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研究杭白菊水萃物的抗發炎效果

得獎獎項

醫學與健康科學科大會獎第三名

候補作品

學校名稱： 臺北市立第一女子高級中學

作者姓名： 邱薰頡　邱薰儀

指導老師： 江伯倫　薛如娟

關 鍵 字： 杭白菊、抗發炎、巨噬細胞

作者簡介

我是邱薰頡，有個雙胞胎妹妹，很高興從小玩在一起的我們上了高中仍舊能一同進行此實驗。若要自我介紹，可能又要和我的partner 重複了！但我也是個喜歡科學的好奇寶寶，喜歡生物的多樣性，也喜歡物理的真與單一。唱歌是我們兩個在課餘時間最常一同度過的消遣，也懷念背著吉他一起上學的好時光。

這一次我們將再用菊花煮出延續高二的好味道！

(左邊是我)

我是邱薰儀，現在就讀北一女中三年級，喜歡唱歌、畫圖，高一時曾經參加過吉他社，對吉他有些許的認識。 在學校選的是生物科專研，喜歡進入生物的世界，也很喜歡科學這大殿堂。 喜歡看網球比賽，希望能利用暑假好好學網球。

媽媽常說西藥治標、中藥治本，我很喜歡中藥那醇厚的味道，或許這也是我為什麼樂於研究此實驗吧！和我的partner 雙胞胎姊姊一同煮著實驗所需的樣品，聞著菊花的香味總令我們樂此不疲呢！

摘要

在炎炎夏日總希望來點可以「降火氣」的食品，依據古籍記載某些中藥材具有降火氣的效果，而中醫所謂的降火氣與西醫的抗發炎反應密切相關。本實驗利用巨噬細胞株受到 LPS (Lipopolysaccharide)刺激後，會分泌發炎介質的現象，再加入傳統降火氣食品----菊花(杭白菊 Dendranthema morifolium)，來評估菊花對巨噬細胞分泌發炎介質能力的影響。

經由實驗，我們發現水萃菊花在適當的濃度下，具有降低 IL-6 和 TNF-α 分泌量的能力，但濃度過高時會使細胞凋亡。 在訊息傳導部分，由於菊花水萃物會阻礙 LPS 訊息傳導過程中對 NF-κB(nuclear factor -κB)的活化而造成下游 cytokine 濃度的下降，因此推論其訊息傳導經由 NF-κB。 另外，我們以微矩陣進行基因分析，比較加入水萃菊花與否對活化之巨噬細胞的影響：發現水萃菊花對大部分的發炎基因有抑制現象，對細胞移動的能力大幅上升，對癌細胞基因有抑制作用。建立在此實驗基礎上，未來可將菊花水萃物應用於抗發炎藥物甚至是抗癌研發之用。

Abstract

According to ancient books, there are some Chinese herbal medicines that were recorded to have the function of cooling us down, which is related to anti-inflammation in the Western. Chrysanthemum tea, a simple drink, is usually taken to refresh ourselves. In our experiment, we use LPS-stimulated macrophage cell lines to test the anti-inflammatory effect of one kind of chrysanthemum---- Dendranthema morifolium(Dm).

In our results, we find Dm which is poached in ancient ways indeed has the ability to show the low dose of IL-6 and TNF-α in appropriate consistency. Luciferase assay indicates that water extracts from Dm may interact with LPS or its receptor and then block the LPS signal transduction pathway of NF-κB activation. We also examined the effects of Dm on macrophages (RAW264.7) with macroarray analysis by Affrymetrix MOE 430_2 GeneChip. In comparing mean signal values between Dm-treated LPS-activated-RAW264.7 with Dm-untreated LPS-activated-RAW264.7, 86 probe sets were up-regulated, and 177 probe sets were down-regulated. These results demonstrate that most of genes associated with inflammation (IL11ra1, IL11ra2, CXCL1, MAPK8, FLT1, PGF, CAMK2D, MALT1, CEBPB, ARF6, TNFAIP3, FAS, DTX2, MYC) were decreased. Genes associated with cellular movement (H3F3B, and etc.) were highly increased and genes associated with neuregulin signal (NRG3, and etc.) were decreased. Genes associated with cancer and cellular proliferation were mostly decreased. Based on the experiment, extracts from Dm can be applied to anti-inflammatory medicine or even the cure of cancers.

