110 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八日

目錄

海報論文發表會議程

110 年 6 月 7 日(星期二) 10:00　直接線上接受提問(一人 6 分鐘 4 分鐘提問)

Teams 代碼 dr5q27f (請參加同學自行加入)

時間	報告講題	學生/指導教授
10:00~10:10	探討皮質類固醇地塞米鬆在斑馬魚胚胎模型中的作用 Exploring the effect of the corticosteroid Dexamethasone in zebrafish embryo model	施冠妤/楊建洲
10:10~10:20	通過製備和分析與白色念珠菌基因缺失和表達抑制相結合的菌株，研究對抗 H3K4 甲基化調節基因 Set1 和 Jhd2 的相互作用 Study of the interplay of the counteracting H3K4 methylation modulators Set1and Jhd2 by making and analyzing strains combined with the gene deletion and expression-repression in Candida albicans	陳彥霖/謝家慶
10:20~10:30	探討新加工製程黑蒜對胃潰瘍模式小鼠的保護效果及分子機制 Deciphering the protective effect and molecular mechanisms of newly processed black garlic on gastric ulcer model mice	陳亭沂/王淑紅
10:30~10:40	探討新小分子藥物對卵巢癌細胞的抑制及其作用路徑 Investigate the inhibition on ovary cancer cells by novel small molecule drugs and their pathways	高偉庭/潘惠錦
10:40~10:50	探討新小分子藥物是否抑制乳癌細胞活性及其背後的分子機轉 Investigate whether novel small molecule drugs inhibit activity of the breast cancer cells and the underlying molecular mechanism	蘇子潔/潘惠錦
10:50~11:00	探討弱化 MBNL3 抑制卵巢癌細胞增生的分子機制 Exploring the molecular mechanisms by which knockdown of MBNL3 inhibits proliferation of ovarian cancer cells	謝善任/潘惠錦
11:00~11:10	抗放射乳癌細胞的胞外泌體功能性分析 Functional analysis of extracellular vesicles in radioresistant breast cancer cells	張毓庭/張文瑋

口頭論文發表會議程

110 年 6 月 7 日(星期三) 13:00　　直接線上接受提問(一人 10 分鐘 5 分鐘提問)

Teams 代碼 dr5q27f (請參加同學自行加入)

時間	報告講題	學生/指導教授
13:00~13:15	沙門氏菌單股 DNA 結合蛋白質的表達、純化、晶體成長與結晶結構之解析 Expression, purification, crystallization, and crystal structure of a single-stranded DNA-binding protein from Salmonella enterica serovar Typhimurium LT2	羅仁宏/黃晟洋
13:15~13:30	對稱性二甲基精胺酸多株抗體及核酸適體之製備並將其分別應用於酵素連結免疫吸附分析法和核酸適體分析法之開發 Production of Polyclonal antibody and Aptamer and Their Application to Enzyme-linked Immunosorbent Assay and Aptamer-based Assay for Symmetric dimethylarginine	林家君/余豐益
13:30~13:45	低劑量山柰酚(Kaempferol)可以抑制 pin1 表現從而降低 pAKT/ERK 來抑制乳癌細胞轉移 Low-dose kaempferol suppresses the migration of breast cancer cells by inhibiting pin1 expression and thus reducing pAKT/ERK	陳慶宇/陳威仁
13:45~14:00	槲皮素藉由影響 PG/β-catenin/c-myc 訊號路徑抑制乳癌細胞的增殖和遷移 Quercetin inhibits the proliferation and migration of breast cancer cells by regulating PG/β-catenin/c-myc signaling pathway.	陳吟佩/陳威仁
14:00~14:15	多媒體影音提升乳癌放射線治療病患自我照護之成效分析 Effectiveness analysis of multimedia audio-visual courses in improving self-care for breast patients with radiotherapy	楊蕙芳/張文瑋
14:15~14:30	蛋白質精胺酸甲基化抑制與核仁壓力對 FUS 蛋白分布之影響及協力降低乳癌細胞存活可能性之探討 The effects of protein arginine methylation inhibition and nucleolar stress on the distribution of FUS protein and putative synergistic effects on reducing the viability of breast cancer cells	劉玉環/李　娟

海報論文摘要

1.

探討皮質類固醇地塞米鬆在斑馬魚胚胎模型中的作用

Exploring the effect of the corticosteroid Dexamethasone in zebrafish embryo model

學生：施冠妤　　指導教授：楊建洲

摘要：

糖皮質激素通常是急性和慢性發炎性疾病的第一線藥物，包括類風濕性關節炎、狼瘡和痛風。儘管其有廣泛使用性和有效的治療成果，但在副作用方面，特別是在懷孕期間的女性中，可能會對心血管發育產生不利影響。因此，本研究的目的是通過進行斑馬魚 (Danio verio) 的體內模型來評估 Dex 對胚胎髮育的影響。連續五天用指定濃度的 Dex（0、5、10 和 15 μg/ml）處理胚胎，並且對斑馬魚的心臟功能和血管生成在心率、心包面積和腸下血管形成 (SIV) 方面進行了評估。我們的研究結果表明，Dex 治療導致 SIV 顯著血管缺損和心包水腫，分別作為第 3 天和第 5 天平均 SIV 面積減少和心包面積增加的證據。但各組間心率無統計學差異。重要的是，這項研究使我們能夠根據我們的實驗條件和對藥物治療產生不良反應的風險，更好地描述 Dex 治療的劑量和時間的長度。

2.

