# 105 學年度第四屆

# 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

中華民國一百零六年五月二十四日

# 目錄

# 海報論文發表

105 年 5 月 24~25 日(星期三~四)正心 1 樓學務處前走廊(12:00~13:00 接受師生提問)

| 序號 | 海報講題 | 學生/指導教授 |
|---|---|---|
| 1 | 探討天然抗氧化合物在 PolyQ 斑馬魚和 N2a 細胞模式的作用<br>Investigate the effects of natural anti-oxidative compounds on the PolyQ zebrafish and N2a cell models | 林冠宇/潘惠錦 |
| 2 | 評估 Retigabine 和 ML213 衍生物保護心臟對抗心肌梗塞傷害的作用<br>The Cardiac Protective Effects of the Derivatives of Retigabine and ML213 onMyocardial Infarction | 張家誠/黃相碩 |
| 3 | Kv7 鉀離子通道開啟劑 #7638692 在於心肌缺血再灌注傷害的心臟保護作用<br>The cardioprotective effect of Kv7(KCNQ) potassium channel opener#7638692 on the myocardial ischemia/reperfusion injury | 劉沛勳/黃相碩 |
| 4 | 利用基因轉殖斑馬魚模式篩檢預防耳毒性藥物造成聽障的保護藥物<br>Utilize the Genetically Modified zebrafish as the model to screen the protective drug which can prevent the damage from aminoglycosides. | 林丞偉/楊建洲 |
| 5 | 利用斑馬魚為模式生物探討乳癌之分子機轉<br>Using zebrafish as a model for investigating the mechanism of breast cancer | 莊詠筌/楊建洲 |
| 6 | 在乳癌幹細胞中 Hsp90 藉由 ezh2/c-myc 使 bmi1 表現<br>Hsp90 involves in EZH2/c-myc mediated BMI1 expression in breast cancer stem cells | 陳怡穎/張文瑋 |
| 7 | 白藜蘆醇順式三甲醚(MR-3)增加人類雌激素受體陽性乳癌細胞對 Tamoxifen 敏感度之分析<br>3,5,4'-trimethoxystilbene (MR-3) sensitizes tamoxifen action in human estrogen receptor-positive breast cancer cells | 張慕亞/陳威仁 |

# 海報發表論文摘要

1

探討天然抗氧化合物在 PolyQ 斑馬魚和 N2a 細胞模式的作用

Investigate the effects of natural anti-oxidative compounds on the PolyQ zebrafish and N2a cell models

學生：林冠宇指導教授：潘惠錦

**Abstract：**

Polyglutamine (polyQ) diseases are inherited neurodegenerative disorders caused by an excessive elongation of a coding trinucleotide (CAG) repeat, which is translated into an abnormally elongated glutamine (Q) tract in the respective mutant proteins. It leads to protein misfolding and aggregation, altering the function of neuronal cells and eventually resulting in neurodegeneration. There are nine known polyQ diseases, including Huntington's disease (HD), spinal-bulbar muscular atrophy (SBMA), and six spinocerebellar ataxias (SCA 1, 2, 3, 6, 7, and 17). However, there is no treatment to prevent or slow the progression of the disease and only symptomatic treatments currently exist. We have established transgenic zebrafish and N2a cell lines expressing polyQ proteins. The polyQ zebrafish showed growth retardation, delayed hatching and increased mortality rate. The escape response and swimming pattern were also affected. In N2a cells, polyQ protein aggregations in the cytoplasm were observed under the fluorescence microscope and the expanded polyQ interrupts normal branching of neuronal processes. Previous studies showed that ROS was increased in cells expressing polyQ proteins. Therefore, we tested the effects of curcumin and resveratrol, both known as anti-oxidative compounds, in rescuing the phenotypes in the polyQ fish and cell models. After treating the Q110 fish with curcumin and resveratrol, the mortality rate, hatching rate, escapes response and swimming pattern were all improved significantly. In addition, both compounds partially revert the formation of neuronal processes and decreased ROS production, as measured by dihydroethidium (DHE) staining in Q200 N2a cells. Further analysis is underway to determine if both compounds could decrease polyQ protein aggregation. These results support that curcumin and resveratrol are beneficial and may be used as a prevention treatment for the progression of the polyQ diseases.