Study on the Anti-inflammatory Effect ofExtracts from Dendranthema morifolium

研究杭白菊水萃物的抗發炎效果

一、背景介紹

(一) 研究動機

在酷熱的暑假裡，總希望來點清涼降火的飲品，品嘗同時也萌生好奇，何謂「降火氣」？食用這些食品是否真能產生『降火氣』的效果？而降火氣在西醫上又與什麼生理機制有關？經過搜尋與查閱相關資料後，我們發現中醫的「火氣」理論由來已久，老祖宗早就發現：上火會使人有嘴巴破、睡不好、心神不寧等症狀。而相對於中醫有關『火氣』的說法，似乎又與西醫的『發炎反應』，有部分的相關性。因此我們便想利用傳統中醫藥，或一般食材中可以清涼降火的食品，來進行相關實驗以評估『降火氣』與『發炎反應』間的關係，最後決定以菊花來做為我們的實驗樣品。

(二) 研究目的

本實驗是利用巨噬細胞株受到 LPS 刺激後會分泌發炎介質的現象，再加入降火氣樣品杭白菊，來評估杭白菊水萃物對巨噬細胞分泌發炎介質能力的影響。 後以 Western blot、Luciferase assay 來推論菊花抗發炎的訊息傳導途徑，並以 Microarray 進行基因分析。

(三) 樣本介紹---杭白菊（Dendranthema morifolium）

1. 菊科(Chrysanthemum)植物，別名有菊華、秋菊、九華、黃花、帝女花、笑靨金、節花。

2. 菊花除可觀賞外也有很高的藥用價值。最為常見的是沖泡「菊花茶」。菊花茶分類主要有：黃山的貢菊，杭州的杭白菊以及山東的野菊花.。本次實驗採用台東地區產的杭白菊 (在後續的實驗中，我們將菊花簡稱為 Dm.，如圖 1 所示)。

圖 1

3. 用途：有的作為藥用或作消暑清涼飲料，如滁菊、杭白菊等，有清涼鎮靜的功效，治頭痛、眩暈、血壓亢進，神經性頭痛及眼結膜炎等症。

(四)偵測指標：生成的介質物

1. IL-6(Interleukin-6)：主要由巨噬細胞和 T 細胞所分泌，可刺激 T 細胞和 B 細胞的增生分化，和急性蛋白質增加，為感染時早期發炎反應 (acute phase reaction)的重要細胞激素。

2. TNF-α(Tumor necrosis factor-alpha)：主要由巨噬細胞和自然殺手細胞所分泌，可活化上皮細胞，以分泌黏附分子吸引淋巴球至感染部位，並增加上皮細胞通透性，是引起局部發炎感染時的早期發炎反應的重要細胞激素。

(五)對照組----Naltrexone :

Naltrexone 是一種鴉片樣受體的拮抗劑 (opioid receptor antagonist)，可降低病患對酒精與鴉片的依賴性，近期研究指出其具有抗發炎的效用。

(六)LPS (Lipopolysaccharide)

是革蘭式陰性菌(Gram-negative bacteria)表面所含有的酯多醣體，可活化巨噬細胞並引發生物體的發炎反應。

(七)Nuclear Factor – kappa B (NF-κ B)

NF-κB 是種轉錄因子，也是細胞受到刺激或感染後產生免疫反應的重要調控角色。 而 Inhibitor of Nuclear Factor(I-κB)可阻礙 NF-κ B 與特定 DNA sequences 的結合，而無法進行轉錄轉譯產生蛋白質。

二、研究方法與過程

(一)細胞培養RAW264.7

RAW264.7 老鼠巨噬細胞株 (購自 ATCC)，以 DMEM+ 10% FBS complete medium 培養。

(二) 配製研究Sample

市售之杭白菊花乾10.00 g+水200 mL 煮沸騰後小火煮 20 分鐘後，以 filter 0.25 μm過濾，得上清液保存於 - 20℃。並測得菊花乾重為7.70 g。並將(10.00-7.70 g)/ 200 mL取為基本濃度，後續實驗濃度以此為基礎稀釋換算得之。

(三)實驗方法

1. 評估 RAW264.7 細胞受到 LPS 刺激後分泌發炎物質的能力與 LPS 刺激的最適當濃度

(1) 取 RAW264.7 細胞調成10^5個/well ，seeding 於 Flat 96 well plate 100 μL/well

(2) LPS stock 1mg/ml，分別稀釋成 4 μg/ml, 1 μg/ml, 400 ng/ml, 200 ng/ml，50 μL/well → final conc. 分別為 1 μg/ml, 250 ng/ml, 100 ng/ml, 50 ng/ml