通過製備和分析與白色念珠菌基因缺失和表達抑制相結合的菌株，研究對抗 H3K4 甲基化調節基因 Set1 和 Jhd2 的相互作用

Study of the interplay of the counteracting H3K4 methylation modulators Set1and Jhd2 by making and analyzing strains combined with the gene deletion and expression-repression in Candida albicans

學生：陳彥霖　指導教授：謝家慶

摘要：

Background

Being is a natural diploid without a complete sexual cycle, the opportunistic human fungal pathogen Candida albicans can cause systemic candidiasis with high mortality. We identified C. albicans Jhd2 as an associated protein with C. albicans Cdc4 whose gene negatively regulates filamentation. C. albicans JHD2, encoding a protein that is homologous to the Saccharomyces cerevisiae histone H3 lysine-4 (H3K4) demethylase, appeared to suppress aggregation and biofilm formation in C. albicans. In contrast, a H3K4 methyltransferase encoded by C. albicans SET1 was found to contribute invasive candidiasis. However, the interplay of JHD2 and SET1 that modulates H3K4 methylation-transcription leads to altered C. albicans virulence traits remains unknown. I aimed to clarify the functional interaction of C. albicans SET1 and JHD2 and the phenotypic consequence.

Methods

I exploited our lab-created Tet-off and deletion system and focused on constructing the strain *SET1*-/- *JHD2* Tet-Off/- where *SET1* is deleted and the expression of *JHD2* is doxycycline (Dox)-repressible. With Dox, the strain mimics the loss of both *SET1* and *JHD2* genes; without Dox, the strain displays loss of *SET1* but overexpression of *JHD2*. Strain *JHD2*-/- *SET1* Tet-Off/- was also created. The control of gene expression is in a contrasting manner.

The *JHD2* deleted strain *JHD2* -/- was previously made. To make *SET1* deleted strain *SET1* -/-, I generated the PCR cassettes for deletion with the long primers that combine complementary sequence to the regions specific to plasmid pSFS2AS (or pHB1S) and to the *SET1* CDS flanking sites. I sequentially electroporation-introduced the two cassettes into the wild-type *C. albicans* strain to select for nourseothricin (Nou^+) or hygromycin B positive ($HygB^+$) where two alleles of *SET1* are replaced with pSFS2AS- and pHB1S-derived cassettes, respectively, followed by maltose induction to pop out the cassettes. I assayed the desired structural alteration of *SET1* locus in the *SET1* -/- by colony PCR with the locus-specific and the cassettes-specific primers. To make *SET1*-/- *JHD2* Tet-Off/- strain, I generated one PCR cassette for deletion with the long primers that combine complementary sequence to the regions specific to plasmid pSFS2AS and to the *JHD2* CDS flanking sites and the other PCR cassette for Tet-off with the long primers combining complementary sequence to the regions specific to plasmid pWTF1 and to the *JHD2* CDS upstream or downstream of the start codon. Subsequent cassettes introduction, selection for Nou^+ or HygB+, and structural verification were done similar to construction of *SET1* deletion. I also made a *JHD2*-/- *SET1* Tet-Off/- strain in a manner similar to that of *SET1*-/- *JHD2* Tet-Off/-.

Results

First, the PCR-amplified cassettes for *C. albicans* transformation were verified by gel electrophoresis. Second, Nou^+ and $HygB^+$ transformants were selected on plates with specific antibiotics

of nourseothrecin and hygromycin B after the cassettes with either *SAT1* or *HygB* resistant gene being transformed into *C. albicans*. Third, the locus-specific PCRs in combination with gel electrophoresis confirmed that the strains *SET1*-/-, *SET1*-/- *JHD2* Tet-Off -/-, and *JHD2*-/- *SET1* Tet-Off -/- constructed were as expected.

Conclusions

I successfully constructed *SET1*-/- strain. The *SET1*-/- *JHD2* Tet-Off/- and *JHD2*-/- *SET1* Tet-Off/- strains were verified structurally.

Key words: *C. albicans*, *JHD2*, *SET1*, histone H3K4 methylation, Tet-Off system

3.

探討新加工製程黑蒜對胃潰瘍模式小鼠的保護效果及分子機制

Deciphering the protective effect and molecular mechanisms of newly processed black garlic on gastric ulcer model mice

學生：陳亭沂　指導教授：王淑紅

摘要:

全世界每年約有 400 萬人罹患消化性潰瘍(Peptic ulcer, PU)，而且 5-10 %的人在一生中會罹患 PU。PU 可分為胃潰瘍(Gastric ulcer, GU)和十二指腸潰瘍。GU 的發病機制是多因素的，尚未完全闡明。目前臨床治療 GU 的藥物主要包括質子泵抑製劑、抗酸劑和抗組胺藥，常引起胃腸道不良反應、肝腎毒性、高胃泌素血症和神經損傷等嚴重副作用，而且目前還沒有完全有效治療 GU 的方法。因此，不同的 GU 實驗動物模型被用於篩選新的 GU 治療，其中以乙醇誘導的 GU 模式動物所造成胃功能和形態變化與 GU 患者相似，所以利用乙醇誘導的 GU 模式動物，可篩選具有胃保護作用和較少副作用的潛在藥物，將可做為保護胃或治療 GU 的替代。本研究主要探討加工食品–黑蒜(Black garlic, BG)與新製程黑蒜(EG)保護酸性酒精(acid ethanol,AE)誘導胃潰瘍的能力。BG 是一種加工食品，是將新鮮大蒜通過高溫和高濕處理而產生的，BG 具有促進胃腸動力、抗糖化和保肝作用。BG 在梅納爾反應(Menard reaction)過程會伴隨 5-羥甲基糠醛(5-Hydroxymethylfurfural, 5-HMF)毒素的產生，而新製程黑蒜(EG)是以大蒜浸漬表沒食子兒茶素沒食子酸酯(Epigallocatechin gallate, EGCG)後製作出的，有降低 50% 5-HMF 產生與提高抗氧化能力效果，由我們的預試驗結果顯示，BG 與 EG 都有明顯降低 GU 的能力，用 BG 與 EG（50,100 和 200 mg/kg）預處理顯著降低了 AE 誘導的黏膜出血、水腫、炎症和 GU 指數。其中 EG 降低 GU 指數的能力優於 BG 組，EG+AE 組的胃黏膜中糖蛋白積累比 BG+AE 組多，EG 預處理還通過降低丙二醛 (MDA) 和恢復超氧化物歧化酶 (SOD) 、 CAT 活性及 GSH 來減少 AE 誘導的氧化壓力。我們將進一步分析 EG 預處理是否降低 AE 誘導的發炎反應來降低 GU 的發生及降低 GU 的可能分子機制。