2

評估 Retigabine 和 ML213 衍生物保護心臟對抗心肌梗塞傷害的作用

The Cardiac Protective Effects of the Derivatives of Retigabine and ML213 on Myocardial Infarction

學生：張家誠指導教授：黃相碩

**Abstract：**

心肌梗塞是由於供應心臟組織的血管阻塞所造成的疾病，臨床上心肌梗塞的病人常會伴隨出現心律不整。缺血區域受損的心肌細胞會不正常去極化，造成心律不整。傳統上，阻斷鈉離子通道，鈣離子通道及鉀離子通道皆為心律不整的主要治療方式之一。然而，開啟鉀離子通道，導致細胞過極化，減少動作電位的發生，也可能可以減少放電異常，改善心律不整的發生。Retigabine和 ML213 是目前已知的KCNQ 鉀離子通道開啟劑，臨床上Retigabine已經用於治療癲癇，本實驗室以Retigabine與ML213 結構為基礎模板合成了38種乙苯胺醯衍生物，評估其開啟鉀離子通道的活性，研究發現相較於控制組#5540 可以使KCNQ 鉀離子電流明顯上升。本研究希望探討 Retigabine衍生物＃5540 是否具有改善心肌梗塞所誘發之心律不整的效果。

研究藉由將SD(Sprague Dawley)大鼠的冠狀動脈左降支(left main coronaryartery)結紮一小時，之後

進行恢復血液再灌流三小時，模擬心肌缺血再灌流之損傷。在梗塞傷害前十五分鐘預處理藥物(0.8μg/kg)，並連續監控動物之血壓及心跳。利用心電訊號判讀並評估心室早期收縮（Ventricular premature beat; VPC）、心室心搏過速(Ventricular tachycardia, VT)以及心室顫動(Ventricular fibrillation,VF)等心律不整指標。最後犧牲動物以TTC 染色評估梗塞大小。實驗結果顯示，＃5540 具有減少心臟缺血再灌流所造成之心肌梗塞的趨勢。

未來本計畫希望確認Retigabine衍生物＃5540 對於心肌缺血再灌流所引發之心律不整之作用，並進一步探討其分子機制，評估其成為臨床心臟保護劑之可能性。

3

Kv7 鉀離子通道開啟劑 #7638692 在於心肌缺血再灌注傷害的心臟保護作用

The cardioprotective effect of Kv7(KCNQ) potassium channel opener #7638692 on the myocardial ischemia/reperfusion injury

學生：劉沛勳指導教授：黃相碩

**Abstract：**

作用在電位依賴型鉀離子通道 Kv7(KCNQ)家族的鉀離子通道開啟劑具有穩定神經元細胞膜電位的作用，目前已知的 Kv7 鉀離子通道開啟劑為 ML213 與 Retigabine，臨床上 Retigabine 已經應用於治療癲癇、疼痛與心律不整。本實驗室以 ML213 與 Retigabine 結構為基礎模板合成了 38 種乙苯胺醯衍生物(相似物)，我們在 HEK293t 細胞株的研究發現其中 methyl{4-[(1-adamantylcarbonyl)amino]phenyl}carbamate (#7638692)具有活化 Kv7 蛋白的作用，可以促進鉀離子通道的開啟。基於#7638692 可以活化 Kv7 蛋白開啟鉀離子通道的作用，本研究藉由心肌缺血再灌流損傷(myocardial ischemia/reperfusion injury)的動物模式，評估#7638692 能否減少心肌缺血再灌流損傷所引起的心律不整，進而達到心臟保護的作用。研究使用 Sprague-Dawley (SD)大鼠，進行一小時左冠狀動脈前降支(the left anterior descending, LAD)結紮及三小時的再灌流，造成心肌缺血再灌流損傷，探討#7638692 是否具有心臟保護作用可以對抗心肌缺血再灌流損傷。