(3) 每個 well 補 50 μL medium

(4) 另外加入已知具有抗發炎效用的Naltrexone 100 μg/ml作為對照組，50 μL/well

(5) Incubation 37 ℃，over night。

(6) 收 supernatant，凍 -20 ℃，之後以 ELISA 測 IL-6, TNF-α。

2. 分析樣品對於受 LPS 刺激後之RAW264.7分泌發炎物質能力的影響

(1) 取 RAW264.7 細胞調成10^5 個/well，seeding 於 Flat 96 well plate 100 μL/well

(2) LPS stock 1mg/ml，稀釋成 1 μg/ml，50 μL/well →final conc. 為 250 ng/ml

(3) 加入樣品：將菊花水萃物分別稀釋使 final conc. 為 1.44 μg/ml、720 ng/ml、360 ng/ml、180 ng/ml、90 ng/ml，50 μL/well

(4) 以已知具有抗發炎效用的Naltrexone 100μg/ml作為對照組，50 μL/well

(5) Incubation 37 ℃，over night。

(6) 收 supernatant，凍 -20 ℃，之後以 ELISA 測 IL-6, TNF-α。

3. 測試經樣品處理後細胞的存活率

(1) 上述實驗，將 supernatant 收集完成後，將每 well 殘餘液體吸乾。

(2) 加入 MTT，以 medium 稀釋五倍。

(3) Incubation 37 ℃，3 小時。

(4) 吸出MTT，加入 100 μL/well DMSO。

(5) Read OD570。

(6) 算出 unknown/LPS only 的比例。

4. 促發炎細胞激素量的測定 (Sandwich ELISA)

(1) 先在 96well ELISA 微量盤上 coating 100μL/well 溶於 PBS 的 capture antibody (IL-6 和 TNF-α)，置於室溫下 overnight

(2) 以 washing buffer 清洗三次後，加入 300μL/well blocking buffer，於室溫下反應至少一小時。

(3) 以 washing buffer 清洗四次後，於各槽 loading 上清液 sample 或 standard 100μL/well，於室溫下反應 2 小時。

(4) 以 washing buffer 清洗五次後，加入以 biotin 連接的 detection antibody (IL-6 和 TNF-α) 100μL/well，室溫下反應 2 個小時。

(5) 以 washing buffer 清洗六次後，加入連接 avidin 的過氧化酶 100μL/well (要稀釋 200 倍)(要避光)，於室溫下反應 30 分鐘。

(6) 以 washing buffer 清洗八次後，加入 100μL/well TMB 基質溶液，進行呈色反應。

(7) 以 ELISA reader 偵測 450nm 吸光值，進而得到細胞激素量。

5. Detection of IκB-α activity(Western Blot)

(1) 取 RAW264.7 細胞調成10^6個/well，seeding 於 Flat 6 well plate 1000 μL/well

(2) LPS stock 1mg/ml，稀釋成 1 μg/ml，500 μL/well →final conc. 為 250 ng/ml

(3) 加入樣品： 將菊花水萃物分別稀釋使 final conc. 為360 ng/ml、180 ng/ml，500 μL/well

(4) Incubation 37 ℃，over night。

(5) 收 protein，加入 Gold lyse buffer，-20℃ 使其作用，之後以超音波震盪機打破細胞。

(6) 以 BCA reagent kit 作蛋白質定量。

(7) SDS-polyacrylamide 電泳分析。

(8) Transfer 至 PVDF membrane 上 (4℃，1hr，400mA)

(9) 以牛奶 blocking 1hr，room temperature。

(10) 先後加入一、二抗 1hr，之間和最後以 TBST wash 3 次，10 min/per。

(11) 加入顯影劑並且壓片。

6. Detection of NF-κB promoter activity(Luciferase assay)

(1) 前一天先取 RAW264.7 細胞 10^5 個/well，seeding 於 Flat 6 well plate，DMEM (含 10％FBS)

(2) 吸掉 complete DMEM，加入 DNA(RL-TK 0.1μg/well、3κB 0.3μg/well)、Opti-MEM (不含血清)、Lipofectamine，共 200 μl /well。

