111 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

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目錄

研究成果論文發表會議程

112.6.02 正心 0325 教室

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9:05~9:20	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/林庭慧
9:20~9:35	白色念珠菌 *JHD2* 通過調節組蛋白 H3 賴氨酸 4 三去甲基化影響毒力 *Candida albicans JHD2* impacts virulence by regulation of histone H3 lysine 4 tri-demethylation	陳彥霖/謝家慶
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11:25	頒獎 合照	
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11:30-12:30	Dasatinib作為三陰性乳癌之放射增敏劑的研究 A radiation sensitizer potential of dasatinb in triple negative breast cancer cells	涂育慈
11:30-12:30	篩選鐮孢菌酸適體和開發基於適體的鐮孢菌酸檢測方法的研究 Selection of aptamer and development of aptamer-based assay for Fusaric acid	林欣潔
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口頭論文摘要

1.

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:林庭慧

摘要:

乳腺癌是女性中最常見的癌症,也是全球癌症死亡的主要原因。乳腺癌發病率每年都在上升,這表明乳腺癌對全球女性的威協日益增長。目前治療的藥物因乳癌細胞是否含有雌激素受體、黃體素受體、及人類表皮生長因子受體而有所不同，而三陰性乳癌因各類荷爾蒙及抗HER2藥物對其是無效的，因此尋找治療或降低乳腺癌風險的藥物是重要的研究課題。在本研究中，使用了MCF-10A、MCF-7和MDA-MB-231细胞系，這三種细胞系被分別用新型小分子藥物f藥和i藥進行處理。在MTT assay，我們觀察到MCF7及MDA-MB-231兩種乳癌細胞的細胞存活率明顯下降，而MCF10A正常細胞細胞存活率下降幅度則沒有乳癌細胞來的大。我們根據MTT assay結果選用合適濃度來做後續實驗，在colony formation實驗，觀察到兩種乳癌細胞經過藥物處理後，細胞增生速度下降。同時，我們想了解細胞轉移情形是否有被影響，使用了wound healing及transwell兩種實驗觀察。實驗結果顯示，MDA-MB-231細胞經過藥物處理後細胞轉移情形有下降。接下來的實驗將進一步了解其分子機轉是否會被影響，我們將使用western blot來了解各個路徑的情形，並同時利用gelatin zymography實驗來觀察是否細胞在經過藥物處理後MMP的表現是否有改變。我們期望在這些初步實驗可看到乳癌細胞的癌症機轉能有效被新型小分子藥物所抑制,並進一步用於動物實驗及臨床實驗。

Abstract:

Breast cancer is the most common cancer among women and the leading cause of cancer-related deaths worldwide. The incidence of breast cancer has been increasing annually, highlighting the growing global threat to women. The treatment options for breast cancer vary depending on whether the cancer cells express estrogen receptors(ER), progesterone receptors(PR), and human epidermal growth factor receptor 2 (HER2). Triple-negative breast cancer does not express these receptors and is resistant to hormone-based and HER2-targeted therapies. Therefore, finding drugs to treat or reduce the risk of breast cancer is an important research topic. In the present study, MCF-10A, MCF-7, and MDA-MB-231 cell lines were used. These cell lines were treated with novel small molecule drugs, namely drug f and drug i. MTT assay was performed to assess cell viability. Colony forming assay was conducted to evaluate cell proliferation and transformation capacity. Wound healing assay was employed to measure two-dimensional cell migration, and transwell migration assay was used to assess cell invasion. Breast cancer is categorized into different subtypes, and the response to treatments can vary. The expression of hormone receptors and HER2 plays a crucial role in determining treatment options. In this study, MTT assay results revealed a significant decrease in cell viability for MCF-7 and MDA-MB-231 breast cancer cells, whereas the decrease in cell viability for MCF-10A normal cells was not as prominent. Based on the MTT assay results, appropriate drug concentrations were selected for subsequent experiments. In the colony formation assay, a reduced cell proliferation rate was observed for both breast cancer cell lines after drug treatment. Additionally, the impact of the drugs on cell migration was investigated using the wound healing and transwell assays. The results indicated a decrease in cell migration for MDA-MB-

231 cells following drug treatment. Further experiments will focus on understanding the molecular mechanisms affected by the drugs. Western blot analysis will be employed to assess the status of various signaling pathways, and gelatin zymography will be used to investigate any changes in matrix metalloproteinase (MMP) expression after drug treatment. These preliminary experiments aim to demonstrate the effective suppression of breast cancer cell carcinogenesis by the novel small molecule drugs. Subsequent animal and clinical studies will be conducted to further validate the findings.

2.

白色念珠菌 *JHD2* 通過調節組蛋白 H3 賴氨酸 4 三去甲基化影響毒力

Candida albicans JHD2 impacts virulence by regulation of histone H3 lysine 4 tri-demethylation

學生:陳彥霖　指導教授:謝家慶

摘要:

背景 – 伺機性人類真菌病原體白色念珠菌可在人類中引起系統性念珠菌病，死亡率很高。 由於白色念珠菌形態發育與毒力相結合，形態可塑性的分子機製成為白色念珠菌研究的重點。 我們已經鑑定出組蛋白 H3 賴氨酸 4 (H3K4) 特異性去甲基化酶 Jhd2，它是一種親和純化的 F-box 蛋白相互作用體，由白色念珠菌 *CDC4* (*CaCDC4*) 編碼的 Skp1-Cullin-F-box (SCF) E3 連接酶抑制白色念珠菌酵母菌到菌絲的轉變。