實驗在左冠狀動脈結紮前十五分鐘經靜脈投予#7638692 10μg/kg 或 100 μg/kg，初步研究結果發現相較於投予 0.1%DMSO 溶劑的對照組，投予#7638692 不會影響動物的心跳速率、平均血壓等血液動力學參數，然而投予 10 μg/kg#7638692 可以減少心肌缺血再灌流損傷引起的動物死亡率。此外，在抗心律不整的作用方面，研究發現投予 100 μg/kg#7638692 相較於對照組，可以減少 VT 和 VF 的發生率。然而，在於心肌缺血再灌流損傷造成的心肌梗塞區域(infarct size)大小，研究發現投予#7638692 相較於對照組並沒有達到統計上的差異。

4

利用基因轉殖斑馬魚模式篩檢預防耳毒性藥物造成聽障的保護藥物

Utilize the Genetically Modified zebrafish as the model to screen the protective drug which can prevent the damage from aminoglycosides.

學生：林丞偉指導教授：楊建洲

**Abstract：**

AGs Aminoglycosides are antibiotics which widely used to treat the infection by the gram-negative bacteria in clinical medicine.Because of the extraordinary features like effective antimicrobial activities and minimal drug-related allergies; however, the treatment of Aminoglycosides usually have many side effects, such as nephrotoxicity and permanent hearing impairment. Among these adverse effects, we will focus on the permanent hearing loss. The impairment of the inner hair cells may even cause the hearing loss when this condition aggravate worsen, resulting in *Ototoxicity*. The hair cells on the fish's lateral line are kind of mechanoreceptor which plays an important role on sensing the external water flows. Furthermore, there are many resemblances between the mammal's inner ear and the fish's lateral hair cell, no matter the morphology structure or characteristic function; therefore, this is the reason why we take zebrafish as an animal model in this experiment. Zebrafish is widely used on the trial of finding preventive drugs that potentially alleviate the ototoxicity of the aminoglycoside treatment. Previously, our laboratory have exploited the zebrafish Tg(*pvalb3b：TagGFP*) to see the protective effect of the drug Ferulic acid on ototoxic damage. Eventually we constructed and proved that Ferulic acid can protect the fish's lateral line from disrupting by neomycin. The Tol2 gene transfer system constructing with the specific promoter *pvalb3b* which fuse with *TagRFP* gene.This design would be utilized for establishing the red fluorscence protein expression hair cell in lateral system.The result is as same as the one established by the previous work. After the correct procedures being set up, then we find two drug, AICAR and AMPK respectively and conduct the ototoxic protection screening experiments with the zebrafish Tg(*pvalb3b：TagRFP*). Observed the fluorescence expression of their lateral line.Additionally, we find another two drugs Gallic acid and Cichoric acid through the access of the papers and other information. In the further experiment, we will utilize Tg(*pvalb3b：TagRFP*) zebrafish as an animal model to screen the effect on ototoxic protection.

**Methods**

The prevention on ototoxicity and the time sequence of the effective drug concentration was evaluated by fluorescence microscope and the total number of the hair cell from six spots are analyzed by GraphPad Prism 5.

**Result**

Neither AMPK nor AICAR showed any hair cells protection on the lateral line.However,we found that both Gallic acid and Cichoric acid manifest great protection on ototoxicity from the preliminary screening trial, thus we will focus on drug Gallic acid first, counting the total hair cells number from the six spots.and conduct the time sequence on gallic acids concentration to see which length of the time is the best option on further related experiments, Gallic acid showed the best protection on ototoxicity at 200μM concentration reacting for one hour.