(3) incubation 37℃，2~24 hr。

(4) 再加入 400μl /well DMEM for transient transfection 24~72hr。

(5) 吸乾 well，加入樣品，incubation 24hr。

(6) 加入稀釋 2 倍的 Dual-Glo luciferase buffer，10 min，吸到白盤測 firefly 冷光值。

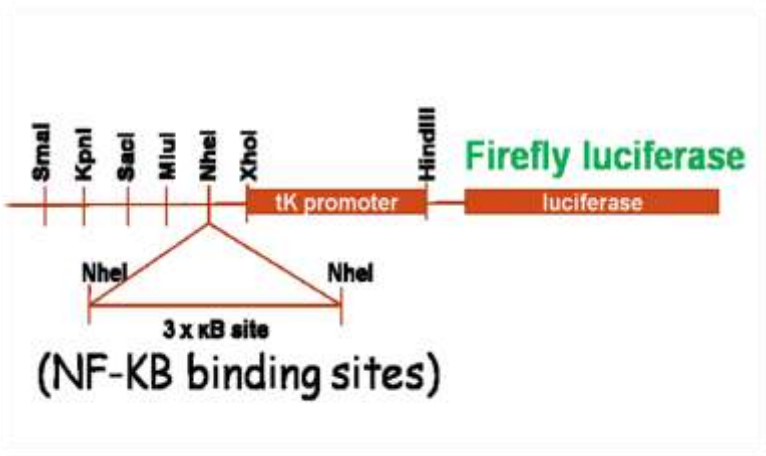
(7) 先配 stop substrate 1/100 + Stop&Glo buffer。

(8) 吸出 55µl，加入 55µl 的 stop&Glo buffer，10 min，測 renilla 冷光值。

(9) 以 relative ratio 分析數據

令 A=每個 well 的 (firefly luminescence) / (renilla luminescence)

B=(sample's A) / (positive control's A) 即為所求，如圖 2 所示。



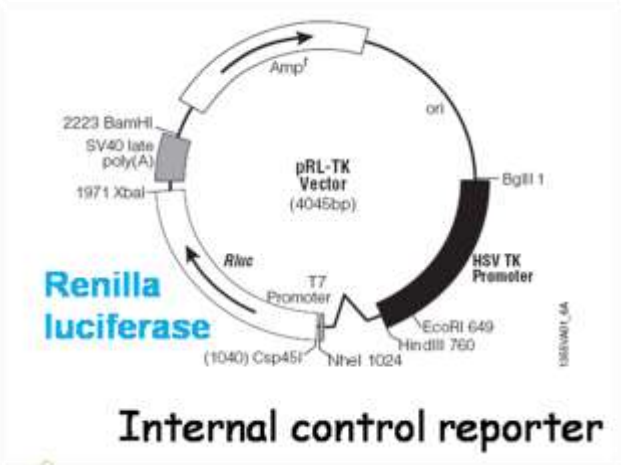


圖 2

7. 以 Microarray Hybridization 分析基因

(1) 以 LPS 250 ng/ml、菊花水萃物 360 ng/ml 刺激 RAW264.7 細胞，incubation 37 ℃，24 hr。

(2) 收取細胞胚，用 HBSS buffer將培養細胞的血清培養溶液（DMEM with FBS）清洗乾淨，再加入TRIzol Reagent將細胞溶碎並有效地使蛋白質變性（denature），之後分別加入酚(phenol)及氯仿(chloroform)將DNA 及蛋白質溶入有機層(organic phase），RNA 則會留在水層（aqueous phase）。離心後小心將水層部分分離並加入異丙醇（isopropanol）將RNA 沉澱出來（precipitation），再經過酒精清洗步驟後，即可得到可供DNA 微陣列分析用的RNA。最後用RNA 分析儀(Bioanalyzer®）來檢測所萃取出total RNA 的品質。