方法 – 為了了解 *JHD2* 控制 H3K4 去甲基化而影響形態發生和相關性狀，我們利用 *SAT1*-flipper 策略構建基於野生型菌株 SC5314 的菌株，並生成 *JHD2* 異型合子和同型合子無效突變株，以及 *JHD2* 互補菌株。 這些菌株用於評估通過特異性抗體的蛋白質西方點法的蛋白 H3K4 甲基化、菌絲誘導劑下的菌落形態和矽基生物膜形成。

結果 – 我們發現 *JHD2* 同型合子無效突變株增強了組蛋白 H3K4 三甲基化、多樣化的菌落形態和生物膜形成。 我們的結果證實 Jhd2 介導的 H3K4 去甲基化導致白色念珠菌的表型後果。

結論和未來展望 – 基於功能抵消 *JHD2* 的 H3K4 甲基轉移酶編碼基因 *SET1* 的菌株和相關菌株正在完成，其中與毒力相關的 H3K4 甲基化的表觀遺傳調控可以在評估菌株時闡明。

關鍵詞：白色念珠菌； *JHD2*； 組蛋白 H3 賴氨酸 4 甲基化； 毒力

Abstract:

Background

The opportunistic human fungal pathogen *Candida albicans* can cause systemic candidiasis in humans with high mortality. Due to *Candida albicans* morphological development being coupled with virulence, the molecular mechanism underlying morphological plasticity becomes focus of *C. albicans* research. We have identified a histone H3 lysine 4 (H3K4)-specific demethylase Jhd2, an affinity-purified interactor of the F-box protein of the Skp1–Cullin–F-box (SCF) E3 ligase encoded by *C. albicans CDC4* (*CaCDC4*) that restrains *C. albicans* yeast-to-hypha transition.

Methods

To understand the influence of *JHD2* on the control of H3K4 demethylation for morphogenesis and associated traits, we took advantage of the *SAT1*-flipper strategy for strain construction based on the wild-type strain SC5314 and generated both the *JHD2* heterozygous and homozygous null mutants, alongside the *JHD2* complementation strain. The strains were used to assess histone H3K4 methylation by western blotting with specific antibodies, colony morphology under the filament-inducing agents and silicone-based biofilm formation.

Results

We found that the *JHD2* homozygous null mutant enhances the histone H3K4 tri-methylation, diversified colony morphology and biofilm formation. Our results verify that Jhd2-mediated H3K4 demethylation leads to phenotypic consequences in *C. albicans*.

Conclusions and future prospects

Strains based on the H3K4 methyltransferase encoded gene *SET1* whose function counteracts *JHD2* and associated strains are in completion in which epigenetic regulation of H3K4 methylation associated with virulence may be elucidated in *C. albicans* upon assessment of the strains.

Key words: *Candida albicans*; *JHD2*; Histone H3 lysine 4 methylation; Virulence

3.

常規製程和兒茶素浸泡製程黑蒜萃取物對酸性酒精誘導胃潰瘍模式小鼠的保護效果與機制

Protective effects and mechanisms of black garlic extracts from conventional and catechin soaking processes on acidic alcohol-induced 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(PU)，PU 可分為胃潰瘍(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。以乙醇誘導胃潰瘍模式動物的形態變化與 GU 患者相似，利用此種模式動物可篩選較少副作用的潛在化合物或萃取物，做為保護胃臟或治療 GU 的替代。本研究主要探討常規製程黑蒜(BG)與表沒食子兒茶素沒食子酸酯浸泡製程黑蒜(EG)萃取物保護酸性酒精(AE)誘導胃潰瘍模式小鼠的效果與可能機制。口服急毒性實驗結果顯示，單一極大劑量(10 g/kg)的 BG 或 EG 處理實驗小鼠，只有造成血清中肌酸酐顯著上升，但是肝臟與腎臟切片的病理分析沒有顯著損傷，顯示本研究使用的 BG 和 EG 沒有顯著肝臟或腎臟的急毒性。分別以 50, 100 和 200 mg/kg BG 或 EG 預處理實驗小鼠七天，再以 AE 誘導產生 GU，EG 與 BG 預處理都顯著降低 AE 誘導的潰瘍指數(UI)，而相同劑量的 EG 降低 UI 效果較 BG 強，EG 預處理組的胃黏膜中糖蛋白積累也比 BG 預處理組多。解析 EG 預處理降低 AE 誘導 GU 的分子機制，EG 預處理可降低 AE 提升的氧化壓力，藉由降低丙二醛含量和恢復穀胱甘肽含量及恢復超氧化物歧化酶及過氧化氫酶活性。EG 預處理可以顯著減少 AE 誘導血清中腫瘤壞死因子-α (TNF-α)、白介素-1β (IL-1β)和白介素-6 (IL-6)含量，並且降低胃組織中 TNF-α、IL-1β、IL-6、環氧合酶-2 和可誘導型一氧化氮合成酶之 mRNA 的表現量和髓過氧化物酶活性。此外，EG 預處理顯著降低 AE 誘導的 p-ERK/ERK、p-JNK/JNK 和 p-p38/p38 比率，以及 p-NF-κB 和 p-IκB 水平。總而言之，本研究使用的兩種黑蒜萃取物都有極低的單一劑量口服急毒性，而 EG 比 BG 對 AE 誘導 GU 有更好的胃保護效果。近一步分析顯示 EG 可通過抗氧化、抗發炎及降低 MAPKs 和 NF-ㄏB 信號傳遞路徑來減緩 AE 所誘導的 GU。

Abstract:

Worldwide, approximately 4 million people suffer from peptic ulcer (PU) every year. PU can be divided into gastric ulcer (GU) and duodenal ulcer. The pathogenesis of GU is multifactorial and has not been fully elucidated. The morphological changes of ethanol-induced GU model animals are similar to those of GU patients. These model animals can be used to screen potential compound or extract with less side effects as a substitute for gastroprotection or treatment of GU. In this study, we explore the gastroprotective effects and possible mechanisms of extracts of black garlic manufactured by conventional (BG) and epigallocatechin gallate soaking (EG) processes on acidic ethanol (AE)-induced GU model mice. Results of orally acute toxicities showed that a single huge dose (10 g/kg) of BG or EG only caused significant increases in serum creatinine levels, but no significant pathological damage was found in both liver or kidney sections. There were no significant acute liver and kidney toxicities for both BG and EG used in this study. Pretreated mice with 50, 100 and 200 mg/kg BG and EG for seven days, respectively, and then AE was used to induce GU. Both EG and BG pretreatments significantly reduced the AE-induced ulcer index (UI), while the same dose of EG has a stronger effect than BG on reducing AE-induced UI. EG pretreatment also showed more glycoprotein accumulations in the gastric mucosa than those of BG

pretreatment. To decipher the molecular mechanisms by which EG pretreatment reducing AE-induced GU, EG pretreatment reduced AE-induced oxidative stress by decreasing malondialdehyde levels and by restoring glutathione contents and superoxide dismutase and catalase activities. EG pretreatment reduced AE-induced serum tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β levels, as well as reduced AE-induced mRNA expressions of TNF-α, IL-6, IL-1β, cyclooxygenase 2, and inducible nitric oxide synthase, and myeloperoxidase activity in gastric tissues. Furthermore, EG pretreatment significantly reduced AE-induced ratios of p-ERK/ERK, p-JNK/JNK and p-p38/p38, and p-NF-κB and p-IκB levels. In summary, two black garlic extracts used in this study have very low single-dose oral acute toxicity. EG has a better gastroprotective effect than BG on AE-induced GU. Furthermore, EG alleviates AE-induced GU through anti-oxidation, anti-inflammation, and inhibiting AE-induced MAPKs and NF-κB signaling pathways.

4.

抗放射三陰性乳癌細胞的細胞外囊泡功能性分析

Functional analysis of extracellular vesicles in radioresistant triple negative breast cancer cells

學生：張毓庭　指導教授:張文瑋

摘要：

細胞外囊泡(Extracellular Vesicles, EVs) 為細胞向外分泌的奈米級囊泡，具有雙層磷脂膜的結構，透過攜帶核酸、蛋白質和脂質等分子，在細胞間溝通扮演重要角色，癌細胞釋放的EVs更被發現可影響癌症的形成及惡化。乳癌(Breast Cancer) 是女性最常見的癌症，而其中大約有15%至20%屬於三陰性乳癌(Triple-Negative Breast Cancer, TNBC)，TNBC細胞表面缺乏荷爾蒙受體及HER2，因此導致標靶治療困難，目前主要以手術合併化學治療和放射線治療為治療手段，然而臨床上時常發現放射線抗性(Radioresistance) 產生，使得治療效果降低和預後不良。β-catenin蛋白已知能被EV包裹並啟動Wnt的癌化訊息，且Wnt/β-catenin路徑之活化也已知與放射線抗性有關，然而由EV攜帶的β-catenin對於TNBC之放射線抗性的影響仍不清楚。因此本研究目的為探討放射線抗性的TNBC細胞是否能透過EV使放射敏感性細胞產生抗性，以及β-catenin在其中扮演的角色。我們過去已經由MDA-MB-231 (231-P) 人類TNBC細胞建立抗放射細胞亞株(231-RR)，並且利用濾膜離心法由細胞培養液中分離EVs，發現比起231-P-EVs，231-RR-EVs中攜帶較多β-catenin蛋白；而且由細胞群落分析(Clonogenic Assay) 可以得知，經過231-RR-EVs處理後，231-P細胞的放射線敏感性會下降；而透過乳腺球體培養(Mammosphere Assay) 實驗，發現處理後的231-P細胞形成乳腺球體的能力提升，表示其癌細胞幹性(Cancer Stemness) 增加；另外利用西方墨點法發現，231-P細胞經過231-RR-EVs處理後，細胞內β-catenin及部分癌幹細胞自我更新相關因子，如c-Myc和Sox2，則有增加的現象。此外，當231-P細胞與231-RR-EVs共處理時同時加入β-catenin抑制劑CCT 031374，則由231-RR-EVs誘導的癌細胞幹性受到抑制。因此我們的結果顯示，來自抗放射TNBC細胞的EVs可透過傳輸β-catenin蛋白，降低鄰近非放射抗性細胞對放射線敏感性，以及其癌幹細胞活性。本研究結果暗示，未來或可利用血清EV中的β-catenin含量作為TNBC患者接受放射線治療的成效評估。

Abstract:

Extracellular Vesicles (EVs) are nanoscale vesicles that cells secrete, with a bilayer phospholipid membrane structure. The cargos contained by EVs including nucleic acids, proteins, and lipids, tha playing a critical role in intrecellular communication. EVs released by cancer cells can influence the formation and progression of cancer. Breast cancer is the most common cancer among women, and approximately 15% to 20% of these cases are Triple-Negative Breast Cancer (TNBC). These TNBC cells lack hormone receptors and HER2 on their surface, creating challenges for the development of targeted therapies. The primary treatment currently involves surgery in combination with chemotherapy and radiation therapy. However, the emergence of radioresistance is frequently observed, leading to poorer prognosis. The protein β-catenin, known to be encapsulated by EVs, activates the Wnt signaling pathway, which has also been associated with radioresistance. However, the influence of EV-carried β-catenin on TNBC radioresistance remains unclear. We previously established radioresistant sub-lines (231-RR) from human TNBC cells, MDA-MB-231 (231-P). In current study, we successfully isolated EVs from the cell culture media using an ultrafiltration method and discovered that 231-RR-EVs contained higher levels of the β-catenin protein compared to 231-P-EVs. Clonogenic assays indicated a decrease in the radiosensitivity of 231-P cells after treatment with 231-RR-EVs. Furthermore, a mammosphere assay demonstrated an increase in the

mammosphere-forming capability of 231-P cells after treatment with 231-RR-EVs, suggesting increased cancer stemness. Western blotting revealed that after 231-RR-EVs treatment, the intracellular levels of β-catenin and some self-renewal-related factors in cancer stem cells, such as c-Myc and Sox2, increased in 231-P cells. When 231-P cells were co-treated with CCT 031374, a β-catenin inhibitor, and 231-RR-EVs, the induced cancer stemness was suppressed. In conclusion, our findings demonstrate that EVs from radioresistant TNBC cells can deliver β-catenin protein to neighboring non-radioresistant cells, reducing their radiosensitivity and enhancing their cancer stem cell activity. These data suggest that the β-catenin content in serum EVs may serve as an indicator to assess the efficacy of radiation therapy in TNBC patients.

5.

篩選鐮孢菌酸適體和開發基於適體的鐮孢菌酸檢測方法的研究

Selection of aptamer and development of aptamer-based assay for Fusaric acid

學生:林欣潔　指導教授:余豐益

摘要:

鐮孢菌酸（Fusaric acid，FA）是由鐮孢菌屬（包括尖孢鐮刀菌和異孢鐮孢菌 ）等真菌產生的古老次生代謝物質。雖然對這種化合物進行了廣泛的研究，但其作用機制仍不清楚。然而，有證據表明鐮孢菌酸能夠抑制多巴胺β-羥化酶酶活性。除了已知的毒性特性外，鐮孢菌酸還能抑制人體細胞增殖和 DNA 合成。然而，缺乏特異性單株抗體對鐮孢菌酸的檢測構成了防止鐮孢菌酸暴露對人體健康產生不良影響的重大挑戰。本研究開發了一種新穎且具有成本效益的方法，利用酵素免疫連結吸附法（ELISA）或基於適體的檢測方法準確檢測鐮孢菌酸水平，旨在防止人類受到鐮孢菌酸毒性的影響。為了生成對鐮孢菌酸具有高特異性的適體，我們首先利用鐮孢菌酸開發了親和管柱，然後使用 SELEX（系統進化配體的指數富集）配體系統從隨機 DNA 庫中選擇單股 DNA（ssDNA）分子。通過七輪迭代選擇步驟，確認出了高度特異性的鐮孢菌酸適體，並透過 ELISA 篩選出與鐮孢菌酸結合特異性最高的最佳候選物。為了更深入了解鐮孢菌酸適體的性質，使用 RNAfold 服務器預測了其二級結構，並選擇 DNA 參數預測選項。為了確認 ELISA 結果的準確性，還採用了西方墨點法作為補充檢測方法。我們已經成功利用適體建立出檢測方法，相比基於抗體的方法，它具有節省時間的優勢。我們未來的工作將集中在開發基於適體的檢測方法。

Abstract:

Fusaric acid (FA) is an ancient secondary metabolite produced by Fusarium species, including Fusarium oxysporum and Fusarium heterosporum. While extensive research has been conducted on this compound, the precise mechanism of action remains unclear. Nonetheless, evidence suggests that Fusaric acid is capable of inhibiting the activity of the dopamine beta-hydroxylase enzyme. In addition to its established toxic properties, fusaric acid (FA) has been found to exert inhibitory effects on cell proliferation and DNA synthesis in humans. However, the lack of a specific monoclonal antibody for FA detection presents a significant challenge in mitigating the adverse health effects of FA exposure. A novel and cost-effective method utilizing ELISA or aptamer-based assay has been developed in this study for accurate detection of FA levels, with the ultimate aim of preventing human suffering from FA toxicity. To generate aptamers with high specificity for fusaric acid, we first developed an affinity column using Fusaric acid, and subsequently employed the SELEX (systematic evolution of ligands by exponential enrichment) ligand system to select single-stranded DNA (ssDNA) molecules from a randomized DNA library. Through seven rounds of iterative selection steps, highly specific aptamers targeting FA were identified, and subsequently screened via ELISA to identify the optimal candidate with the highest binding specificity for FA. To gain further insights into the properties of the FA aptamer, its secondary structure was predicted using the RNAfold server with DNA parameter prediction options. To confirm the accuracy of the ELISA results, western blotting was employed as a complementary detection method.

We have identified aptamers suitable for use in an aptamer-based assay, which offer a time-saving advantage compared to antibody-based assays. Our future efforts will be focused on developing aptamer-based assays, including FA detection strips, to enable rapid and sensitive detection of FA in various settings.

6.