**Conclusion**

We are looking forward to seek the drug which can protect the fish's lateral line from damaging by aminoglycosides. We also expect that one day the drug could go through a series of trials, like animal trial and human trial in future. Finally they can treat and cure human diseases and make a dedication on clinical application.

5

利用斑馬魚為模式生物探討乳癌之分子機轉

Using zebrafish as a model for investigating the mechanism of breast cancer

學生：莊詠筌指導教授：楊建洲

**Abstract:**

Breast cancer is one of the most prevalent cancers in female. Even though there are many diagnosis strategies and remedies have been implemented to cope with this disease, several obstacles still distress the scientists. After patients finish the surgery, the chemotherapy and radiotherapy will be practice in the further treatment. However, some cases show that the cancer cell could revitalize after the standard treatment and lead to the deterioration of the patient's situation. The further researches manifest that the property of resistant to therapy could contribute to the overexpression of Hsp27(Heat Shock Protein 27).

Hsp (Heat Shock Protein) family is the first kind of the chaperone protein been discovered. The expression of the Heat Shock Protein could associate with the ability of enduring the extracellular stresses in the cell. It could increase the stability of client protein through the folding consolidation mechanism. Other research also vindicates that the overexpression of the Heat Shock Protein has high correlation with the viability of the patient. The Hsp27 has the characteristic of anti-apoptosis, cell cycle regulation, and treatment resistance. The activation of downstream mechanism depends on the different type of the Hsp27 conformation (phosphorylation or non-phosphorylation). Moreover, Hsp27 also regulate cell motility and even induce angiogenesis. On the other hand, Hsp27 seems to be the upstream protein of Hsp90 which had been verified that it is related to the cancer stem cell formation. Accordingly, the investigation of Hsp27 is urgent and imperative.

The aim of theexperiment is to investigate the role of the Hsp27 that plays in the metastatic behavior. The vector of the Hsp27, Hsp27D (mimic phosphorylated), and Hsp27A (mimic non-phosphorylated) had been established,dispensed to the BT-474 and MDA-MB-231 breast cancer cell and injected into the zebrafish(3 days post-fertilization). Since the vector could also expresstRFP, the cancer cell which entered the zebrafish circulation will be observed and recorded though CHT (caudal homatopoitictissue) region with fluorescence microscope.

The experiment of BT-474 shows that the cell which expresses Hsp27D seems to have more aggressive property in metastasis. However, since the BT-474 cell line has inferior cancer characteristic, the pathologic phenomenon isn't enough significant for analysis. Thus, the further studies resort to use the MDA-MB-231 breast cancer cell. The fluorescenceintensity alteration of control group (MDA-MB-231) had been established chronologically from 1dpi (day post-injection) to 5dpi. Other samples will be compared with the control group in 1dpi to 3dpi for the metastasis assessment.

6

在乳癌幹細胞中 Hsp90 藉由 ezh2/c-myc 使 bmi1 表現

Hsp90 involves in EZH2/c-myc mediated BMI1 expression in breast cancer stem cells

學生：陳怡穎指導教授：張文瑋

**Abstract:**
Cancer stem cells (CSCs) are a subpopulation of cancer cells with self-renewal and differentiation capabilities and play an important in tumor initiation and metastasis.Thus,targetingCSCs is considered as a key forsuccessfulcancer therapy.Heat shock protein 90(Hsp90) is anintracellular chaperon protein to stabilizethe structure of its clients which include many oncogenes and is often overexpressed in cancers.17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17-DMAG) is aHsp90 inhibitor throughthe competitively binding to the ATP binding region of Hsp90.Our lab previously found that 17-DMAG could inhibit BMI1 expression through decreasing the binding of c-mycto BMI1 promoter in breast CSCs.Here we furtherdiscovered thatenhancer of zeste homolog 2 (EZH2), a histone methyltransferase, participatedin 17-DMAG induced downregulation of BMI1. We first observed that 17-DMAG treatment caused the downregulation of EZH2 in breast CSCs. The overexpression of EZH2 abolished the inhibitory effect of 17-DMAG in BMI1 expression. By chromatin immunoprecipitation assay, we found that EZH2 and c-myc formed as a complex binding to BMI1 promoter and the binding capacity was suppressed by 17-DMAG. Furthermore,Hsp90 complexed with EZH2/c-myc in nucleus of breast CSCs and the nuclear translocation of EZH2/c-myccould be inhibited by 17-DMAG treatment. Our data indicate that Hsp90 involves in EZH2/c-myc mediated BMI1 expression in breast CSCs. In the future, we will examine the involvement of Elk1 or NF-κB in 17-DMAG mediated downregulation of EZH2 in breast CSCs.