(3) 送至台大基因體中心微矩陣實驗室進行 array：

晶片類別：Affrymetrix MOE 430_2

以 Ingenuity Pathway Analysis 分析我們所篩選基因得到的 data。

三、研究結果與討論

(一) 評估 RAW264.7 細胞受到 LPS 刺激後分泌發炎物質的能力與 LPS 刺激的最適當濃度

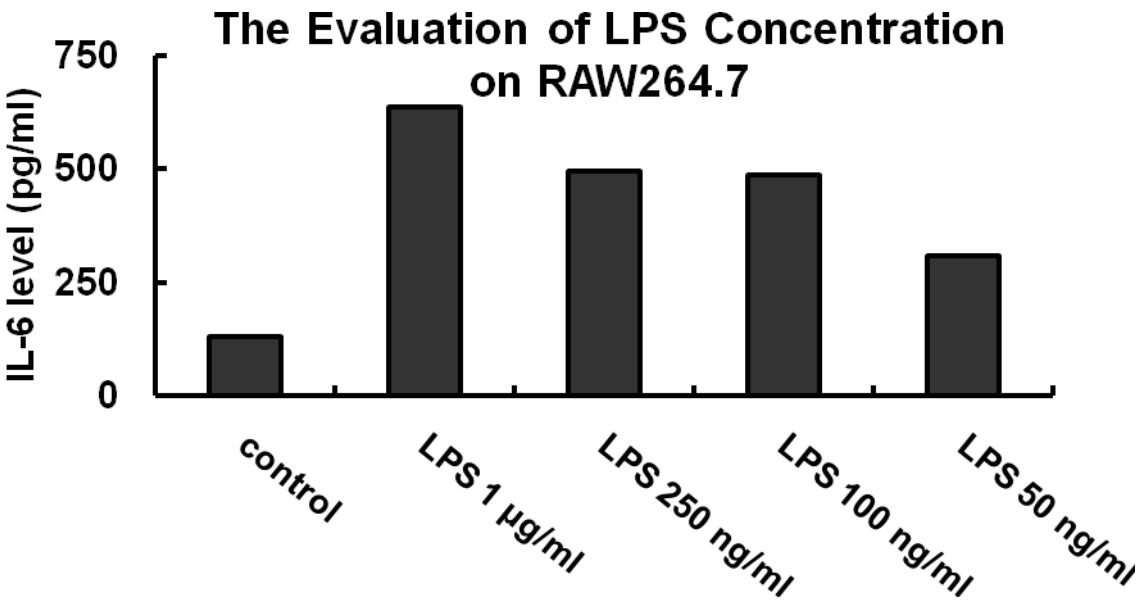


圖 3

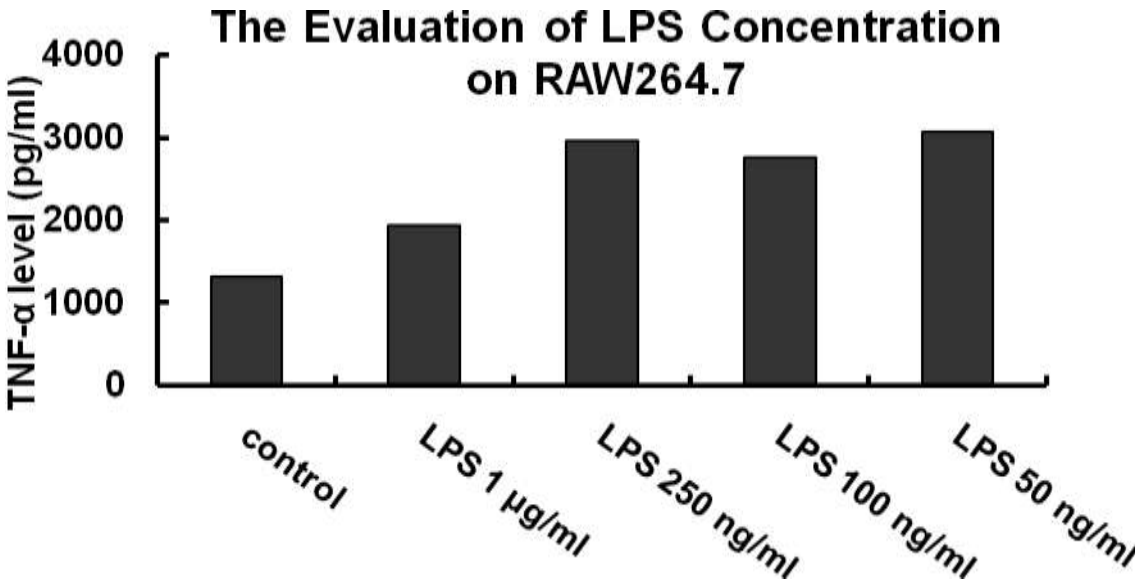


圖 4

以上(如圖 3，圖 4 所示)綜合不同濃度之 LPS 對細胞分泌 cytokine 的影響，我們選用 LPS 250 ng/ml 進行後續實驗。

(二)測試經樣品處理後細胞的存活率

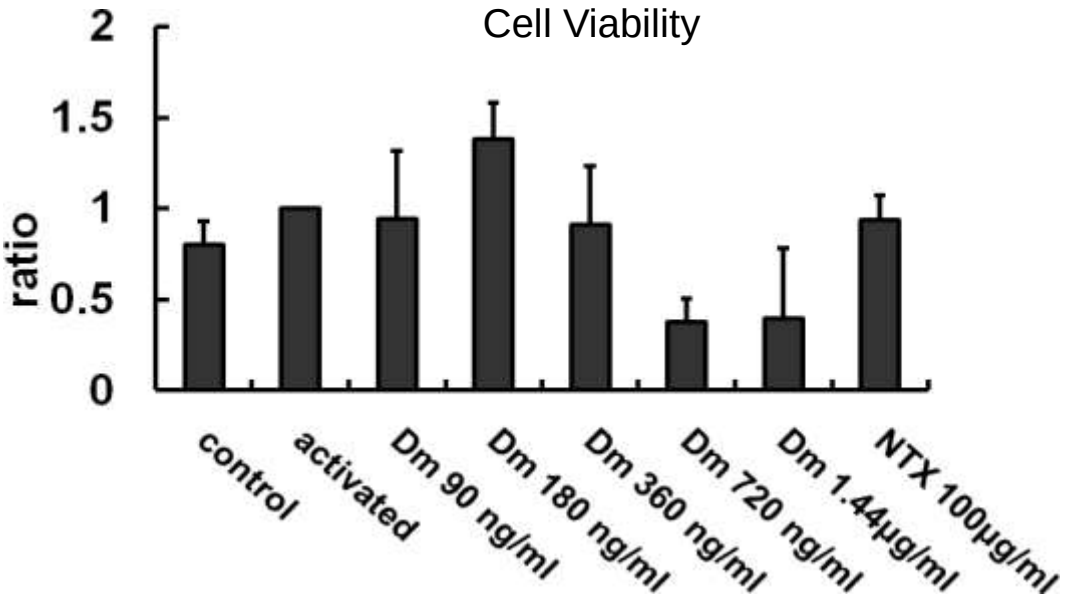