利用斑馬魚動物模型探討地塞米松藥物的副作用

Exploring the effect of the side effects of corticosteroid Dexamethasone in zebrafish embryo mode

學生:施冠妤 指導教授:楊建洲

摘要:

糖皮質激素通常是治療發炎性疾病的一線藥物，包括類風濕性關節炎、紅斑狼瘡和近年來流行Covid-19感染。儘管其被廣泛的使用和傑出的治療效果，但其產生的副作用，特別是在懷孕期間的婦女，可能會對其胎兒的心血管系統發育產生不利的影響。因此，本研究的目的是通過進行斑馬魚（Danio rerio）的動物模型來評估地塞米松（Dex）對胚胎發育的影響。

本研究透過將胚胎暴露於地塞米松進行斑馬魚胚胎毒性試驗。從受精後4小時（hpf）開始，並且在24、48、72、96和120hpf評估其存活率、全身血管型態和總體表型變化。另外，分子機制方面我們分析了3dpf時斑馬魚的血管生成相關基因的表達來評估Dex對胚胎帶來的影響。我們的結果表示，Dex處理後斑馬魚的平均SIV面積及心跳數會減少；心包膜面積和眼球玻璃體血管數量則會增加；血管生成相關基因vegfaa、vegfkdr、pfkfb3、angpt-1和tie-2會呈現濃度依賴性上升；nrarpb則是濃度依賴性下調，在斑馬魚中nrarp的下調導致心包水腫和心率降低。從上述結果可以得知以Dex處理會導致顯著心血管發育缺損。

總之，我們的研究顯示地塞米松可能導致斑馬魚胚胎毒性，地塞米松還會誘導VEGF和血管生成素等基因過度表達並促進血管生成，造成胚胎產生異常表型。由於糖皮質激素在醫療方面被廣泛用於治療各種疾病，然而其對於心血管系統發育的副作用已經成為另一個需要關注的問題，目前仍有許多潛在機制有待闡明。

Abstract:

Glucocorticoids are often first-line drugs for acute and chronic inflammatory diseases, including rheumatoid inflammation, lupus, and the Covid-19 infection that has been prevalent in recent years. However, the side effects of glucocorticosteroids, especially in women during pregnancy, may have adverse effects on the development of the cardiovascular system of their fetuses. Therefore, the aim of this study was to evaluate the effects of dexamethasone(Dex)on embryonic development by performing an animal model of zebrafish(Danio rerio).

In our study, zebrafish embryotoxicity assays were evaluated by exposing embryos to dexamethasone(a synthetic glucocorticoid). Start at 4 hpf, and assess their survival rate, systemic blood vessels, and phenotypic changes at 24, 48, 72, 96, and 120 hpf. In terms of molecular mechanism, we analyzed the expression of angiogenesis-related genes in zebrafish at 3dpf to evaluate the effects of Dex on embryos.Our results show that the average SIV area and heartbeat number of zebrafish after Dex treatment will decrease; the pericardium area and the number of hyaloid vessel will increase; Angiogenesis-related genes vegfaa, vegfkdr, pfkfb3, angpt-1, and tie-2 showed a concentration-dependent increase; nrarpb was concentration-dependent down-regulation, and the down-regulation of nrarp in zebrafish led to pericardial edema and decreased heart rate. From the above results, it can be concluded that treatment with Dex leads to significant defects in cardiovascular development.

In conclusion, our study shows that dexamethasone may cause zebrafish embryotoxicity, and that Dex also induces the overexpression of genes such as VEGF and angiopoietin and promotes angiogenesis, resulting in abnormal embryonic phenotypes. Since glucocorticoids are widely used medically to treat various diseases,

their side effects on the development of the cardiovascular system have become another concern, and many underlying mechanisms remain to be elucidated.

7.

Oncogenic role of MBNL3 in ovarian cancer cells.

MBNL3 在卵巢癌細胞中的致癌作用

學生:謝善任　指導教授:林庭慧教授

摘要:

MBNL3（Muscleblind-like protein 3）是一種調節選擇性剪接的RNA 結合蛋白。關於MBNL3 在人類癌症中是否扮演致癌角色的訊息很少。先前的研究表明，MBNL3 在卵巢癌細胞中表達是增加的，使用專一性敲低MBNL3 的表達減少了卵巢癌細胞增殖、遷移和聚落的形成。本研究旨在探討MBNL3在卵巢癌進展中致癌作用的分子機制。使用表達sh-Luc 對照或sh-MBNL3 的慢病毒感染卵巢癌Hey C2 細胞來降低MBNL3之表達。使用RT-PCR 和西方點墨法來確認sh-MBNL3 慢病毒對Hey C2 細胞中MBNL3 的抑制效率。Western blot檢測MBNL3是否參與細胞凋亡、自噬和上皮-間質轉化（EMT）。凝膠酶譜法用於檢測基質金屬蛋白酶(MMP) 的表達。在本研究中，我們闡明了MBNL3 對HeyC2 細胞的細胞生長、凋亡、自噬和遷移中的影響。數據表明MBNL3 在人類卵巢癌中心扮演致癌基因角色，並且MBNL3 介導的抗細胞凋亡、自噬和EMT 促進了卵巢癌的進展。抑制MBNL3 的作用可能有助於作為治療卵巢癌的新策略。

Abstract:

MBNL3 (Muscleblind-like protein 3) is an RNA-binding protein that regulates alternative splicing. There is little information on whether MBNL3 plays an oncogenic role in human cancers.Previous studies showed that MBNL3 is increased in ovarian cancer cells and specific knockdown of MBNL3 reduced ovarian cancer cell proliferation , migration and the colony formation ability. This study aimed to investigate the molecular mechanism of the oncogenic role of MBNL3 in the progression of ovarian carcinoma.Ovarian cancer Hey C2 cells were infected with lentivirus expressing sh-Luccontrol or sh-MBNL3 to reduce the expression of MBNL3. RT-PCR and Western blot were used to confirm the efficiency of sh-MBNL3 lentivirus on inhibition of MBNL3 in Hey C2 cells.Western blot was used to detect whether MBNL3 participated in apoptosis, autophagy and epithelial–mesenchymal transition (EMT). Gel zymography was used to examine matrix metalloproteinases (MMP) expression.In the present study,we elucidated the effects of MBNL3 on cell growth, apoptosis, autophagy and migration of Hey C2 cells.Data suggested that MBNL3 plays an oncogene role in human ovarian cancer, MBNL3-mediated anti-apoptosis, autophagy and EMT contributed to the progression of ovarian cancer. Inhibiting the effects of MBNL3 may be useful as a new strategy in treatment of ovarian cancer.