**7**

白藜蘆醇順式三甲醚(MR-3)增加人類雌激素受體陽性乳癌細胞對 Tamoxifen 敏感度之分析
3,5,4'-trimethoxystilbene (MR-3) sensitizes tamoxifen action in human estrogen receptor-positive breast cancer cells

學生：張慕亞指導教授：陳威仁

**Abstract:**
乳癌是女性常見癌症之一。而 Tamoxifen 是一種廣泛使用於乳癌治療的藥物，初期接受治療的乳癌患者雖然有顯著效果，但在治療後五年常出現抗藥性及高度的復發率。許多研究指出白藜蘆醇能夠恢復癌細胞對 tamoxifen 的敏感度，且有研究表明白藜蘆醇能透過調控 sirt1 來抑制轉錄功能和上皮-間質細胞轉換(epithelial-mesenchymal transition，EMT)，但其生物利用率低，影響其臨床用藥價值。3,5,4'-trimethoxystilbene (MR-3)為白藜蘆醇結構相近的甲基衍生物，具有相當於白藜蘆醇之生物活性且生物利用率較高。近期研究發現 MR-3 能透過抑制 PI3K/Akt/GSK-3β 訊號路徑之活化，來抑制乳癌細胞 EMT 轉換。而 EMT 轉換與雌激素受體陽性乳癌獲得 tamoxifen 抗性有關，故我們推測 MR-3 或許能增加雌激素受體陽性乳癌細胞對於 tamoxifen 治療的敏感性。為證明此點，我們將採用傷口癒合試驗、MTT assay 和西方點墨法探討 tamoxifen 配合 MR-3 處理是否增強 tamoxifen 抑制雌激素受體陽性乳癌細胞株 MCF-7 之生長並探討其作用途徑。初步研究結果中，不同濃度的 MR-3 處理會促使 MCF-7 乳癌細胞株 epithelial-like marker E-cadherin 表現逐步上升。傷口癒合試驗也觀察到 MR-3 處理後的 MCF-7 乳癌細胞株，其傷口癒合現象也會隨著 MR-3 濃度增加而有明顯的抑制。接著透過 MTT 分析，將不同濃度 MR-3 處理後的 MCF-7 細胞加入相同濃度的 tamoxifen，並與僅加入 tamoxifen 但未處理 MR-3 之 MCF-7 細胞進行比對，發現經 MR-3 處理過的 MCF-7 細胞存活率比未處理 MR-3 之 MCF-7 細胞存活率有顯著的降低。因此，我們推

論 MR-3 可增強 tamoxifen 抑制 MCF-7 乳癌細胞增生之效果。進一步地，我們將探索 PI3K/Akt/GSK-3β 訊號路徑之活性調節是否參與在 MR-3 促進 tamoxifen 細胞生長抑制作用之機制中。