4

探討新小分子藥物對卵巢癌細胞的抑制及其作用路徑

Investigate the inhibition on ovary cancer cells by novel small molecule drugs and their pathways

學生：高偉庭　指導老師：潘惠錦

摘要：

卵巢癌是女性十大癌症之一，初期徵狀並不明顯，且死亡率高。目前治療方法除了手術之外，仍需化學治療。大約 80%新診斷的卵巢癌對一線化學治療藥物有明顯反應；然而，大多數病患在一段時間後仍會復發，且二線治療藥物的療效多相當短暫，因此研發新的維持療法及副作用較低的標靶藥物相當重要。本研究所使用的新小分子藥物主要是針對血管新生因子及受體酪胺酸激酶(receptor tyrosine kinase，RTK)來合成的，我的目標是探討這些藥物是否可以有效抑制卵巢癌的進展，並分析這些藥物在卵巢癌細胞的作用途徑。我使用 OVCAR3 以及 HeyC2 兩種卵巢癌細胞做實驗。首先，利用不同濃度的藥及不同時間點做 MTT assay，觀察 IOSE(正常卵巢上皮細胞)、HeyC2、OVCAR3 細胞存活是否被抑制，找出合適藥物濃度及處裡時間後續做 colony formation assay，觀察細胞增生被藥物抑制的情況，並做 wound healing 及 transwell 分析，觀察藥物抑制細胞的爬行狀況。之前實驗已知卵巢癌細胞含有較高的 MBNL3 表達量，在 knockdown MBNL3 後則細胞生有被抑制。因此我將觀察新小分子藥物是否會影響 MBNL3 的表達，並檢查一些癌細胞常見活化的訊號傳導途徑是否有受抑制。我也會分析不同細胞程序如細胞凋亡、細胞增生、及 RTK 相關路徑等的蛋白質表現量是否有變化。另外為進一步確認小分子藥物的作用路徑，我將使用一些路徑蛋白分子的抑制劑做實驗，觀察細胞生長狀況及爬行能力等。若這些小分子藥物可以有效抑制卵巢癌細胞生長，也能確定其分子機制，將能提供卵巢癌治療藥物另種選擇與方向。

Abstract：

Ovarian cancer is one of the top ten cancers in women, with few early symptoms and a high mortality rate. In addition to surgery, the current treatment methods still require chemotherapy. Approximately 80% of newly diagnosed ovarian cancers respond significantly to first-line chemotherapy; however, most patients relapse after a period of time, and the effects of second-line treatments are often quite short-lived, thus the development of new maintenance therapies and target drugs with lower side effects are quite important. The novel small-molecule drugs used in this study are mainly synthesized against angiogenesis factors and receptor tyrosine kinase (RTK). My goal is to explore whether these drugs can effectively inhibit the progression of ovarian cancer, and analyze the pathways through which these drugs work. I used OVCAR3 and HeyC2 ovarian cancer cells for experiments. First, I will use different concentrations of drugs and different time points to do MTT assay to observe whether the survival of IOSE (normal ovarian epithelial cells), HeyC2, and OVCAR3 cells is inhibited, find out the appropriate drug concentration and treatment time, and then do colony formation assay to see if cell proliferation is inhibited by drugs. Wound healing and transwell analysis will be performed to observe the crawling ability of the drug-inhibited cells. It was known from previous experiments that ovarian cancer cells contained high levels of MBNL3 expression, and cell proliferation was inhibited after knockdown of MBNL3. So I will see if new small molecule drugs affect the expression of MBNL3 and examine whether some of the commonly activated signaling pathways in cancer cells are inhibited. I will also analyze whether there are changes in protein expression for different cellular processes such as apoptosis, cell

proliferation, and RTK-related pathways. In addition, in order to further confirm the action pathway of the small molecule drugs, I will use inhibitors of the pathway protein molecules to observe cell growth and crawling ability. If these small-molecule drugs can effectively inhibit the growth of ovarian cancer cells, and their molecular mechanisms can be determined, they will provide alternative options and directions for ovarian cancer treatment.

5

探討新小分子藥物是否抑制乳癌細胞活性及其背後的分子機轉

Investigate whether novel small molecule drugs inhibit activity of the breast cancer cells and the underlying molecular mechanism

學生：蘇子潔　指導老師：潘惠錦

摘要：

乳癌是全世界最常見的癌症，目前治療的藥物因乳癌細胞是否含有雌激素受體（ER)、黃體素受體（PR）、及人類表皮生長因子受體（HER2)而有所不同。三陰性乳癌因各類荷爾蒙及抗 HER2 藥物對其是無效的，因此研發新型的標靶藥物成為未來的發展方向。本研究所使用的新小分子藥物主要是針對血管新生因子及受體酪胺酸激酶(receptor tyrosine kinase，RTK)來合成的，我們使用兩種乳癌細胞做實驗，MCF7 及 MDA-MB-231。MCF7 為 ER+ PR+ HER2-，而 MDA-MB-231 為三陰性乳癌細胞(ER- PR- HER2-)。

首先，利用不同濃度的藥及不同時間點做 MTT assay，觀察 MCF10A(正常乳腺細胞)、MCF7、MDA-MB-231 細胞存活是否被抑制，找出合適藥物濃度及處理時間後續做 colony formation assay，觀察細胞增生被藥物抑制的狀況，並做 wound healing 及 transwell 分析，觀察藥物抑制細胞的爬行狀況。

其次，根據之前實驗結果已知乳癌細胞的 MBNL3 表達量是高的，並在 knockdown MBNL3 後觀察到細胞生長狀況有被抑制。為了觀察新小分子藥物是否會影響 MBNL3 及一些訊號傳導路徑的表達而抑制乳癌細胞，我將使用 PCR 及 western blot 實驗，分析加入藥物後 MBNL3 是否有被抑制，並分析不同路徑如細胞凋亡、細胞存活、及 RTK 相關路徑等的蛋白質表現量是否有變化。