8.

開發針對Moniliformin的適體及其應用於基於適體的檢測方法的研究

Developing of an aptamer against moniliformin and its application to aptamer-based assay

學生:戴偉宏　指導教授:余豐益

摘要:

Moniliformin是由不同鐮孢菌屬（Fusarium）菌株產生的一種真菌毒素，可以透過競爭性抑制呼吸途徑中的丙酮酸脫氫酶複合物，從而阻礙丙酮酸和糖解產物轉化為乙醯輔酶A，進而發揮其毒性作用。這種毒素常見於玉米和小麥等人類主要食物作物中，且最近的研究還顯示Moniliformin是一種致癌物質。為了降低Moniliformin的暴露風險並保護公眾健康，開發一種快速且具有成本效益的檢測方法至關重要。在文中，我們報告了一種基於選擇性進化（SELEX）的配體系統的Moniliformin檢測方法的開發。具體而言，我們通過化學衍生Moniliformin以增加其分子量並建立親和管柱。隨後，使用SELEX從隨機DNA庫中選擇了適體並識別為單股DNA分子。在進行了七輪的SELEX之後，識別出了兩個適體，Apt 4和Apt 5，它們對Moniliformin具有高度特異性，並且都能用作西方墨點法的探針。值得注意的是，Apt 5表現出最高的特異性並且與其他化合物幾乎不發生交叉反應。為了更深入地了解Moniliformin適體的性質，使用RNAfold服務器預測了Apt 5的二級結構。與基於抗體的方法相比，基於適體的檢測方法的開發可以更快地實現，為Moniliformin的檢測提供了一種替代方法。未來，我們的目標是開發更多基於適體的檢測方法，如ALISA或免疫層析試紙，以實現更便捷和可行的Moniliformin檢測。

Abstract:

Moniliformin is a mycotoxin produced by various Fusarium species that exerts its toxic effects by competitively inhibiting the pyruvate dehydrogenase complex in the respiratory pathway, thereby preventing the conversion of pyruvic acid and glycolytic products to acetyl CoA. This toxin can be commonly found in corn and wheat, which are critical staples in the human diet, and recent studies have also shown that Moniliformin is a carcinogen. To mitigate the risk of Moniliformin exposure and safeguard public health, it is crucial to develop a rapid and cost-effective detection method. Here, we report the development of an aptamer-based detection method for Moniliformin using a ligand system that employs systematic evolution of ligands by exponential enrichment (SELEX). Specifically, we chemically derivatized Moniliformin to increase its molecular weight and establish affinity columns. Subsequently, aptamers were selected from a randomized DNA library using SELEX and were identified as single-stranded DNA (ssDNA) molecules. Following seven rounds of SELEX, two aptamers, Apt 4 and Apt 5, were identified with high specificity for Moniliformin, and both were able to serve as probes for western blotting. Notably, Apt 5 exhibited the highest specificity and negligible cross-reactivity with other compounds. To gain further insight into the properties of the Moniliformin aptamer, the secondary structure of Apt 5 was predicted using the RNAfold server. Compared to antibody-based methods, the development of aptamer-based assays can be achieved more rapidly, providing an alternative approach for Moniliformin detection. In the future, we aim to develop additional aptamer-based assays such as ALISA or strips to enable even more convenient and feasible Moniliformin detection.

海報論文摘要

1.

茶多酚作為神經保護劑去抑制毛果芸香鹼誘導的 N2a 細胞神經毒性

Tea Polyphenols as Neuroprotective Agents: Inhibition of Pilocarpine-Induced Neurotoxicity in N2a Cells

學生:陳又榕　指導教授:王祖興

摘要**:**

活性氧 (Reactive oxygen species, ROS) 及炎症因子在神經系統疾病中起著至關重要的作用，活性氧及炎症因子會引起氧化損傷及炎症。過量 ROS 累積會導致神經元產生嚴重的氧化壓力，而炎症反應會損害神經元活性和血腦屏障完整性。因此，針對氧化壓力和細胞因子的產生是神經疾病的關鍵治療方法。茶多酚 (Tea polyphenols, TP) 具有抗氧化、抗發炎和其他有益的特性，也顯示出作為神經保護劑的潛力。本研究主要在評估茶多酚抑制毛果芸香鹼 (Pilocarpine, PILO) 所誘導之小鼠神經母細胞瘤細胞 (Neuro-2a, N2a) 神經毒性的能力。首先， 評估 PILO 和 TP 對於細胞的毒性。用 4 mM PILO 處理的 N2a 細胞存活率有 53%；而處理 10 μg/mL TP 的 N2a 細胞存活率則為 83%。另一方面，同時在 N2a 細胞處理 PILO 及不同濃度的 TP，結果顯示 TP 能提高細胞存活率。此外，考慮到 1 – 4 mM PILO 與 ROS 生成的關聯，本研究還檢查了 1 – 4 mM PILO 對粒線體氧化磷酸化的影響，還使用 DCFH-DA 染色觀察用 PILO 處理的 N2a 細胞中 ROS 產生的情況。未來本研究將探討 TP 對戊四唑 (Pentylenetetrazol, PTZ) 誘導的神經毒性的保護作用，並將其與 PILO 誘導的神經毒性進行比較。 總結來說，這項研究證明 TP 具有神經保護劑的潛力，可用來對抗神經疾病中的神經損傷。