# 口頭論文發表大會議程

106 年 5 月 26 日(星期五) 正心 0313 教室

| 9:50~10:00 主任致辭 | | |
|---|---|---|
| 時間 | 報告講題 | 學生/指導教授 |
| 10:00~10:20 | Klotho 抑制γ型干擾素誘導之 SAMHD1 表現於腎絲球系膜細胞中之研究<br>Klotho suppresses IFNγ- induced SAMHD1 expression in glomerular mesangial cells | 梁珊瑗/林庭慧 |
| 10:20~10:40 | 吲哚胺 2,3-雙加氧酶蛋白在子宮頸癌幹細胞表現的調節機制研究<br>The regulatory mechanism of indoleamine 2,3-dioxygenase 1 expression within cervical carcinoma stem-like cells | 陳裕益/張文瑋 |
| 10:40~11:00 | 乳癌細胞內熱休克蛋白 27 之磷酸化對於其參與之訊息傳遞網路的影響<br>The impact of Hsp27 phosphorylation in the maintenance of breast cancer stem cells | 張庭瑜/張文瑋 |
| 11:00~11:20 | 萊克多巴胺多株抗體之製備及其酵素連結免疫分析法之開發<br>Production of Polyclonal Antibody and Development of Enzyme-Linked Immunosorbent assay for Ractopamine | 鐘唯恆/余豐益 |
| 11:20~11:40 | 硫酸鎵誘導神經細胞 N2a 神經毒性路徑之研究<br>Mechanism of $Ga_2(SO_4)_3$ Induced Neurotoxicity inN2a Cells. | 梁清惠/楊建洲 |
| 11:40~12:00 休息(備茶點、飲品) | | |
| 12:10 頒獎 | | |

# 口頭報告論文摘要

1

Klotho 抑制γ型干擾素誘導之 SAMHD1 表現於腎絲球系膜細胞中之研究

Klotho suppresses IFNγ- induced SAMHD1 expression in glomerular mesangial cells

學生：梁珊瑗指導教授：林庭慧

**Abstract：**

SAMHD1 is a disease-related protein involving in restricting HIV-1 infection, Aicardi-Goutières syndrome, cerebral vasculopathy, cancer therapy and inflammaging. Expression of SAMHD1 has been identified in myeloid lineage cells and information regarding the responsiveness of SAMHD1 to proinflammatory cytokines in different immune cells is controversial. In kidney, SAMHD1 is expressed in cells of glomerulus but not tubules. Information regarding the function and underlying mechanisms of SAMHD1 gene regulation in kidney is meager. Klotho is an anti-aging protein exhibits multiple biological activities. Predominant expression of the Klotho protein in the kidneys was severely reduced in patients with chronic renal failure. The well-known functions of Klotho include suppression of oxidative stress, inhibition of insulin/IGF-1, WNT signaling, participation in Ca2+ and phosphate homeostasis. In the present study, the effect of LPS and IFN-γ on SAMHD1 and Klotho expression in glomerular mesangial cells was investigated. IFN-γ significantly upregulates SAMHD1 expression while LPS downregulates Klotho expression in glomerular mesangial cells in a concentration-dependent manner. IFN-γ-induced SAMHD1 expression was modulates via JAK-STAT and NF-κB signaling. LPS -suppressed Klotho expression was through NF-κB signaling. Pre-treatment of glomerular mesangial cells with recombinant Klotho suppresses IFN-γ-induced SAMHD1 expression through blocking NF-κB nuclear translocaton. Our results suggest the anti-aging protein Klotho may modulate inflammaging marker SAMHD1 through its inhibitory effect on NF-κB activation.

2

吲哚胺 2,3-雙加氧酶蛋白在子宮頸癌幹細胞表現的調節機制研究

The regulatory mechanism of indoleamine 2,3-dioxygenase 1 expression within cervical carcinoma stem-like cells

