且 NO 及發炎相關因子 IL6 及 IP10 也有上升的現象。因此，我們在後續實驗以此條件來探討洋蔥萃取物(Onion)和槲皮素(Quercetin)對抗 HSV 誘發炎症的情形。

我們先以洋蔥萃取物和槲皮素預先處理 BV2 細胞 1.5 小時後，再感染 0.5 (M.O.I)的 HSV-1，於培養 15 小時後觀察細胞型態，並收取上清液及細胞萃取物，再利用細胞毒性、酵素免疫分析(Enzyme-Linked Immunosorbent Assay)等方法來檢測病毒誘導產生的發炎相關機制。結果顯示，HSV-1 感染後會誘導 BV2 細胞產生細胞膜融合的現象，並且有細胞數量減少的趨勢，利用洋蔥萃取物與槲皮素進行預先處理後，細胞數量有明顯的回升，且病毒誘導的 NO、IL6 及 IP10 表現量顯著地下降。

本研究目前證實了 HSV-1 感染 BV2 誘導產生炎症反應下會導致細胞死亡，因此未來將探討洋蔥萃取物及槲皮素是否具有保護功能及其相關路徑為何。

3

用碘酸鈉建立老年性黃斑部病變的斑馬魚動物模式

Sodium iodate induced age-related macular degeneration in zebrafish.

學生:周佩璇　指導教授:楊建洲

摘要:

近年來斑馬魚經常被當作研究藥物及疾病的動物模式。老年性黃斑部病變(AMD)是一種常見的眼睛疾病，主要因感光細胞的死亡導致中心的視力喪失，可分成兩種型態：一種為乾性 AMD，通常是無症狀的，會有玻璃疣(drusen)的出現；另一種為溼性 AMD，中心視力開始下降，有新生血管的出現。根據之前的研究指出碘酸鈉($NaIO_3$)會誘導視網膜色素上皮(RPE)變性使感光細胞死亡，可模擬 AMD 的病徵，因此本研究主要利用 $NaIO_3$ 建立 AMD 的斑馬魚動物模式。

首先我們先建立了3個月至12個月野生型斑馬魚的眼壓值,之後拿三個月大的公魚,用 0.5mM 和 1.0mM 的 $NaIO_3$ 去誘導斑馬魚 30 天、60 天及 90 天，先用眼壓計檢測眼壓，再將魚犧牲後取出眼球，包埋於石蠟並進行視網膜組織切片，之後進行蘇木精和伊紅（H&E）染色觀察。

結果顯示：在野生型斑馬魚的眼壓值模型中，四個月、五個月及七個月公魚的眼壓顯著高於十個月的公魚。在加入 NaIO3 誘導的組別中，眼壓並沒有產生顯著差異，大多位於 21~24mmHg。0.5mM 的 $NaIO_3$ 誘導 90 天後，RPE 厚度顯著低於 1.0mM 的組別。在 1.0mM 的 $NaIO_3$ 誘導 90 天後，感光細胞外節(outer segment, OS)的厚度顯著低於控制組，而 RPE 厚度顯著高於控制組。

根據以上結果推斷 $NaIO_3$ 引起的視網膜厚度的變化可能與劑量和時間有關，使用 1.0mM 的 $NaIO_3$ 誘導 90 天可能是最適合做為 AMD 的斑馬魚動物模式，但還需經後續的實驗驗證。

4

Study the interaction between MBNL3 and miRNA in ovarian cancer

Student：陳虹均　　Professor：潘惠錦

Abstract：

MBNL3 is an RNA-binding protein that regulates alternative splicing and is mainly expressed in proliferative tissues. Previously, our laboratory has found higher MBNL3 expression in ovarian cancer cell lines than in normal ovarian cell lines and MBNL3 knockdown decreased the growth rate of ovarian cancer cells. As cancer and miRNAs are significantly related, we would like to know whether MBNL3 participates in specific miRNA regulations, thus affecting the growth of the ovarian cancer cells. It has been reported that miR-21 and miR-221 are downregulated, while miR-34 is upregulated in ovarian cancers. Their corresponding downstream target genes (such as apoptosis inhibitors or apoptosis stimulating factor) would change accordingly, thereby promoting the apoptosis and inhibiting the proliferation of cancer cells, thus inhibiting tumor growth. We cultured normal IOSE and ovarian cancer OVCAR3 cells and transfected the cells with si-MBNL3 to knockdown MBNL3, as well as with si-NC as a control group. We confirmed decreased MBNL3 expression after MBNL3 knockdown using PCR and Western blotting. We then extracted total RNA, reverse transcribed miRNA, and then used qPCR to measure miRNA expression. However, the expression level of miRNA was not significantly up- or down-regulated in ovarian cancer cells compared with that of normal ovarian cells as reported. After MBNL3 knockdown, the expression levels of the three miRNAs also did not change as expected. It is likely that the characteristics of the cell lines have been changed, or that these miRNAs were not regulated by MBNL3. Instead, it is possible that the miRNAs may act upstream of MBNL3. We performed bioinformatics analysis and found that MBNL3 is a predicted target for miR-21. In the future, we will examine the relationship of MBNL3 and miR-21, and reveal their regulation mechanism in ovarian cancer cells.