關鍵字:茶多酚、神經毒性、毛果芸香鹼、小鼠神經母細胞瘤細胞、抗氧化

Abstract:

Reactive oxygen species (ROS) and inflammatory cytokines play a crucial role in neurological disorders, causing oxidative damage and inflammation. Excessive ROS accumulation leads to neuronal oxidative stress, while inflammatory responses compromise neuronal survival and blood-brain barrier integrity. Targeting oxidative stress and cytokine production is a critical therapeutic strategy for neurological diseases. Tea polyphenols (TP), possessing antioxidant, anti-inflammatory, and other beneficial properties, show potential as neuroprotective agents. This study aims to evaluate the ability of TP to inhibit pilocarpine (PILO)-induced neurotoxicity in N2a mouse neuroblastoma cells. The cytotoxicity of PILO and TP was assessed. The results showed N2a cells treated with 4 mM PILO exhibited a 53% survival rate, whereas 10 μg/mL TP displayed an 83% survival rate. Subsequent treatment of N2a cells with PILO followed by various concentrations of TP significantly enhanced cell survival. Additionally, this study examined the impact of 1 – 4 mM PILO on mitochondrial oxidative phosphorylation, given its association with ROS generation. ROS production in N2a cells treated with PILO was also observed using DCFH-DA staining. Future investigations will explore protective effects of TP against neurotoxicity induced by pentylenetetrazol (PTZ) and compare them to PILO-induced neurotoxicity. Overall, this study demonstrates the potential of TP, a natural compound extracted from green tea leaves, as a neuroprotective agent against neurological damage in various diseases.

Keyword: Tea polyphenols, Neurotoxicity, Pilocarpine, N2a Cells, Antioxidant

2.

Dasatinib作為三陰性乳癌之放射增敏劑的研究

A radiation sensitizer potential of dasatinb in triple negative breast cancer cells

學生:涂育慈　指導教授:張文瑋

摘要:

乳癌是全世界主要的女性癌症之一，其中三陰性乳癌(triple negative breast cancer, TNBC)佔所有乳癌的15-20%，具有易轉移、疾病進展快速、生存期短等特性，亦因其缺乏賀爾蒙受體(包括雌激素受體與黃體素受體)及Her2上皮生長因子受體，發展標靶治療不易，雖能進行放射線治療，但常見抗放射線細胞的出現。因此，發展有效的放射增敏劑對TNBC的治療非常重要。本研究選用人類TNBC細胞株MDA-MB-231，成功建立了三陰性乳癌抗放射線細胞(稱為231-RR)。初步結果顯示，231-RR細胞內的Src磷酸激酶活性上升。Src是一種非受體型酪氨酸激酶，在乳癌的進展中扮演了重要的角色，包含腫瘤起始、生長及轉移。因此，我們推測Src可能涉及到TNBC對放射線治療的抗性，因而使用dasatinib，一種已經用於慢性骨髓血癌治療的Src小分子抑制劑，探討Src活化對於TNBC抗放射反應的重要性。結果顯示，dasatinib對231-RR細胞產生特異性的增生抑制，並能提高231-RR細胞對放射治療的敏感性。我們進一步發現dasatinib能抑制Signal transducer and activator of transcription 3 (STAT3)的磷酸化，而STAT3抑制劑C188-9，也能提升231-RR細胞對放射線的敏感性。透過γ-H2AXser139染色，發現抑制Src/STAT3路徑在231-RR細胞中可直接造成DNA損傷反應。綜合以上結果，我們認為Src/STAT3路徑的活化是TNBC產生抗放射治療的機制之一，抑制Src/STAT3路徑可做為TNBC的放射增敏策略。未來研究將著重於探討Src/STAT3的抑制對於TNBC細胞內DNA修復路徑，以及癌幹細胞活性與癌幹細胞因子表現的影響，以完整闡釋Src/STAT3路徑在TNBC抗放射反應的分子機制。

3.

篩選鐮孢菌酸適體和開發基於適體的鐮孢菌酸檢測方法的研究

Selection of aptamer and development of aptamer-based assay for Fusaric acid

學生:林欣潔　指導教授:余豐益

摘要:

鐮孢菌酸（Fusaric acid，FA）是由鐮孢菌屬（包括尖孢鐮刀菌和異孢鐮孢菌 ）等真菌產生的古老次生代謝物質。雖然對這種化合物進行了廣泛的研究，但其作用機制仍不清楚。然而，有證據表明鐮孢菌酸能夠抑制多巴胺β-羥化酶酶活性。除了已知的毒性特性外，鐮孢菌酸還能抑制人體細胞增殖和 DNA 合成。然而，缺乏特異性單株抗體對鐮孢菌酸的檢測構成了防止鐮孢菌酸暴露對人體健康產生不良影響的重大挑戰。本研究開發了一種新穎且具有成本效益的方法，利用酵素免疫連結吸附法（ELISA）或基於適體的檢測方法準確檢測鐮孢菌酸水平，旨在防止人類受到鐮孢菌酸毒性的影響。為了生成對鐮孢菌酸具有高特異性的適體，我們首先利用鐮孢菌酸開發了親和管柱，然後使用 SELEX（系統進化配體的指數富集）配體系統從隨機 DNA 庫中選擇單股 DNA（ssDNA）分子。通過七輪迭代選擇步驟，確認出了高度特異性的鐮孢菌酸適體，並透過 ELISA 篩選出與鐮孢菌酸結合特異性最高的最佳候選物。為了更深入了解鐮孢菌酸適體的性質，使用 RNAfold 服務器預測了其二級結構，並選擇 DNA 參數預測選項。為了確認 ELISA 結果的準確性，還採用了西方墨點法作為補充檢測方法。我們已經成功利用適體建立出檢測方法，相比基於抗體的方法，它具有節省時間的優勢。我們未來的工作將集中在開發基於適體的檢測方法。