學生：陳裕益指導教授：張文瑋

**Abstract：**

Cervical cancer is a disease with a partial, slow and gradual progression and is the fourth common cancer in female around the world. According to the statistical data from the Department of Health and Statistics, the mortality of cervical cancer is ranked as tenth in Taiwanese female. There are evidences suggesting that cancer stem cells (CSCs) are responsible for the initiation, maintenance, drug resistance and metastasis of tumors and these particular cancer cells are considered as an effective therapeutic target. Indoleamine 2,3-dioxygenase 1 (IDO1) is a single chain oxidoreductase that catalyzes tryptophan metabolizing to kynurenine and plays a role in immunosuppressive microenvironment within cancers through diminishing T cell activation.Notch1 plays an important role in development and determination of cell fate. The recent study shows that when Notch1 overexpression in cancer cells will lead to decreasing radiation sensitivity. The purpose of this study is to explore the molecular mechanism in regulating of IDO1 expression in cervical CSCs. We first found that the IDO1, as well as Notch

intracellular domain (NICD) was increased in sphere cells by western blot analysis. Using the IDO1 activity assay, we also found that the IDO1 activity in total cell lysates was significantly increased in sphere cells than those of parental cells. Using Jurkat T cells as a model, the growth rate of T cells was significantly decreased in conditional medium after cervical cancer sphere cultivation when compared to those from parental adherent culture. In addition, IDO1 expression was suppressed in Notch 1 knockdown SiHa cervical cancer cells. Our data indicated that Notch1 signaling positively regulated IDO1 expression in cervical CSCs. In the future, we will examine if IDO1 inhibitor or knockdown of IDO1 causes enhancement of radiation sensitivity of cervical CSCs. We also plan to find out which soluble factors secreted by cervical CSCs is responsible for IDO1 induction. We hope these results will provide new insights in the development of immunotherapy in against cervical cancer.

3

乳癌細胞內熱休克蛋白 27 之磷酸化對於其參與之訊息傳遞網路的影響

The impact of Hsp27 phosphorylation in the maintenance of breast cancer stem cells

學生: 張庭瑜指導教授: 張文瑋

**Abstract：**

Heat Shock Protein 27(Hsp27) acts as a cellular chaperon to maintain stability of its client proteins. Hsp27 also functionsinthe cell migration and anti-apoptosis in cancer cells. Wepreviously found thatHsp27 and its phosphorylation were increased in breast cancer stem cells (BCSCs) and inhibition of Hsp27 expression suppressedthe tumorigeicity of these particular breast cancer cells. In addition, we also found that vasculogenic mimicry activity of BCSCs was mediatedby Hsp27 phosphorylation. In this study,we try to understand impactof Hsp27 phosphorylation in the maintenance of BCSCs. By overexpression of the phosphorylation mutant forms of Hsp27 (Hsp27A for phosphor dead form and Hsp27D for phosphor mimic form) in MDA-MB-231 or Hs578t breast cancer cells,the mammosphere formation was increased in Hsp27D overexpressed cells than wildtype ones and was decreased when overexpressed Hsp27A. By quantitative RT-PCR method, the expression of insulin-like growth factor receptor-1, Nanog and Oct4 was decreased in mammospheres from Hsp27A overexpressed MDA-MB-231 sphere cells but upregulated in Hsp27D ones. Through immunoprecipitation and mass spectrometry analysis, we found that the interaction with MeCP2, XRCC1 or UBAP2L was increased in Hsp27D cells but decreased in Hsp27A ones. In addition, we also found that the expression of XRCC1 in MDA-MB-231 cells was induced by overexpression of wildtype Hsp27 and Hsp27D, but not Hsp27A.In conclusion, Hsp27 regulates self-renewal of BCSCs through its phosphorylation and may involve the regulation of stemness genes or the interaction with XRCC1.

4

萊克多巴胺多株抗體之製備及其酵素連結免疫分析法之開發

Production of Polyclonal Antibody and Development of Enzyme-Linked Immunosorbent assay for Ractopamine

學生: 鐘唯恆指導教授: 余豐益

**Abstract：**

Background

Ractopamine (Rac), a synthetic beta-adrenergic agonist, first designed as a therapeutic drug for asthma. As a beta-agonist, Rac was also found to have the ability to promote animal weight gain and enhance leanness ratio of fat. In spite of advantage effects of Rac on livestock, it was still banned in many countries due to its adverse effects on cardiovascular and central nervous system of human.