5

SET7/9 在抗老化蛋白質表現，細胞衰老及粒線體自噬作用之角色探討

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。我們先前的研究結果顯示, SET7 / 9 可調節小鼠腎小球細胞（MES-13）中誘導型一氧化氮合酶（iNOS）的蛋白質表達。此結果顯示 SET7 / 9 在發炎反應中扮演重要角色。由於慢性發炎反應與衰老導致的相關疾病有關，因此，我們目前的研究目標，旨在闡明 SET7 / 9 在抗衰老蛋白質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 分析檢測抗衰老蛋白質（包括 Klotho，sirtuin1 和 HSP70）的蛋白質表達 。同時， 也檢視了細胞衰老標記，例如 P16, P21，P53 的表現變化。此外，也研究了 IS 對 PINK1 / PARKIN 蛋白質的影響。當我們使用 set7/9 抑制劑，降低 NRK-52E 細胞中 SET7 / 9 蛋白質表達時,我們發現被 IS 抑制的抗衰老蛋白表達可被恢復。這部份實驗結果說明，SET7 / 9 蛋白質在抗衰老蛋白表達上扮演重要角色。未來將進一步研究 SET7 / 9 在調節抗衰老蛋白表達的分子作用機制。此外，SET7 / 9 是否參與細胞衰老和線粒體自噬作用將一併檢視。

6

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

學生:陳柏宇　　指導教授:林庭慧

摘要:

Indoxyl Sulfate (IS)是一種尿毒素，是造成慢性腎臟病之致病因子。在尿毒素處理的大鼠腎細胞中，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 基因的表達。在我們之前的研究中，我們證明了 PRMT6 和 NF-κB 的相互作用導致精氨酸在 NF-κB 上甲基化並進入細胞核以抑制 Klotho 表達。為了確認 Klotho 蛋白質的新分子機制，我們研究了 NF-κB 上的賴氨酸和精氨酸甲基化是否會影響 Klotho 蛋白質，以及確認 Klotho 蛋白質本身是否會受到甲基化的調控進而影響其穩定性。我們使用 IS 來治療大鼠腎臟近端小管細胞(NRK-52E cells)，觀察到 IS 增加了細胞甲基化反應中心酶的蛋白質表達，包含精氨酸甲基轉移酶 4（PRMT4）和精氨酸甲基轉移酶 6（PRMT6）。而經 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) 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底物，在免疫沉澱實驗中，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 抑制 PRMT 活性，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，使 Klotho 蛋白質的穩定度變高。這些發現提供了新穎的 Klotho 蛋白質調控之分子機轉，以治療缺乏 Klotho 蛋白質而以引起之腎臟疾病。

7

BisGMA- induced cytotoxicity and genotoxicity in macrophages. Pretreatment of macrophage RAW264.7 cells with NAP inhibits cytotoxicity and increases survival rate.

Student:楊孟蓁　Advising professor:關宇翔 楊建洲

Abstract：

Bisphenol-A-glycidyldimethacrylate (BisGMA) is a resin frequently used in dental restorative. It is widely used in the repair process of caries treatment. But after a period of time for caries treatment, BisGMA could leach from dental restorative resins after polymerization leading to inflammation in the peripheral environment. NAP is the organic compound and mainly accumulates in plants such as potato, and tomato. Chemical synthesis of NAP is difficult; therefore, NAP is primarily extracted from plants. NAP possesses antibacterial, antiviral, anti-inflammatory, and NAP derivatives also have anti-oxidant. NAP derivatives can also be used for the treatment of cardiovascular disease, and wound healing. The aim of this study was to explore whether NAP can inhibit cytotoxicity and genotoxicity induced by BisGMA in macrophages. In this research, MTT Assay was first used to measure the survival rate of macrophages after pretreatment with NAP. Next, the detection of NO and LDH confirmed the toxicity of BisGMA. Then using Annexin-V to detect whether the cell apoptosis has improved. JC-1 detects whether the mitochondrial membrane potential is unbalanced. ROS detection of damage to nucleic acids, proteins and membrane lipids. These results demonstrated that macrophages pretreated by NAP can reduce the damage caused by BisGMA and the survival rate of macrophages will also increase.

8

芹菜素(Apigenin)藉由抑制 Wnt/β-catenin 和 PI3K/AKT/mTOR 訊號路經進而產生對三陰性乳癌增生,侵襲的抑制

Apigenin suppresses triple-negative breast cancer cell proliferation and, invasion via inhibition of the Wnt/β-catenin and, PI3K/AKT/mTOR signaling pathways

Student:廖泯嘉　Professor:陳威仁

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 progressions in human cancers. Apigenin is a kind of flavonoids abundant in vegetables and fruits. Apigenin has been reported to possess anti-cancer activity with low cytotoxicity and mutagenic activity, but the mechanism by which apigenin inhibits triple-negnative breast cancer cell proliferation and invasion still remains unclear. To this end, we investigate whether apigenin inhibits proliferation and EMT of triple-negnative breast cancer via modulation of the Wnt/β-catenin and PI3K/AKT/mTOR signaling pathways.

Methods: In this study, we conducted MTT and wound healing assay to evaluate cytotoxicity, proliferation and cell migration. Western blotting were performed to monitor the protein levels of active molecules involved in WNT/β-catenin and PI3K/AKT/mTOR pathways.

Results: The results of wound healing assay demonstrated that apigenin inhibits cell migration in a dose-dependent manner. Western blot analysis revealed that apigenin decreases the protein level of phosphor-AKT, β-catenin, and phosphor-GSK3β, and concomitantly suppresses the expression of EMT-associated proteins such as ZEB1, Slug, Snai1 and vimentin.

Conclusion: Apigenin can regulate β-catenin level to inhibit EMT through inhibiting PI3K/AKT/mTOR signaling pathway. We thus suggest that apigenin may act as a potential therapeutic agent for triple-negative breast cancer patients.