第三，根據上述的 western blot 實驗，進一步確認小分子藥物是否會透過某個 pathway 來影響乳癌細胞，我將使用小分子藥物及其路徑蛋白分子的 inhibitor 做實驗，觀察細胞生長狀況及爬行能力是否有差別。此研究希望能觀察到這些小分子藥物會透過調控某個 pathway 進而達到抑制乳癌細胞的效果，特別是希望可以有效對付三陰性乳癌細胞。

6

探討弱化 MBNL3 抑制卵巢癌細胞增生的分子機制

Exploring the molecular mechanisms by which knock down of MBNL3 inhibits proliferation of ovarian cancer cells

學生:謝善任　　指導教授:潘惠錦

摘要：

MBNL3（Muscleblind-like protein 3） 是一種調節選擇性剪接的 RNA 結合蛋白，在許多癌症中扮演著促進的角色，例如乳腺癌、肝癌、非小細胞肺癌等。先前實驗室的研究中發現卵巢癌細胞株中的 MBNL3 表現量高於正常卵巢細胞，並且弱化 MBNL3 會降低卵巢癌細胞的生長速度。弱化 HeyC2 細胞中的 MBNL3 降低了細胞的遷移能力，以及細胞聚落的形成。因此我想更進一步觀察，弱化 MBNL3 在 HeyC2 細胞中造成那些生物過程的改變。我使用 sh-MBNL3 慢病毒感染的卵巢癌 HeyC2 細胞降低 MBNL3 表現量，並以 sh-Luc 作為對照組。使用西方點墨法觀察細胞凋亡以及自噬相關蛋白的表達量。目前結果顯示，弱化 MBNL3 在 HeyC2 細胞中造成細胞凋亡上升，而自噬作用則有減少的現象。許多研究指出 mTOR 在各種細胞過程中被激活，比如腫瘤形成、血管生成、胰島素抵抗、脂肪形成，並在多種癌症中表達失調。mTOR 抑制劑已經應用於實體瘤、器官移植、類風濕性關節炎等疾病的治療研究中。本實驗也將同時探討 mTOR pathway 在 MBNL3 弱化的 HeyC2 細胞扮演之角色。

7

抗放射乳癌細胞的胞外泌體功能性分析

Functional analysis of extracellular vesicles in radioresistant breast cancer cells

學生：張毓庭　　指導教授：張文瑋

Abstract:

Extracellular vesicles(EVs) are nanoparticles released by most cell types. These nanoparticles have a lipid bilayer structure and cargos proteins, DNA, RNA and lipids. Recently found that EVs play an important role in many biological processes such as cancer progression. It also has been reported that cancer cells release more EVs than normal cells. In addition, some studies indicate that the cargo contents of EVs are variable and related to the original cells. Triple-negative breast cancer (TNBC) cells account for 15-20 % of breast cancer, which does not express estrogen receptor, progesterone receptor, and Her2, and the poor prognosis is observed in TNBC. Due to this property, there is few targeted theray for TNBC and radiotherapy is one of the main treatments for this type of breaats cancers but radioresistance is common. Wnt/β-catenin signaling has been linked to cancer radioresistance but if exosomal β-catenin participates in radioresistance is still unclear. Our lab previously have established a radioresistance TNBC cell line (called 231-RR) from the parental MDA-MB-231 cells (called 231-P). In this study, we isolated EVs from the TNBC cell culture medium by differential centrifugation followed by ultrafiltration with centrifugal filters based on a molecular weight of 100KDa. We found that β-catenin expression of 231-RR-EVs was higher than 231-P-EVs. After treatment with 231-RR-EVs, the radioresistance, β-catenin protein level, and cancer stemness of 231-P cells were increased. Furthermore, the induction activity of cancer stemness genes by 231-RR-EVs was disrupted after the treatment of β-catenin inhibitor. In conclusion, EVs secreted from radioresistant TNBCs could carry β-catenin to induce radioresistance and to increase cancer stem cell activity of radiosensitive neighbor cancer cells.

口頭論文摘要

1.

沙門氏菌單股 DNA 結合蛋白質的表達、純化、晶體成長與結晶結構之解析

Expression, purification, crystallization, and crystal structure of a single-stranded DNA-binding protein from Salmonella enterica serovar Typhimurium LT2

學生:羅仁宏　指導教授:黃晟洋

摘要:

鼠傷寒沙門氏菌 (*Salmonella enterica* serovar Typhimurium) 是人類胃腸炎的主要原因，也作為是人類傷寒的小鼠研究模型。非傷寒沙門氏菌病的發病不斷增加，每年導致了數百萬人感染和許多人的死亡。此外，此細菌的抗生素耐藥性病例正在驚人地增加。因此，迫切需要開發臨床上有用的小分子抗生素或是找到針對此微生物的新標靶，以對抗此日益嚴重的耐藥細菌之威脅。單股 DNA 結合蛋白質 (SSB) 對於 DNA 複製和細胞存活至關重要，因此 SSB 可能是潛在的抗病源菌的化學攻擊標靶蛋白質。在此研究中，我們解出了此細菌 SSB 的晶體結構，其解析度為 2.8 Å (PDB ID 7F25)。此結晶結構表明此 SSB 單套體在其 N 端具有寡核苷酸/寡糖結合折疊功能區 (OB fold)，而在其 C 端則具有動態的尾端。此結構的 OB fold 核心是由一 6 個 β 平板的桶狀結構組成，其上有一個 α 螺旋。與其他形成四套體的細菌 SSB 不同，此 SSB 的晶體結構中每個不對稱單元僅包含兩個單套體。通過 MOE 對接分析，我們提出了各黃酮醇類，即 myricetin、 quercetin、kaempferol 與 galangin 對此沙門氏菌 SSB 的結合/抑制模式。在這些對接模式中，主要的結合氨基酸為 Gln52、Leu84、Tyr98 與 Asn103。這些初步結果將有助於開發針對此沙門氏菌 SSB 的新抑製劑，亦可能用於進一步臨床的化學治療。

Abstract:

Salmonella enterica serovar Typhimurium is a leading cause of human gastroenteritis and is used as a mouse model of human typhoid fever. The incidence of non-typhoid salmonellosis is increasing worldwide, causing millions of infections and many deaths in the human population each year. In addition, cases of antibiotic-resistant infections from this bacterium are alarmingly increasing. Therefore, developing clinically useful small-molecule antibiotics and identifying new target(s) in this microorganism are urgently needed to fight the growing threat of drug-resistant bacteria. The single-stranded DNA-binding protein (SSB) is essential for DNA replication and cell survival and, thus, is an attractive target for potential antipathogen chemotherapy. In this study, we determined the crystal structure of an SSB from *S. enterica* serovar Typhimurium LT2 (SeSSB). The crystal structure was solved at a resolution of 2.8 Å (PDB ID 7F25), indicating that the SeSSB monomer possesses an oligonucleotide/oligosaccharide-binding (OB) fold domain at its N-terminus and a flexible tail at its C-terminus. The core of the OB-fold in the SeSSB is made of a six-stranded β-barrel capped by an α-helix. Unlike other bacterial SSBs forming tetramers, the crystal structure of the SeSSB contained two monomers per asymmetric unit. Through MOE analysis, the binding/inhibition mode of the flavonols, namely myricetin, quercetin, kaempferol, and galangin to SeSSB, was suggested. In these docking models, Gln52, Leu84, Tyr98 and Asn103 in SeSSB are predicted to interact with these flavonols. These preliminary findings may facilitate the development of new inhibitors to target SeSSB for further clinical chemotherapies.

2.

對稱性二甲基精胺酸多株抗體及核酸適體之製備並將其分別應用於酵素連結免疫吸附分析法和核酸適體分析法之開發

Production of Polyclonal antibody and Aptamer and Their Application to Enzyme-linked Immunosorbent Assay and Aptamer-based Assay for Symmetric dimethylarginine

學生:林家君　指導教授:余豐益

摘要：

腎臟疾病是國人十大死因之一，也是伴侶動物疾病中的隱藏殺手，其中對稱性二甲基精胺酸 (Symmetric dimethylarginine, SDMA) 僅能藉由腎臟來代謝排除，是由 L-精胺酸經由蛋白質精胺酸甲基轉移酶轉化而來，而且不會受到生理狀態影響，因此被認為是一種腎功能衰竭之生物標記物。因此本研究期望能製備對 SDMA 具有專一性的多株抗體和適體，並且利用多株抗體與適體分別建立一套快速且靈敏的分析法，用以檢測 SDMA 的含量，以期能為民眾與動物健康問題來進行預防篩檢。由於 SDMA 是一種小分子化合物，因此需與載體蛋白質接合後進而對實驗動物進行免疫，以製備出對 SDMA 具有專一性的抗體，並以此抗體來建立免疫分析法；然而為了避免製備抗體時再現性不佳以及減少實驗動物用量等問題，本計畫也利用了配體指數擴增的系統進化技術 (Systematic Evolution of Ligands by Exponential enrichment, SELEX) 來篩選出對 SDMA 具有專一性的適體，並以此適體建立適體分析法來檢測 SDMA。在抗體製備部分，本研究雖然嘗試多種接合載體蛋白質的方式，但是進行實驗動物免疫後並無專一性抗體產生。然而在適體開發上，本研究成功找出一種對 SDMA 具有高度專一性的適體 (Apt #8)，其與其他載體蛋白質或接合物之間並無交叉反應的出現，也將此適體建立在以適體為基礎的西方點墨法，其檢測極限可達 0.5 μg 的 SDMA-SH-OVA。本研究成功篩選對 SDMA 具有專一性的適體，並且建立一適體分析法，但在多株抗體的生產上尚須改善 SDMA 接合載體蛋白質的方法，使其專一性與敏感度更加提升。

Abstract:

Renal disease is one of ten leading causes of death in Taiwan as well as the deadly disease of companion animal. However, chronic kidney failure is an irreversible disease progression. Symmetrical dimethylarginine (SDMA), derived from L-arginine by protein-arginine methyltransferase 5, is strictly excreted by kidney, and the early appearance of SDMA during renal failure makes it a potential biomarker for detecting renal function of animals. Therefore, to protect human and animal health, a high sensitive and rapid detection methods such as enzyme-linked immunosorbent assay (ELISA) and aptamer-based assay is developed herein to examine the SDMA level. SDMA belongs to a small molecule weight compound, needs to conjugate with carrier proteins in order to render their immunogenicity. SDMA-protein conjugates as antigens were immunized to the animal to produce the antibody against SDMA for establishment of the ELISA methods. In order to prevent the poor reproducibility of antibody production and reduce the use of the animals, an alternative choice such as aptamer was used to develop the aptamer-based assay. In the antibody section, although several methods for antigen preparation had been carried out, there was no specific antibody produced for SDMA after immunization. In the aptamer section, selected aptamers #8 (Apt #8) showed the highest specificity against SDMA in the aptamer-based Western blotting; additionally, Apt #8 did not cross-react with other protein or conjugate, indicating its high specificity of Apt #8. A dose-dependent manner were successful developed with detection limit of 0.5 μg SDMA-SH-OVA. In this study, we have successfully established the aptamer-based assay for

detecting SDMA. However, the currently produced polyclonal antibodies still need to improve the method of SDMA conjugation to the carrier proteins to make it more specific and sensitive for detection the SDMA level.

3.