圖 5

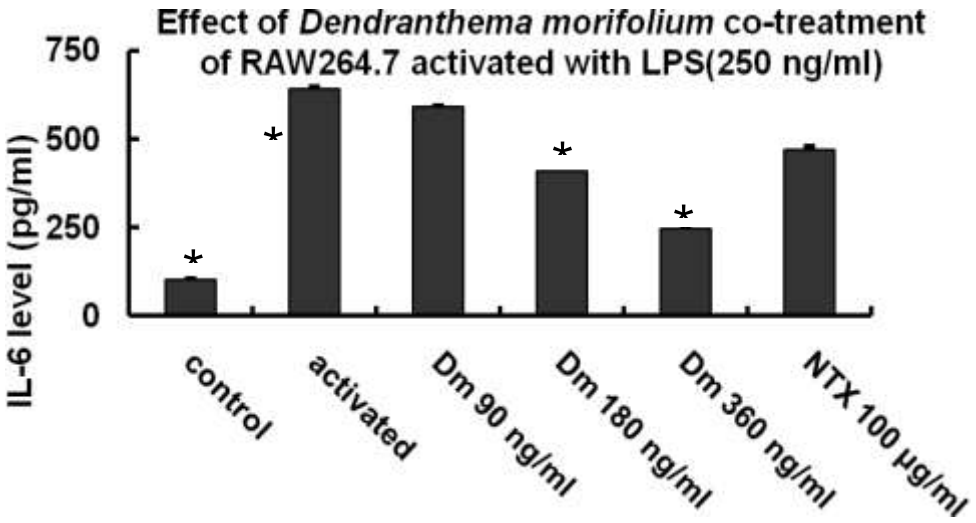
如圖 5 所示濃度太高的樣品會造成細胞的死亡，故探討樣品對於受 LPS 刺激後所產生的抑制效果時，sample 1.44 μg/ml, 720 ng/ml 不予探討。

(三)分析樣品對於受 LPS 刺激後之 RAW264.7 分泌發炎物質能力的影響(如圖 6，圖 7 所示)

1.在 IL-6 的表現上，菊花水萃物在 360 ng/ml、180 ng/ml 下，有良好抑制 IL-6 分泌的效果，90 ng/ml 效果較不顯著。

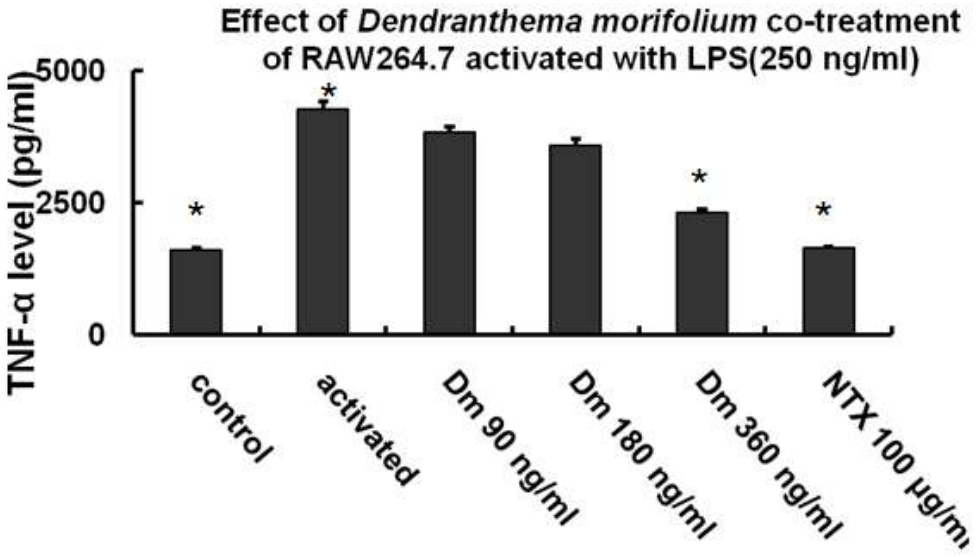
2.在 TNF-α 的表現上，菊花水萃物在 360 ng/ml 下，有良好抑制 TNF-α 分泌的效果，180 ng/ml、90 ng/ml 效果較不顯著。