9

N-乙醯半胱氨酸治療舒疼消熱劑誘導急性肝損傷的劑量效應與可能機制

(The dosage effect of Nacetylcysteine therapy in propacetamol-induced acute liver damage and possible mechanisms)

學生:謝承綺　　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，但臨床上對於 NAC 治療 APAP 所引起的肝損傷並沒有最佳處置方式，且 NAC 可能會阻礙肝臟修復或造成其他副作用。舒疼消熱劑(propacetamol)是 APAP 的前驅物，舒疼消熱劑引起的急性肝衰竭的作用機制與 APAP 一樣，因此，我們研究 NAC 治療舒疼消熱劑引起肝中毒的使用劑量與可能作用機制。

我們首先測試不同品系小鼠在不同劑量舒疼與 NAC 治療下的 168 小時存活率.發現 BALB/c 品系小鼠對於舒疼具有高敏感性，而 C57BL6/J 小鼠較不敏感,因此我們選用了 BALB/c 作為我們後續實驗的實驗動物.我們分析不同時間點的肝臟外觀、血清肝、腎功能指數、組織抗氧化指數及發炎因子，透過這些結果我們發現，給予 275 mg/kg NAC 是一個較好的治療劑量，而給予過高劑量的 NAC 進行治療，會造成小鼠死亡，過多的 NAC 也會使發炎因子 IL-6 大量上升，接下來我們將進一步研究過量 NAC 導致發炎因子 IL-6 大量上升的原因，及其對肝中毒後期肝臟組織修復再生的影響，與高劑量 NAC 治療造成小鼠死亡的原因。

10

The specificity non-Th2 airway inflammatory endotypes in the cell-based using crude allergens from *Dermatophagoides microceras*

Student：馮天浩　Professor：劉玉凡

Abstract：

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. We proposed the RNA-Seq approach to discover the *Der m* crude allergens to triggers sensitization mechanisms in the normal human bronchial epithelial cells (BEAS-2B) compared with *Der p* and *Der f* treated. In this study, we constructed five samples including a control group, three crude allergens protein extracts (such as *Der m*, *Der p* and *Der f*), in addition to *Der p* combined with Dexamethasone treatment group. Dexamethasone is a synthetic corticosteroid to current treat allergies, asthma, rheumatic diseases, and skin diseases. Then, provided total RNAs preparation for next generation sequence (NGS) technology and then bioinformatic analysis. We offered the Hisat2 and StringTie public packages for RNA-seq analysis, R Studio presented charts for statistics analysis, GSEA, in additional to the Cytoscape, KEGG (Kyoto Encyclopedia of Genes and Genomes) databases for pathways analysis, respectively. In conclusion, we found three pathway: Toll-like receptor signaling pathway, mTOR signaling pathway and MAPK signaling pathway unique for *Der m* crude. In the future, we will verify the results of OMICs approach with real-time qPCR and ELISA methods, and find the novel genes in the non-Th2 immune pathway to induce a specificity sensitization endotype mechanism in the *Der m* crude allergens-based exposure environment.

1

脆葉馬蘭水萃液抑制子宮肌瘤之分子機轉研究

Molecular mechanisms of the anti-uterine fibroid activity of Strobilanthes crispus water extract

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摘要:

子宮肌瘤是一種良性的腫瘤，也是女性在生育年齡中最好發的婦科疾病。根據研究，美國有70-80%的女性曾經罹患子宮肌瘤，而衛福部的資料顯示 2018 年在台灣有超過 36 萬人被診斷為此疾病。子宮肌瘤的症狀包括經期異常出血、子宮壓力升高、下背痛、甚至是胚胎著床不易。Strobilanthes crispus 脆葉馬蘭是一種原生於馬達加斯加的植物，也被稱為黑面將軍，在新馬地區的傳統醫學上使用於癌症、糖尿病及子宮肌瘤的治療。某些研究指出脆葉馬蘭的萃取物可以抑制癌細胞增生、抗氧化及抑制血管增生，但目前沒有研究分析脆葉馬蘭萃取物對於子宮肌瘤的分子機轉。首先，本研究發現脆葉馬蘭水萃液可以抑制大鼠子宮肌瘤細胞株 ELT3-Luc 的生長，並且可以造成細胞自噬的標誌蛋白 LC3-II 以及 ATG5-ATG12 複合體的增加；反之，以氯喹(chloroquine)抑制細胞自噬作用則可以部份回復脆葉馬蘭水萃液的抑制效果。我們使用 DCFDA 活性氧化物質偵測套組進行分析，發現脆葉馬蘭水萃液會增加細胞內活性氧化物質的產生，而彗星試驗則證明其可以誘導細胞 DNA 的損傷；此外，以 N-acetyl-L-cysteine 抑制細胞內活性氧物質產生則可完全消除脆葉馬蘭水萃液對細胞的生長抑制以及 DNA 損傷的產生，顯示脆葉馬蘭水萃液可誘導活性氧物質進而造成子宮肌瘤細胞產生 DNA 損傷。接著我們使用流式細胞儀分析細胞週期，PI 染色的結果指出脆葉馬蘭水萃液會造成細胞濃度依賴性地累積於 G1 期，同時也確認了細胞週期 G1 檢查點蛋白 cyclin D1/CDK6 複合體的表現量會在處理後下降。為了確認脆葉馬蘭水萃液藉由何種機制導致 G1 期停滯，我們使用了西方點墨法分析了細胞經過治療後的蛋白表現，發現 ATM、IGF1R、以及 Akt 的磷酸化形式蛋白表現量都會下降。而 IGF1R 以及 ATM 的抑制劑也分別能夠抑制 p-Akt/cyclin D1/CDK6 的表現量；我們更進一步發現 ATM 的抑制劑 ku-55933 也同樣會導致細胞的 G1 期停滯。總結本研究結果，我們確認了脆葉馬蘭水萃液會藉由抑制 p-ATM/p-IGF1R/p-Akt 的路徑導致 cyclin D1/CDK6 複合體的表現量下降，進而誘導大鼠子宮肌瘤細胞株 ELT3-Luc 的 G1 期停滯；脆葉馬蘭水萃液也會誘導細胞內活性氧化物質的產生導致 DNA 損傷。未來本研究將以氣相層析質譜儀鑑定脆葉馬蘭水萃液的有效成分，以期能進而開發以純化之脆葉馬蘭植化素作為子宮肌瘤之單方治療藥物。

Abstract:

Uterine fibroids (UF), the benign tumors of the uterus, are one of the most common diseases among women in the 40s and early 50s. UF may lead to heavy bleeding, pelvic pressure, pain, or even reproductive dysfunction. The percentage of women who suffered from UF is up to 70-80% in the USA. Moreover, there are over 360,000 women diagnosed as uterine fibroids in Taiwan in 2018. Strobilanthes crispus (also called black face general, BFG) is a traditional medication in Singapore and Malaysia. It's used for the treatment of tumor, diabetic, and UFs. Several studies have confirmed that the extraction of BFG displays anti-cancer, anti-oxidant, and anti-angiogenic activities. However, how BFG extraction influences UFs is yet to be elucidated. We firstly found that the BFG water extract inhibited the proliferation of a rat UF cell line, ELT3-Luc. The autophagy marker, LC3-II, and the autophagosome elongation factor, ATG5-ATG12 complex, were increased. Treatment of chloroquine, the autophagy

inhibitor, partially recovered the inhibitory effect of BFG extract. Furthermore, the intracellular production of ROS was increased in BFG treated ELT3 cells, as well as the enhanced DNA damage was observed by comet assay. The treatment of N-acetyl-L-cysteine abolished the inhibitory effect of BFG in proliferation or the induction of DNA damage indicating that BFG extract could cause ROS mediated DNA damage. With flow cytometric analysis of propidium iodide (PI) staining, treatment of BFG in ELT3 cells induced G1 arrest, as well as the reduction of cyclin D1 and CDK6 expression, in a dose-dependent manner, which was. The downregulation of p-ATM, p-IGF1R, and p-Akt proteins in ELT3 cells was also observed in BFG-treated ELT3 cells. In addition, the treatement of ku-55933, an ATM inhibitor, caused G1 arrest at the similar level with BFG treatment. In conclusion, we demonstrate that BFG water extracts display a growth inhibition effect to a rat UF cell line, which is associated with the induction of G1 arrest through the downregulation of p-ATM and the ROS mediated DNA damage. In the future, we will further identify the functional component of BFG extract in the UF inhibition by Gas chromatography–mass spectrometry, which may lead to the development of phytochemicals for UF treatment.

2

L-type amino acid transporter 1 在肺癌幹細胞自我更新的角色探討

L-type amino acid transporter regulates self-renewal capability of lung cancer stem cells

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摘要:

肺癌，衛福部統計為國人十大死因之首，近數十年來在臺灣地區的肺癌病人有顯著增加的趨勢。癌症幹細胞(cancer stem cells)，為癌細胞中有一群具有自我更新和分化能力的細胞，其在腫瘤生成和抗藥性扮演著重要的角色。L-type amino acid transporter 1 (LAT1)，主要轉運大分子支鏈胺基酸和芳香族中性胺基酸，其中包括必需胺基酸白胺酸(leucine)。近年來研究發現，LAT1 在許多癌症中都有較高的表現量，包含食道癌、口腔癌、乳癌及肺腺癌，並且可藉由抑制 LAT1 表現量來抑制癌細胞的生長。

本研究首先發現肺癌細胞株 A549 之 Pemetrexed 抗藥性細胞 A400 細胞中自我更新相關基因及 LAT1 的表現較 A549 細胞為高，因此我們推測 LAT1 可促進肺癌幹細胞的自我更新能力。除此之外，從 Human Protein Atlas 資料庫中顯示，高表現 LAT1 的肺癌病患其總生存期間較低表現者短。不論藉由小分子抑制劑 JPH203 或以慢病毒傳送專一性 shRNA 來抑制 LAT1 的活性或蛋白表現，都能有效抑制 A400 細胞中自我更新相關基因的表現和癌症球體(tumorsphere)的形成，並且造成細胞內 mTOR 和 Akt 磷酸化程度下降。為更進一步探討 LAT1 在肺癌幹細胞所扮演的角色，我們使用可穿透細胞之 Leucine 類似物(L-Leucyl-L-Leucine methyl ester, Leu-Leu-OMe)處理 A400 細胞，則發現可以增加 mTOR 或 Akt 的磷酸化，提高其癌症球體形成能力，並且 Leu-Leu-OMe 處理可降低 JPH203 對癌症球體形成的抑制效果，這些結果顯示 LAT1 在肺癌幹細胞的自我更新能力中扮演著重要角色。未來我們將在 JPH203 處理下的肺癌細胞中過度表現穩定活化態之 Akt，以探討 Akt 在 LAT1 所調控的肺癌幹細胞自我更新中所扮演的角色。總結本研究結果，LAT1 可能是藉由 Akt/mTOR 路徑來調控肺癌幹細胞的自我更新，且抑制 LAT1 的活性可能做為肺癌病患的潛在治療策略。

Abstract:

Lung cancer is the leading cause of death among cancers in Taiwan. Cancer stem cells (CSCs) are a subpopulation of cancer cells which participate in tumor initiation, drug resistance, and metastasis. L-type amino acid transporter 1 (LAT1/SLC7A5) is a membrane transporter, which delivers neutral amino acids into cells, such as leucine. Recent studies indicate that LAT1 is highly expressed in a variety of cancers, such as esophageal carcinoma, oral cancer, breast cancer, and lung adenocarcinoma, and the inhibition of LAT1 reduces cell proliferation.