低劑量山柰酚(Kaempferol)可以抑制 pin1 表現從而降低 pAKT/ERK 來抑制乳癌細胞轉移

Low-dose kaempferol suppresses the migration of breast cancer cells by inhibiting pin1 expression and thus reducing pAKT/ERK

學生:陳慶宇　指導教授:陳威仁

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。後期乳癌經常會伴隨著癌細胞轉移的風險，除了使用藥物治療之外，以天然化合物作為藥物佐劑也是一種研究方針。山柰酚為天然黃酮類化合物存在於許多植物中，許多研究表明山柰酚可以抑制癌細胞的增生和轉移，但沒有對於 pin1 蛋白表現的相關研究。Pin1(peptidyl-prolyl cis/trans isomerase 1)經常在癌細胞中高度表達，可以促進細胞增並抑制細胞凋亡，尤其在大多乳腺癌細胞中表現，Pin1 可以促進 AKT 的磷酸化並使 pAKT 不容易被降解來活化下游蛋白，其中包含促進轉移的 ERK。

本次實驗起初利用組織癒合實驗觀察到低劑量山柰酚可以抑制乳癌細胞癒合能力，後續利用 migration assay 觀察到在 24 小時反應後乳癌細胞轉移效率隨著山柰酚濃度增加而下降，除了轉移外利用 western blotting 也觀察到影響乳癌細胞侵襲能力的 mmp2 和 mmp9 有下降的趨勢。觀察 Pin1 蛋白表現量時發現 Pin1 蛋白在 24 小時有些微下降而在 48 小時隨著藥物濃度下降的幅度增加，然後觀察 Pin1 下游蛋白 AKT 表現後發現 pAKT 也隨著藥物濃度上升而下降，並且使影響轉移的 ERK 蛋白表現量下降。其他方面在間質細胞表現的相關蛋白(例:vimentin、twist)也有下降的趨勢。

在本次實驗中隨著山柰酚的濃度上升 Pin1 有下降的趨勢，而從 AKT/ERK 路徑可以觀察到抑制 Pin1 蛋白後可以使下游的 pAKT 和 ERK 表達下降，了解到山柰酚可能透過該路徑來抑制細胞轉移能力，山柰酚在抑制癌症轉移方面有很好的潛力。

Abstract：

Breast cancer has always been in the top three in Taiwan's female cancer rankings,and is one of the major cancers in humans. Terminal breast cancer is often accompanied by the risk of metastasis of cancer cells, and in addition to using drug therapy, it is also a research policy to use natural compounds as drug adjuvants. Kaempferol is a natural flavonoid found in many plants. Many studies have shown that kaempferol can inhibit cancer cell proliferation and metastasis, but there is no relevant study on the performance of the pin1 (peptidyl prolyl cis/trans isomerase 1) protein. Pin1 is often highly expressed in cancer cells, which can promote cell proliferation and inhibit apoptosis, especially in most breast cancer cells, Pin1 can promote the phosphorylation of AKT and make it difficult for pAKT to be degraded to activate downstream proteins, which contain ERK that promote metastasis.

In this experiment, we used the tissue healing experiment to observe that low-dose kaempferol could inhibit the healing ability of breast cancer cells, followed by the migration assay to observe that the effect on the migration of breast cancer cells decreased with increasing kaempferol concentration after 24 hours of reaction.

In addition to metastasis, MMP-2 and MMP-9, which affect the invasive ability of breast cancer cells, were also observed to decrease by using Western blotting. When observing the amount of Pin1 protein, it was found that the Pin1 protein slightly decreased with increasing drug concentration in 24 hours and was severely reduced in 48 hours. Then, after observing AKT, the protein downstream of Pin1, it was found that pAKT also decreased with increasing drug concentration, and the expression of the ERK protein

promoting single cell migration decreased. Other related proteins (e.g. vimentin and twist) expressed in mesenchymal cells also showed a downward trend.

In this experiment, as the concentration of kaempferol increased, the Pin1 protein also decreased gradually. From the AKT/ERK pathway, it can be observed that inhibition of Pin1 protein can reduce the expression of downstream protein pAKT and ERK . It is understood that kaempferol may inhibit cell metastasis through this pathway, and kaempferol has good potential in inhibiting cancer metastasis.

4.

槲皮素藉由影響 PG/β-catenin/c-myc 訊號路徑抑制乳癌細胞的增殖和遷移

Quercetin inhibits the proliferation and migration of breast cancer cells by regulating PG/β-catenin/c-myc signaling pathway.

學生：陳吟佩　指導教授：陳威仁

摘要：

槲皮素是一種在植物中被發現的類黃酮。文獻中的證據證明了槲皮素具有廣泛的生理活性，包括抗發炎、抗氧化以及抗血管生成，並且表明了槲皮素可以預防各種疾病，例如某些癌症。因此，槲皮素在預防疾病以及促進健康方面發揮著重要作用。Plakoglobin (PG, γ-catenin)是犰狳蛋白家族的成員之一，也是 β-catenin 的結構同源物。PG 除了在細胞中發揮重要的結構作用之外，還會參與細胞的訊號調節。而 PG 在 Wnt/β-catenin 這個訊號路徑中的作用跟 β-catenin 不同。事實上，多項研究都表示 PG 在多種癌症中具有抑制的作用，並且對於 β-catenin 有拮抗的作用。PG 甚至會透過與細胞內蛋白質的相互作用或是藉由隔離擔任轉錄因子的 β-catenin，與其競爭來達到調節的功能。

我們使用 MTT 分析法來檢測槲皮素對於人類乳癌細胞 MDA-MB-231 存活的影響。我們的結果表明，槲皮素透過濃度依賴性的方式抑制 MDA-MB-231 的細胞活性。為了進一步了解槲皮素對乳癌細胞增殖和遷移的抑制是否伴隨著 PG 蛋白表達的上調，我們進行 western blot 分析以及細胞免疫螢光染色評估槲皮素處理後的 MDA-MB-231 細胞中 PG 的表達。我們發現槲皮素上調 MDA-MB-231 細胞中 PG 蛋白的表達，同時分別抑制細胞週期進程所需的下游蛋白如 Cyclin D1 和 β- catenin 的蛋白表達。我們目前的數據表明，槲皮素可能透過上調 PG 使得 PG 能夠抑制 c-myc/β-catenin 來阻止乳癌細胞的增殖和遷移，並希望能夠將槲皮素開發為治療乳癌的前導藥物。

Abstract：

Quercetin is a flavonoid found in plants. Evidence in the literature proves that quercetin has a wide range of physiological activities, including anti-inflammatory, antioxidant, and anti-angiogenesis, and shows that quercetin prevents various diseases, such as some forms of cancer. Therefore, quercetin plays an important role in the prevention and the promotion of health. Plakoglobin (PG, γ-catenin) is a member of the Armadillo proteins family and a structural homolog of β-catenin. In addition to playing a significant structural role in the cell, PG participates in cell signaling regulation. The role of PG in the Wnt/β-catenin pathway is different from that of β-catenin. In fact, the plurality of studies suggest that PG has a tumor suppressor role in various cancers and, furthermore, it is an antagonistic effect to β-catenin. PG even executes the regulatory function competing with β-catenin that interacts with intracellular proteins or sequestering transcription factors.