Abstract:

Fusaric acid (FA) is an ancient secondary metabolite produced by Fusarium species, including Fusarium oxysporum and Fusarium heterosporum. While extensive research has been conducted on this compound, the precise mechanism of action remains unclear. Nonetheless, evidence suggests that Fusaric acid is capable of inhibiting the activity of the dopamine beta-hydroxylase enzyme. In addition to its established toxic properties, fusaric acid (FA) has been found to exert inhibitory effects on cell proliferation and DNA synthesis in humans. However, the lack of a specific monoclonal antibody for FA detection presents a significant challenge in mitigating the adverse health effects of FA exposure. A novel and cost-effective method utilizing ELISA or aptamer-based assay has been developed in this study for accurate detection of FA levels, with the ultimate aim of preventing human suffering from FA toxicity. To generate aptamers with high specificity for fusaric acid, we first developed an affinity column using Fusaric acid, and subsequently employed the SELEX (systematic evolution of ligands by exponential enrichment) ligand system to select single-stranded DNA (ssDNA) molecules from a randomized DNA library. Through seven rounds of iterative selection steps, highly specific aptamers targeting FA were identified, and subsequently screened via ELISA to identify the optimal candidate with the highest binding specificity for FA. To gain further insights into the properties of the FA aptamer, its secondary structure was predicted using the RNAfold server with DNA parameter prediction options. To confirm the accuracy of the ELISA results, western blotting was employed as a complementary detection method. We have identified aptamers suitable for use in an aptamer-based assay, which offer a time-saving advantage compared to antibody-based assays. Our future efforts will be focused on developing aptamer-based assays, including FA detection strips, to enable rapid and sensitive detection of FA in various settings.

4.

開發針對Moniliformin的適體及其應用於基於適體的檢測方法的研究

Developing of an aptamer against moniliformin and its application to aptamer-based assay

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摘要:

Moniliformin是由不同鐮孢菌屬（Fusarium）菌株產生的一種真菌毒素，可以透過競爭性抑制呼吸途徑中的丙酮酸脫氫酶複合物，從而阻礙丙酮酸和糖解產物轉化為乙醯輔酶A，進而發揮其毒性作用。這種毒素常見於玉米和小麥等人類主要食物作物中，且最近的研究還顯示Moniliformin是一種致癌物質。為了降低Moniliformin的暴露風險並保護公眾健康，開發一種快速且具有成本效益的檢測方法至關重要。在文中，我們報告了一種基於選擇性進化（SELEX）的配體系統的Moniliformin檢測方法的開發。具體而言，我們通過化學衍生Moniliformin以增加其分子量並建立親和管柱。隨後，使用SELEX從隨機DNA庫中選擇了適體並識別為單股DNA分子。在進行了七輪的SELEX之後，識別出了兩個適體，Apt 4和Apt 5，它們對Moniliformin具有高度特異性，並且都能用作西方墨點法的探針。值得注意的是，Apt 5表現出最高的特異性並且與其他化合物幾乎不發生交叉反應。為了更深入地了解Moniliformin適體的性質，使用RNAfold服務器預測了Apt 5的二級結構。與基於抗體的方法相比，基於適體的檢測方法的開發可以更快地實現，為Moniliformin的檢測提供了一種替代方法。未來，我們的目標是開發更多基於適體的檢測方法，如ALISA或免疫層析試紙，以實現更便捷和可行的Moniliformin檢測。

Abstract:

Moniliformin is a mycotoxin produced by various Fusarium species that exerts its toxic effects by competitively inhibiting the pyruvate dehydrogenase complex in the respiratory pathway, thereby preventing the conversion of pyruvic acid and glycolytic products to acetyl CoA. This toxin can be commonly found in corn and wheat, which are critical staples in the human diet, and recent studies have also shown that Moniliformin is a carcinogen. To mitigate the risk of Moniliformin exposure and safeguard public health, it is crucial to develop a rapid and cost-effective detection method. Here, we report the development of an aptamer-based detection method for Moniliformin using a ligand system that employs systematic evolution of ligands by exponential enrichment (SELEX). Specifically, we chemically derivatized Moniliformin to increase its molecular weight and establish affinity columns. Subsequently, aptamers were selected from a randomized DNA library using SELEX and were identified as single-stranded DNA (ssDNA) molecules. Following seven rounds of SELEX, two aptamers, Apt 4 and Apt 5, were identified with high specificity for Moniliformin, and both were able to serve as probes for western blotting. Notably, Apt 5 exhibited the highest specificity and negligible cross-reactivity with other compounds. To gain further insight into the properties of the Moniliformin aptamer, the secondary structure of Apt 5 was predicted using the RNAfold server. Compared to antibody-based methods, the development of aptamer-based assays can be achieved more rapidly, providing an alternative approach for Moniliformin detection. In the future, we aim to develop additional aptamer-based assays such as ALISA or strips to enable even more convenient and feasible Moniliformin detection.