In this study, we first discovered that the CSC activity of pemetrexed-resistant A549 cells (called A400) was higher than the parental A549 cells, including the increased number of tumorspheres and the upregulation of BMI1, Sox2, Oct4, and c-Myc. We also found that LAT1 expression in pemetrexed A400 cells was higher in comparison to the parental A549 cells. From Human Protein Atlas database, the expression level of LAT1 is positively associated with the shorter overall survival of lung cancer patients. Inhibition of LAT1 by a small molecule inhibitor, JPH203, or by lentiviral delivery of specific shRNAs led to a significant reduction in tumorsphere formation and downregulation of cancer stemness genes including BMI1, Sox2, Oct4, and c-Myc. We also found that the activation of Akt/mTOR was

declined after LAT1 inhibition. The treatment of Leu-Leu-OMe, a cell-permeable leucine derivative, in A400 cells promoted mTOR/Akt phosphorylation and CSC activity in A400 cells. Leu-Leu-OMe also reduced the inhibitory effect of JPH203 to A400 cells in tumorsphere formation. In the future, we attempt to perform the overexpression of constitutively active form of Akt followed by JPH203 treatment to demonstrate the involvement of Akt in the LAT1 mediated self-renewal of lung CSCs. In conclusion, our data reveal that LAT1 regulates self-renewal of lung CSCs through Akt/mTOR pathway and its inhibition may offer an effective therapeutic strategy in lung cancer patients.

3

探討 miR-145-5p 在非小細胞肺癌對於愛寧達藥物的抗藥機制所扮演的角色

The involvement of miR-145-5p in pemetrexed resistant non-small-cell lung cancer cell (NSCLC)

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摘要:

肺癌長年居於國人癌症死亡首位，其中非小細胞肺癌 (non-small-cell lung cancer, NSCLC)更是佔了肺癌的 85%。Pemetrexed (Alimta)是一種葉酸抑制劑被用於治療局部晚期或轉移性非小細胞肺癌的二線以上化療藥物。然而長期使用 Pemetrexed 治療可能會造成癌細胞對藥物產生抗藥性。其中，Thymidylate synthase (TS)的高表達被認為是肺癌細胞對 Pemetrexed 藥物產生抗藥性的主要機制，但臨床研究卻發現 TS 表現程度與 pemetrexed 抗藥性無顯著相關，顯示可能有其他途徑會導致 pemetrexed 抗藥性。

在本研究中發現 miR-145-5p 在 pemetrexed 抗藥性 A549 細胞 (稱為 A400 細胞)中表現較 A549 細胞低。透過轉染方式將 miR-145-5p mimic 送入 A400 細胞使細胞中的 miR-145-5p 高表達，抑或是透過 miR-145-5p 抑制劑降低 A549 細胞中 miR-145-5p 的表現，皆會使細胞對於 pemetrexed 藥物的敏感性受到影響。另外，我們也發現在 pemetrexed 抗藥株 A400 細胞中，細胞的 BMI1 (B Lymphoma Mo-MLV insertion region 1 homolog)、Sp1 與 TS 蛋白表現都比 A549 細胞來的高。透過結合位預測網站資源 (TargetScan database)我們發現 miR-145-5p 可能會與 Sp1 的 3 端非轉譯區(3’-UTR)結合。而利用西方點墨法分析非小細胞肺癌中 miR-145-5p 表現受到抑制或高表達時，Sp1 蛋白表現會受到調控。除此之外，透過 Sp1 3’UTR 報導基因分析，我們確立在非小細胞肺癌中，Sp1 為 miR-145-5p 的目標基因。

另一方面，在 A549 細胞中當 Sp1 高表達時，細胞對於 pemetrexed 藥物的敏感性下降，同時誘導細胞產生上皮間質轉換 (epithelial-mesenchymal transition, EMT)，並上調控 EMT 相關轉錄因子，如: Snai1, ZEB1 與 Twist，並增加細胞轉移能力。更重要的是，我們也發現當 A549 細胞 BMI1 高表達時，Sp1 會被上調控，而 BMI1 的高表達也會誘導 A549 細胞產生 EMT，並且細胞的增生能力也會增加。然而，透過 Sp1 inhibitor (Mithramycin A)抑制 Sp1 表現可以逆轉此一現象。

總而言之，本研究結果顯示，在 pemetrexed 抗藥性非小細胞肺癌中，BMI1 表現提升會藉由下調控 miR-145-5p 以提升 Sp1 的表現。而隨著 pemetrexed 抗藥性非小細胞肺癌的 BMI1 與 Sp1 表現增加，會促使細胞發生 EMT，並且減低細胞的藥物敏感性。這些結果表明，透過 RNA 藥物增加細胞中 miR-145-5p 表現可能可以做為 pemetrexed 抗藥性非小細胞肺癌患者的增敏劑。

Abstract:

Lung cancers are the leading cause of death among cancer patients in Taiwan and non-small-cell lung cancer (NSCLC) accounts for 85% of all lung cancers. Pemetrexed is an antifolate drug and is the second line chemotherapy drug for NSCLCs. However, the long-term pemetrexed treatment may cause resistance. The high expression of thymidylate synthase (TS) has been suggested to be one of the mechanisms in pemetrexed resistance in cell study but the clinical observations indicate the involvement of other factors.

Here, we firstly found that miR-145-5p was decreased in pemetrexed resistant A549 cells (called A400 cells). The overexpression of miR-145-5p by a specific mimic to A400 cells or the knockdown of miR-145-5p by a specific inhibitor to A549 changed the pemetrexed sensitivity of the corresponding

cells. We also found the increased expression of BMI1 (B Lymphoma Mo-MLV insertion region 1 homolog), Sp1, and TS in pemetrexed A400 cells. Using TargetScan database, we found that there was a putative miR-145-5p binding site within the 3'UTR (3'-untranslated region) of Sp1 gene. The Sp1 3'UTR reporter assay and western blot analysis of Sp1 protein expression after manipulation of miR-145-5p expression by the transfection of mimic or inhibitor further confirmed that Sp1 is a direct target of miR-145-5p in NSCLC cells.

On the other hand, the Sp1 overexpression in A549 cells decreased their pemetrexed sensitivity and induced epithelial-mesenchymal transition (EMT) process by upregulation of EMT related transcription factors, such as Snail1/Twist1/ZEB1, as well as the increased cell migratory ability. We further found that Sp1 could be upregulated and the miR-145-5p expression could be downregulated by BMI1 overexpression in A549 cells. The BMI1-induced cell proliferation or EMT in A549 cells could be abolished by the treatment of Mithramycin A, a Sp1 inhibitor.