We used the MTT assay to detect the effect of quercetin on the survival of human breast cancer MDA-MB-231 cells. Our results show that quercetin inhibited the cell viability of MDA-MB-231 cells in a dose-dependent manner. To understand whether inhibition of quercetin in breast cancer cell proliferation and migration is accompanied by up-regulation of PG protein expression, we performed Western blot analysis and immunofluorescence staining to evaluate PG expression in quercetin-treated MDA-MB-231 cells. We found that quercetin increased PG protein expression in MDA-MB-231 cells, while inhibiting the protein expression of PG substrate proteins such as c-myc and β-catenin required for cell cycle progression, respectively. Our current data suggest that quercetin may prevent proliferation and migration by up-regulating the PG, while inhibiting c-myc/β-catenin signaling pathway and we hope to develop

quercetin as a leading drug for the treatment of breast cancer.

5.

多媒體影音提升乳癌放射線治療病患自我照護之成效分析

Effectiveness analysis of multimedia audio-visual courses in improving self-care for breast patients with radiotherapy

學生:楊惠芳　指導教授:張文瑋

關鍵字：放射線治療、多媒體影音、**照護品質**

摘要:

【目的】接受放射線治療的患者，約有 9 成會產生副作用。因臨床業務繁忙，使護理人員衛教時間被壓縮，易產生片段性指導內容缺乏完整性，進而導致病患自我照護認知錯誤，副作用程度加重也因自我照護執行不確實。因此，此研究將護理衛教製作成多媒體，與傳統紙本單張比較，不同教材是否能提升病患自我照護執行正確率並減輕病患之焦慮。研究設計的衛教方式結果可以廣泛應用於放射治療病患的自我照護參考。

【方法】於 2020 年 1 月 1 日至 12 月 31 日，隨機將患者分為兩組實驗組與控制組，取樣選取放射腫瘤科乳癌初次接受放射線治療患者為對象，實驗組共 55 人使用多媒體影音，控制組共 55 人使用傳統紙本衛教單張。兩組於首次治療衛教前與治療後滿第 10 天，給予放射線自我照護認知問卷（前後測），比較兩組對放射線治療自我照護認知指標之差異程度。

【結果】統計 2020 年共收案 110 人次、影音 55 人次，衛教單張 55 人次，實驗組完整看完教材 100%，而對照組看完衛教單張內容僅 45%。**放射線治療病患對治療自我照護**研究結果發現，對照組正確認知率由 10.9%提升至 79.1%，實驗組正確認知率由 24.8%提升至 98.5%，結果顯示兩組間均有提升認知率，但兩組認知評核後測答對題數比較，介入前兩組 6 題全對皆為 0%(0 人) ，介入後 6 題全對對照組為 32.73%(18 人)、實驗組為 94.55%(52 人)，P＜0.001，達顯著差異。研究顯示介入多媒體影音衛教後可提升執行自我照護品質之成效。

【結論】初次放射線療病患於治療前運用多媒體 QR code 衛教模式，比傳統紙本衛教模式較能提升治療自我照護的正確認知率。基於本研究成果，未來亦可針對不同族群語言或將癌症別做分類設立，建立以病患為中心的醫療服務症狀衛教網站，平時調查病患對於衛教內容需求，藉由提供更切合病患需要的服務，除了放射線治療延伸將副作用作一系列多媒體影音作為臨床實用衛教工具，包含化學藥物、免疫治療、標靶治療等等一系列癌症治療相關知識，亦可建立衛教專欄或影音資庫，以供大眾查詢。若病患對自己接受的服務(疾病治療認知、自我照護衛教滿意度)感到滿意，會對於醫生或護理人員的指導更遵從，進而減少降低因知識吸收不完整造成面對治療不安感或因自我照護執行不確實而引發副作用加重，長久下來可以節省不少醫院對病患的醫療成本。

Abstract:

(Purpose)About 90% of patients receiving radiation therapy experience side effects. Due to the busy clinical business, the nursing staff's health education time is compressed, and it is easy to produce fragmented guidance content that lacks integrity, which leads to patients' self-care cognition errors, aggravated side effects, and inaccurate self-care implementation. Therefore, this study made nursing education into multimedia, and compared with traditional paper sheets,whether different teaching materials can improve the accuracy of patients' self-care implementation and reduce patients' anxiety.The results of the health education method of the study design can be widely used for self-care reference of radiotherapy patients.(Methodology)From January 1st to December 31st, 2020, the patients were randomly divided into two groups: the experimental group and the control group. The patients with breast

cancer who received radiation therapy for the first time in the radiation oncology department were selected as the subjects. A total of 110 people, 55 people in experimental group used multimedia audio and video, and another 55 people in the control group used traditional paper health education leaflets. Before the first treatment of health education and the 10th day after treatment, the two groups were given the radiotherapy self-care cognitive questionnaire (pre- and post-test), and the differences in the cognitive indicators of radiotherapy self-care between the two groups were compared.(Results)In 2020, a total of 110 cases, 55 videos and 55 health education leaflets were received. The experimental group completed 100% of the textbooks, while the control group read only 45% of the health education leaflets. The results of the study on self-care of radiation therapy patients found that the correct cognition rate of the control group increased from 10.9% to 79.1%, and the correct cognition rate of the experimental group increased from 24.8% to 98.5%. Comparing the number of correct questions in the two groups after the cognitive assessment, before the intervention, all 6 questions in the two groups were 0% (0 people), and after the intervention, the control group was 32.73% (18 people), and the experimental group was 94.55% (52 people), $P<0.001$, a significant difference. Studies have shown that intervention in multimedia audio-visual education can improve the performance of self-care quality.(Conclusion)Compared with the traditional paper health education model, the use of multimedia QR code health education model for first-time radiotherapy patients can improve the correct cognition rate of self-care treatment. According to the results of this study, a patient-centered medical service symptom health education website was established to investigate patients' needs for health education content. Or a different type of cancer is causing it. In addition to the side effects of radiation therapy, it can also expand the knowledge of a series of cancer treatments including chemical drugs, immunotherapy, and targeted therapy. Multimedia audio and video can be used as a clinical and practical health education tool, and a health education column or audio-visual database can also be established. public inquiries.