Materials and methods

To generate the antibody specific to Rac, Rac was first derivatized with succinic anhydride (SH), and the result was confirmed by thin layer chromatography (TLC). After the confirmation of Rac-SH, bovine thyroid thyroglobulin (BTG) was conjugated to Rac-SH and BALB/c mice were immunized with Rac-SH-BTG by intraperitoneal injection. A competitive direct enzyme-linked immunosorbent assay (cdELISA) was developed based on the antibody describe above. The specificity and titer of Rac antibody were monitored via cdELISA every week.

Result

During the TLC confirmation, the result showed that there was shifts appeared comparing to the standard. The concentration causing 50% inhibition (IC50) of binding of Rac-horseradish peroxidase (Rac-HRP) to the antibody by Rac was calculated to be 22.7 ng/mL.

Conclusion

According to the result, a high sensitive polyclonal antibody based cdELISA for Rac was developed. For the further approach, monoclonal antibody of Rac is going to be generated using the hybridoma technique and the immunochormatographic strip (immunostrip) will be developed based on this monoclonal antibody.

5

硫酸鎵誘導神經細胞 N2a 神經毒性路徑之研究

Mechanism of $Ga_2(SO_4)_3$ Induced Neurotoxicity inN2a Cells.

學生：梁清惠指導教授：關宇翔、楊建洲

**Abstract：**

**Backgrounds**

Apart from the radio gallium (67 Ga) scan for the detection of cancers, gallium is used extensively in the electronics industry as a component of semiconductors, light emitting diodes, and solar energy applications. Recent reports on neurodegenerative diseases have shown that alterations in protein kinase expression and activity can modify the downstream activation of signaling proteins and trigger neuronal loss. N2a neuron cells have been used extensively to screen novel compounds for neurotoxic properties and associated mechanisms. Gallium compounds have shown therapeutic activity in cancers, infections, and inflammatory conditions, but the mechanism of neurotoxicity still unclear. The aim of this paper is to estimate the neurotoxicity of semiconductor metals $Ga_2(SO_4)_3$ in neuron cells.

**Materials & methods**

The cytotoxicity was evaluated by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The induction of DNA damage and apoptosis were analyzed by reactive oxygen species

(ROS) activity kits and flow cytometry and caspase activity assay kits. The results of anti-oxidative enzymes were analyzed by superoxidase dismutase (SOD) activity kits and catalase activity kits and glutathione detection kits. The intracellular molecules of $Ga_2(SO_4)_3$inducing DNA damage and apoptosis in N2a cells wereanalyzed by western blot assay andreal-time PCR assay.

**Results**

At present, the results were shown that $Ga_2(SO_4)_3$ induced cytotoxicity in N2a cells. We also demonstrated that $Ga_2(SO_4)_3$induced cytotoxicity might be via generation of ROS and activation of caspase-3, -8 and -9. Therefore, in this study, we further demonstrated that $Ga_2(SO_4)_3$ enhances the phosphorylation of p38 MAPK, JNK and p53 via generation of ROS. There is a decrease in mitochondria membrane potential via regulation of Bcl-2 family, causing release of cytochrome c then combined with apaf-1 and caspase-9 to form an apoptosome, followed by activating caspase-3 and cleaved PARP-1 which resulted in apoptosis. We also showed that AIF translocated into nuclear and cause DNA damage. On the other hand, we found that $Ga_2(SO_4)_3$ induced ROS production triggers HO-1 expression by activating Nrf-2.

**Conclusion**：

The studies demonstrated that pretreatment of $Ga_2(SO_4)_3$ induced cytotoxicity and DNA damage of N2a neuron cells via up-regulation of ROS generation through activated the caspase pathway. On the other hand, $Ga_2(SO_4)_3$ could also lead to antioxidant defense by up-regulating HO-1 expression by activating Nrf-2.