Taken together, our data suggest that the increased expression of BMI1 in pemetrexed resistant NSCLC cells causes the upregulation of Sp1 through the downregulation of miR-145-5p. The increased expressions of BMI1 and Sp1 in pemetrexed resistant NSCLC cells also promote EMT process, which may also enhance the drug resistance. These data also suggest that the RNA drug to increase cellular expression of miR-145-5p is potentially to be developed as a sensitizer for NSCLC patients with pemetrexed resistance.

4

組蛋白 H3 賴胺酸 4 去甲基酶 Jhd2 和 E3 泛素連接酶 Not4 編碼基因之間的功能交互作用對白色念珠菌表型的影響

Phenotypic consequences of the functional interaction between histone H3 Lys 4 demethylase Jhd2 and E3 ubiquitin ligase Not4 encoded genes in *Candida albicans*

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摘要:

白色念珠菌 (*Candida albicans*)是一種缺乏完整有性生殖週期的自然界二倍體生物。作為伺機性的人類真菌病原菌，白色念珠菌能夠引發宿主黏膜及組織的表層感染，或是在免疫失調患者中導致伴隨高致死率的系統性念珠菌血症 (Systemic candidiasis)。其型態可塑性 (Morphological plasticity)被認為是造成白色念珠菌致病的關鍵能力，故作為白色念珠菌研究的重點之一。先前我們揭露進化保守的有絲分裂細胞週期正調節因子，Skp1-Cullin-1 / Cdc53-F-box (SCF) Cdc4泛素連接酶，具有抑制白色念珠菌酵母菌到菌絲型態轉變的新角色。通過對白色念珠菌Cdc4的親和純化實驗，我們得到並鑑定白色念珠菌Jhd2為*Ca*Cdc4的相關蛋白，並發現*CaJHD2*編碼組蛋白H3第四離胺酸去甲基酶 (Histone H3K4 demethylase)且可抑制白色念珠菌細胞聚集及生物膜形成。已知白色念珠菌*NOT4*編碼E3泛素連接酶 (E3 ubiquitin-protein ligase)，參與白色念珠菌芽管延長維持及菌絲形成，故對白色念珠菌致病力極為重要。已證明在釀酒酵母 (*Saccharomyces cerevisiae*)中Not4可透過對Jhd2的多泛素化-蛋白酶體依賴降解 (Polyubiquitin-proteasome dependent degradation)途徑，調控組蛋白H3K4的三甲基修飾狀態及部分外表型。然而藉由*NOT4*與*JHD2*之間功能性交互作用，導致組蛋白H3K4甲基化改變以及造成致病相關特徵等外表型變化，在白色念珠菌中仍待釐清。

我的目的是研究白色念珠菌*NOT4*是否可藉與*JHD2*交互作用，改變組蛋白H3K4甲基化狀態而造成白色念珠菌外表型變化。我利用透過實驗室新建立的系統建構而成的「NOT4-/-JHD2Tet-Off/-」白色念珠菌品系，透過在白色念珠菌*NOT4*同型合子無效突變珠 (NOT4-/-)中建構JHD2的四環黴素誘導表達關閉系統 (Tet-Off system)。當存在四環黴素的類似物去氧羥四環素(Doxycycline, Dox)時，此品系可模擬*NOT4*、*JHD2*雙剔除突變珠；當不存在Dox時則可呈現*NOT4*缺失而*JHD2*過量表現。*NOT4*和*JHD2*之間功能性交互作用的結果可透過對組蛋白H3K4甲基化狀態進行分析，並評估其可能的外表型結果變化。

我們發現當缺少*JHD2*時 (JHD2-/-)可增加白色念珠菌的細胞表面疏水性 (Cell surface hydrophobicity, CSH)；*NOT4*缺失 (NOT4-/-)則結果與之相反。而NOT4-/- JHD2Tet-Off/-細胞，在無Dox的條件下疏水性顯著降低，可能是*JHD2*過量表現與NOT4缺失結合的結果；相反的，當細胞加入Dox培養時則顯示能使疏水性恢復至野生型水準，被認為是*JHD2*表現受抑制所導致。此結果表明*JHD2*、*NOT4*在功能上與白色念珠菌致病力相關，同時也有針對其他致病力相關表徵的分析正在評估。透過NOT4-/- JHD2Tet-Off/-品系搭配Dox的使用來控制*JHD2*基因表現，似乎可揭示Not4對於Jhd2之抑制性的部分關聯。

Abstract:

Candida albicans is a natural diploid without a complete sexual cycle. As an opportunistic human fungal pathogen, *C. albicans* can trigger either superficial infection or systemic candidiasis with high mortality in immunocompromised patients The morphological plasticity is the critical virulent attribute to the pathogenesis of *C. albicans*, hence one of the center point in *C. albicans* research. We uncovered

that the evolutionary conserved positive regulator of the mitotic cell cycle, Skp1-Cullin-1/Cdc53-F-box $(SCF)^{Cdc4}$ ubiquitin ligase, has a novel role in suppressing the yeast-to-hypha transition in *C. albicans*. We identified *C. albicans* Jhd2 as a *C. albicans* Cdc4-associated protein by affinity purificationand found that *C. albicans JHD2* encodes a histone H3 lysine 4 (H3K4) demethylase and suppresses aggregation and biofilm formation in *C. albicans*. *C. albicans NOT4* is known to encode an E3 ubiquitin ligase that participates in germ tube extension and biofilm formation in *C. albicans*, which are important in virulence. The polyubiquitin-proteasome dependent degradation of Jhd2 by Not4 regulating the tri-methylation status of histone H3K4 for some phenotypes in *Saccharomyces cerevisiae* is known. However, the functional interaction between *CaNOT4* and *CaJHD2* in *C. albicans* leading to change in H3K4 methylation along with expression of genes and the phenotypic consequences, particularly features in virulence, remains to be clarified.