3.故由圖 6，圖 7 顯示：菊花水萃物對 cytokine IL-6、TNF-α 表現呈現一致性，皆有抑制其分泌的能力。



(*表 $p<0.05$)

圖 6



(*表 $p<0.05$)

圖 7

(四)Detection of IκB-α activity(Western Blot)

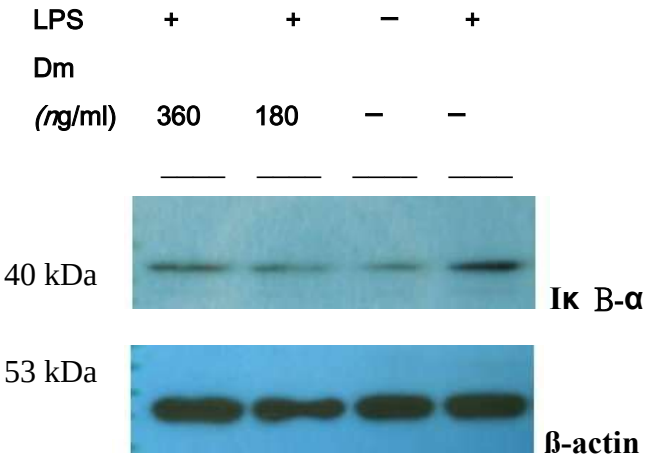


圖 8

以上如圖 8 所示表現經三次相同實驗皆有相同結果。在 I-κB 的表現上，positive control 和 negative control 的結果與理論不符，推斷是因為訊息傳導多不超過數小時，刺激 24hr 可能使蛋白質的磷酸化效果回復，而與理論狀況不符。因此我們改以人工添加 DNA 的方式，以 Luciferase assay 來測定並評估 NF-κB 在細胞受刺激後的表現。

(五) 將 positive control 的 relative ratio 定為標準 1，由實驗中可知細胞經樣品處理之後，NF-κB 的表現下降，推論樣品進行抗發炎的反應途徑會經由 NF-κB。(如圖 9 所示)

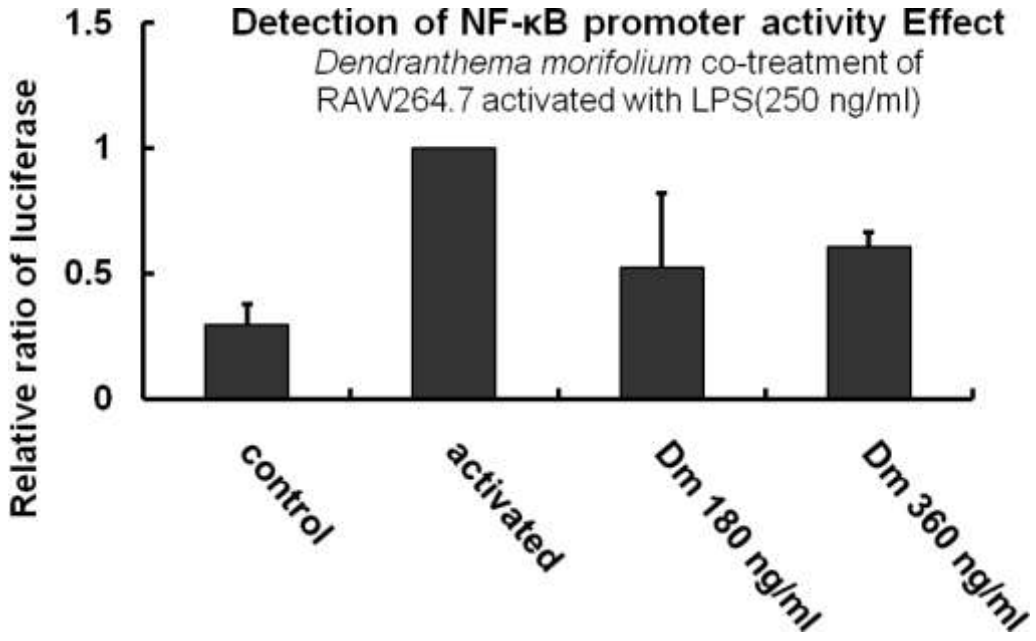


圖 9

(六)以 Microarray Hybridization 分析基因

Changes in gene expression were analyzed using the Affrymetrix MOE 430_2 GeneChip, which contains ~45081 probe sets. Comparing mean signal values between Dm-treated LPS-activated-RAW264.7 with Dm-untreated LPS-activated-RAW264.7, 86 probe sets were up-regulated (significantly more than 2-fold), and 177 probe sets were down-regulated (significantly more than 2-fold).