Keyword: Radiation therapy、Multimedia、 Self-care education

6.

蛋白質精胺酸甲基化抑制與核仁壓力對 FUS 蛋白分布之影響及協力降低乳癌細胞存活可能性之探討

The effects of protein arginine methylation inhibition and nucleolar stress on the distribution of FUS protein and putative synergistic effects on reducing the viability of breast cancer cells

學生:劉玉環　指導教授:李娟

摘要:

蛋白質精胺酸甲基轉移酶(PRMTs)在許多癌症中都是過表達的狀態，因此調節蛋白質精胺酸甲基化可以作為一種治療方向。研究顯示，RNA 結合蛋白 FUS 經 PRMT1 精胺酸甲基化後，會調節其於「次細胞結構」的分布，當癌細胞處於低甲基化環境時，可以在細胞核內觀察到 FUS 的顆粒狀結構生成。根據核仁蛋白質體動力學，FUS 在核仁壓力藥物的處理下會往核仁的位置聚集，然而文獻中並未提及這些次細胞結構是位在核質還是核仁。因此我想知道，癌細胞在受到「蛋白質精胺酸甲基化抑制」與「核仁壓力」共處理後，癌細胞的「細胞存活率」以及「次細胞結構」生成會受到哪些影響。本研究透過 lentivirus infection 下調人類口腔癌細胞 HSC-3 的甲基化表現，或是選擇具有 MTAP 基因缺失特性而使得細胞本身處於低甲基化狀態的乳腺癌細胞 MCF-7，進行核仁壓力藥物的處理。免疫螢光染色的結果證實，低甲基化會導致細胞中生成 FUS granules，然而這些 FUS granules 並不會聚集在核仁。此外，我們分別在核仁壓力藥物處理與甲基轉移酶抑制劑處理的試驗中，都觀察到乳腺癌細胞的細胞存活可能受到抑制，所以我想知道甲基轉移酶抑制劑與核仁壓力藥物對於抑制乳腺癌細胞的細胞存活率是否具有協同作用。因此，我選擇同為 MTAP 基因缺失的乳腺癌細胞 MDA-MB-231，MTAP 基因正常的乳腺癌細胞 MDA-MB-435、MDA-MB-468 以及抗輻射乳腺癌細胞 231-RR 進行實驗。結果顯示，廣效型的甲基轉移酶抑制劑 AdOx 在低劑量時即可抑制 MTAP 基因缺失的乳腺癌細胞，但與核仁壓力藥物 CX-5461 共處理無法更有效抑制乳腺癌細胞生長。然而，CX-5461 與 PRMT1 特異性抑制劑 K313 共處理卻可以有效抑制 MDA-MB-231 和 231-RR 的細胞存活率，其中又以 231-RR 細胞的抑制效果最佳。後續免疫螢光染色顯示，經 K313 處理後，231-RR 細胞內 DNA 損傷的情形顯著增加，顯示 K313 增強了 231-RR 細胞對輻射線的敏感度。根據研究結果，我們認為「蛋白質精胺酸甲基轉移酶 PRMT1 的活性抑制」與「核仁壓力」具有協同作用會共同抑制乳腺癌細胞生長，而且 PRMT1 特異性抑制劑 K313 還可能作為 231-RR 細胞的放射增敏劑，這對具有抗輻射特性的乳腺癌細胞而言可能會是一個有效的治療策略。

Abstract:

Protein arginine methyltransferases (PRMTs) are often upregulated in cancer. Thus, modulation of protein arginine methylation can be a therapeutic strategy. Recent studies revealed that an RNA-binding protein FUS can be arginine methylated by PRMT1 to modulate the subcellular distribution and forms nuclear granules in a hypomethylated environment. According to the nucleolar proteome dynamics, FUS is likely to move into nucleolus when treated with nucleolar stress inducers. I would like to know if inhibition of protein arginine methylation and nucleolar stress might affect the subcellular distribution of FUS. I thus treated an oral cancer HSC-3 cell line with PRMT1 knockdown, or a MTAP-deficient hypomethylated breast cancer cell line MCF-7 with a nucleolar stress drug. Immunofluorescent staining showed that under hypomethylation, FUS granules formed in cancer cell. However, these granules did not co-localize with the nucleolar marker fibrillarin but co-localize with a paraspeckle marker. Moreover, we observed that treatment of nucleolar stress drugs and methyltransferase inhibitors might reduce the

viability of breast cancer cells. I would like to know whether methyltransferase inhibitors would work with nucleolar stress drugs to suppress breast cancer cells. We used MCF-7, together with another MTAP-deficient MDA-MB-231 and two MTAP normal MDA-MB-435 and MDA-MB-468 cell lines and also radio-resistant MDA-MB-231 cells (named as 231-RR). MTT assay revealed that though an indirect methyltransferase inhibitor AdOx significantly reduced the survival of MTAP-deficient cells, co-treatment with nucleolar stress drug CX-5461 could not suppress breast cancer cells growth further. However, a PRMT1-specific inhibitor K313 worked synergistically with CX-5461 in MDA-MB-231, especially in 231-RR cells. Indeed, we demonstrated that the DNA damage level in 231-RR cells was significantly increased after K313 treatment, indicating that K313 might enhance the radiation sensitivity in 231-RR cells. Our study suggests that inhibition of PRMT1 and nucleolar stress can suppress breast cancer cell growth synergistically and K313 might function as a radiosensitizer to 231-RR cells. The treatment might be an effective therapeutic strategy to radio-resistant breast cancer cells.