I aimed to investigate whether *CaNOT4* interacts with *CaJHD2* to alter H3K4 methylation status that affects phenotypic changes in *C. albicans*. I took advantage of a *C. albicans* strain called "NOT4-/-JHD2Tet/-", which was constructed with our newly established system. This strain was made by constructing tetracycline-repressible (Tet-Off system) *JHD2* expression into a *CaNOT4* homozygous null mutant (*NOT4*-/-). In the presence of doxycycline (Dox), the tetracycline analog, the strain mimics the loss of both *NOT4* and *JHD2* genes. In the absence of Dox, the strain displays loss of *NOT4* but overexpression of *JHD2*. The outcome of the functional interaction between *CaJHD2* and *CaNOT4* can be assayed for the status of H3K4 methylation and the possible alteration of phenotypic consequences can be assessed.

We found that while *C. albicans* cells lacking *JHD2* (*JHD2* -/-) increase the cell surface hydrophobicity (CSH), those lacking *NOT4* (*NOT4* -/-)show the opposite. Cells of NOT-/-JHD2Tet-Off/- strain grown without Dox in exhibited significantly decrease in the CSH, presumably due to the overexpressed Jhd2 in combination with *NOT4* deficiency. On the other hand, cells of NOT-/- JHD2Tet-Off/- strain grown with Dox showed restoring the CSH level comparable to the wild-type one, likely resulted from the repressed expression of *JHD2*. . The result indicates that *CaJHD2* and *CaNOT4* are functionally associated with *C. albicans* virulence and assays of other virulence related features are under assessment. The use of NOT-/- JHD2Tet-Off/- strain coupled with the usage of Dox for control of *JHD2* expression appears to reveal the inhibitory nature of Not4 to Jhd2 at least in part.

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以碘酸鈉誘導之老年性黃斑部病變模型探討槲皮素之保護功效

Protective effects of Quercetin on sodium iodate-induced age-related macular degeneration model

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摘要:

老年性黃斑部病變(Age-related Macular Degeneration, AMD)是造成失明的主要疾病之一。雖然已有眾多文獻指出：AMD 的發病情形與年齡及氧化堆積呈正相關，但目前對於 AMD 的發病機制與進程仍未完全探明，並且缺乏有效的治療策略。但在前人的文獻中指出：碘酸鈉(sodium iodate,NaIO3)這種天然強氧化劑在多種哺乳類動物中，可通過誘導視網膜色素上皮(Retinal Pigment Epithelial cells, hRPE)的氧化堆積，造成繼發性的感光細胞與視網膜病變，並且症狀與人類的 AMD 極為相似。因此，在目前探討 AMD 的相關研究中，碘酸鈉已廣泛地被各大期刊認同，用來建構 AMD 模型。本篇的研究目的是通過碘酸鈉誘導的 AMD 模型，探討槲皮素這種具有穿透血液視網膜屏障(Brain-blood barrier)功能的天然抗氧化劑對 AMD 可能的保護效果。

為了探討槲皮素的預防效果，我們對 ARPE-19 細胞株(人類 RPE 細胞)預先處理槲皮素 1.5 小時後，再添加碘酸鈉反應 15 或 24 小時，再藉由免疫染色、JC-1 染色、流式細胞儀、酵素活性分析及西方墨點法等來評估槲皮素對於碘酸鈉誘導之 ARPE-19 細胞的氧化壓力及細胞凋亡的保護效果。在動物實驗我們先以腹腔注射給予小鼠槲皮素(100 mg/kg/day)，2 小時後再以尾靜脈給予碘酸鈉(40 mg/kg)，之後每日再給予槲皮素，於第 7 天經斷層掃描(optical coherence tomography, OCT)後犧牲，分析血清中抗氧化酵素的表現量，並以免疫染色來探討其保護效果。

在 in vitro 實驗中，結果顯示：槲皮素可下降 pNrf-2、PGC-1α 及 SOD-2，以及增加 Catalase 與 GSH 的表現量，並減緩碘酸鈉誘導的 H2O2 堆積，降低了細胞內 ROS 的含量。除此之外，槲皮素通過增加 Bcl-2 的表現量，降低了碘酸鈉誘導的 Bax、cleaved RARP 及 caspase-3 上調，並減少了碘酸鈉誘導的粒線體的損傷及細胞凋亡的情況。最後，我們在小鼠模型中同樣觀察到：槲皮素增加了血清中 Catalase 與 GSH 的表現量，並減緩了小鼠視網膜中 Caspase-3 的增加，顯著地降低了碘酸鈉誘導的視網膜扭曲及變薄的情形。

我們的結果顯示：槲皮素可以通過減緩 RPE 細胞內的氧化壓力，與降低碘酸鈉誘導的細胞凋亡，達到保護 RPE 及視網膜損傷的功效。這個研究顯示了槲皮素作為 AMD 或其他氧化相關疾病的保健食品或預防藥物的潛力。

Abstract:

Background:

Age-related Macular Degeneration (AMD) is one of the major blinding diseases. Though AMD is widely accepted to be positively related to aging and oxidative accumulation, but the mechanism and treatment still remain unclear. Sodium iodate ($NaIO_3$), a natural oxidant, has been reported with capability to induce retinal pigment epithelial cells (RPE) oxidative stress and AMD-liked retinal degeneration in a variety of mammls, and widely accepted as an AMD model in many articles. The aim of this study was to evaluate the protective effects of Quercetin (Que), a natural antioxidant with Blood-Retinal barrier (BRB) penetration capability, on AMD disease.

Methods:

For preventive effects, the ARPE-19 cells (human RPE) were pre-treated with Que 1.5h and after

treated with $NaIO_3$ for 15h or 24h, to screen the influence of Que on $NaIO_3$-induced oxidative stress and apoptosis by JC-1 staining, flow cytometry, anti-oxidative enzyme assay and Western blotting. In mice model, we used intravenous injection of $NaIO_3$ (40 mg/kg) to induce retinal damage in mice after intraperitoneal injection of Que (100 mg/kg/days) for 2h, then provided Que daily until sacrifice on the 7th day, after observation by OCT (optical coherence tomography), to measure the expression of antioxidative enzymes in serum, and evaluate the protective effects by immunohistochemistry.

Results:

In vitro, our data indicated that Que could decrease $NaIO_3$-induced H_2O_2 and ROS via reduced the expression of pNrf-2, PGC-1α and SOD-2, with enhanced the activity of Catalase and GSH. Besides, Que reduced $NaIO_3$-induced up-regulation of Bax, cleaved RARP and caspase-3 through enhanced Bcl-2 expression, as well as relieved mitochondrial damage and the expression of apoptosis induced by $NaIO_3$. Finally, *in vivo*, we also observed that Que improved the expression of Catalase and GSH in mice serum, decreased the expression of Caspase-3 in mice retina, and then significantly reduced $NaIO_3$-induced retinal distortion and thinning.

Conclusions:

Our data indicated that Que could protect RPE and retina form $NaIO_3$-induced apoptosis and damage through down-regulated the RPE oxidative stress induced by $NaIO_3$. This study supports that Que may have potential to be healthy foods or medicines on preventing and delaying AMD or other retinal diseases involving in oxidative stress.

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利用斑馬魚探討 GJB2 基因突變造成聽損的機轉和菊苣酸對毛細胞的保護效果

Using zebrafish model to investigate the machanisms of GJB2 gene mutation caused hearing loss and protective effect of cichoric acid in hair cells

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摘要:

非症候群聽障 non-syndromic hearing loss (NSHL)跟由 GJB2 基因轉譯的人類連接蛋白 26 CONNEXIN 26(CX26)突變有密切的關係，先前實驗室透過 NSHL 病患發現 CX26 p.R184Q 這個體染色體顯性遺傳突變點。斑馬魚(Daino rerio)是一種經常使用在脊椎動物發育和藥物篩選的動物模式，且之前實驗室也發現斑馬魚 Cx30.3 與人類 CX26 高度同源，所以在本研究中我們透過 Tol2 transposon system 和 agr2 promoter 建構出在內耳支持細胞特異性表現突變 Cx30.3 的基因轉殖斑馬魚動物模式，用來探討人類 CX26 p.R184 突變導致 NSHL 的機制。

我們的結果發現 Cx30.3 p.R186Q 不會被送到細胞膜上而是會堆積在細胞核周邊，且與野生型斑馬魚相比，基因轉殖斑馬魚的內耳結構發生顯著改變，也透過斑馬魚游動行為分析發現基因轉殖斑馬魚行為上的改變，並從 Cx30.3 胺基酸的突變假設了可能影響 Cx30.3 蛋白功能的原因。

感音神經型聽力損失的原因有老化、基因和各種外部因素造成，例如噪音汙染、化療藥物和胺基糖苷類抗生素(例如新黴素)等，為了篩選出對耳毒性有保護效果的藥物我們使用基因轉殖斑馬魚(pvalb3b:TagGFP)，此種斑馬魚的內耳和側線毛細胞會表現出綠色螢光，我們使用菊苣酸 Circhoric acid(CA)來探討對新黴素誘導的毛細胞損傷的影響，CA 是一種由菊苣(Cichorium intybus)萃取出的酚酸類化合物，它具有抗氧化和增強免疫系統等功能。

我們的體外實驗發現，CA 減少了由新黴素誘導的毛細胞損傷，並找出保護效果最佳的 CA 濃度和處理時間，也透過 TUNEL 染色發現 CA 可以減少新黴素誘導的毛細胞凋亡。

Abstract:

Non-syndromic hearing loss (NSHL) has close relationship with mutated human CONNEXIN 26(CX26),which is coded by GJB2 gene,the CX26 p.R184Q chromosome dominant mutation was previously detected in NSHL patients in the laboratory. Zebrafish(*Danio rerio*) is an animal model commonly used for vertebrate development and drug screening. Previously, we also demonstrated that zebrafish Cx30.3 is orthologous to human CX26. In the present study, we established transgenic zebrafish models with mutated Cx30.3 specifically expressed in the supporting cells of zebrafish inner ears driven by the Tol2 transposon system and agr2 promoter to demonstrate and understand the mechanism by which the human CX26 R.184 mutation causes NSHL.

Our results indicated that Cx30.3 p.R186Q was not sent to the cell membrane but accumulated at the periphery of the nucleus. And it demonstrated significant structural changes in the inner ears of transgenic lines with mutations, which were compared to wild-type zebrafish. Simultaneously, significant alterations of transgenic lines with mutations in swimming behavior were analyzed with the zebrafish behavioral assay, and from the mutation of Cx30.3 amino acid, it is assumed that it may affect the function of Cx30.3 protein.

Sensorineural hearing loss is a permanent consequence of aging and gene mutation, and it results from a variety of extrinsic factors, including excessive noise, chemotherapy drugs and and ototoxic

drugs such as aminoglycoside antibiotics. Transgenic zebrafish (*pvalb3b:TagGFP*), which inner ear and lateral hair cells exhibit green fluorescence, was used to screen out drugs that have a protective effect on ototoxicity. Then, we used Cichoric acid (CA) to explore the effect on neomycin-induced hair cell damage. Cichoric acid is a phenolic compound extracted from chicory(*Cichorium intybus*). It also has the functions of antioxidation and strengthening the immune system.

For our in vitro studies, CA reduces hair cell damage induced by neomycin, and we find out the best protection effect of the concentration and processing time, TUNEL assay also shows that CA reduced neomycin-induced apoptosis of hair cells.