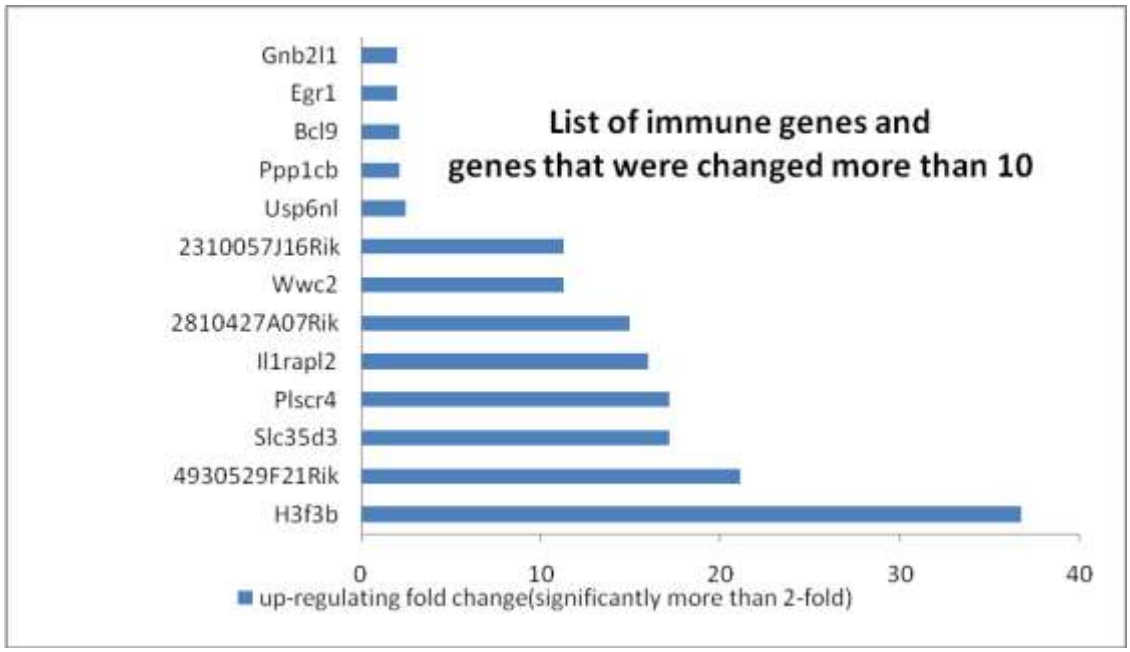


Figure10.

Figure10. We list the genes that were changed more than 10 and some genes which were related to immune system.

1. The highest is H3F3B (H3 histone, family 3B). Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes. We inferred that extracts from Dm may help the cellular development and restoration after inflammation.

2. Slc35d3 (solute carrier family 35, member D3), encoding an orphan nucleotide sugar transporter, regulates platelet-dense granules(Chintala S, et al.) Thus, we inferred that extracts from Dm may help the restoration after inflammation.

3. Plscr4 (phospholipid scramblase 4): Phospholipid scramblase is a multiply palmitoylated, calcium-binding endofacial membrane protein proposed to mediate transbilayer movement of plasma membrane phospholipids. A study indicated Plasma membrane phospholipid scramblase 1 promotes EGF-dependent activation of c-Src through the epidermal growth factor receptor(_Nanjundan M, Sun J, Zhao J, et al. 2003).

We infered extracts from Dm are likely to cellular growth.

4. Wwc(WW,C2 and coiled-coil domain containing 2) didn't have related study.

5. Genes related to immune system are IL1rapl2(interleukin 1 receptor accessory protein-like 2)、Usp6nl(USP6 N-terminal like)、Ppp1cb(protein phosphatase 1, catalytic subunit, beta isoform)、Bcl9(B-cell CLL/lymphoma 9)、Egr1(early growth response 1)、Gnb2l1(Guanine nucleotide binding protein (G protein), beta polypeptide 2 like).In the table1, we list pathways and related molecules of immune system.

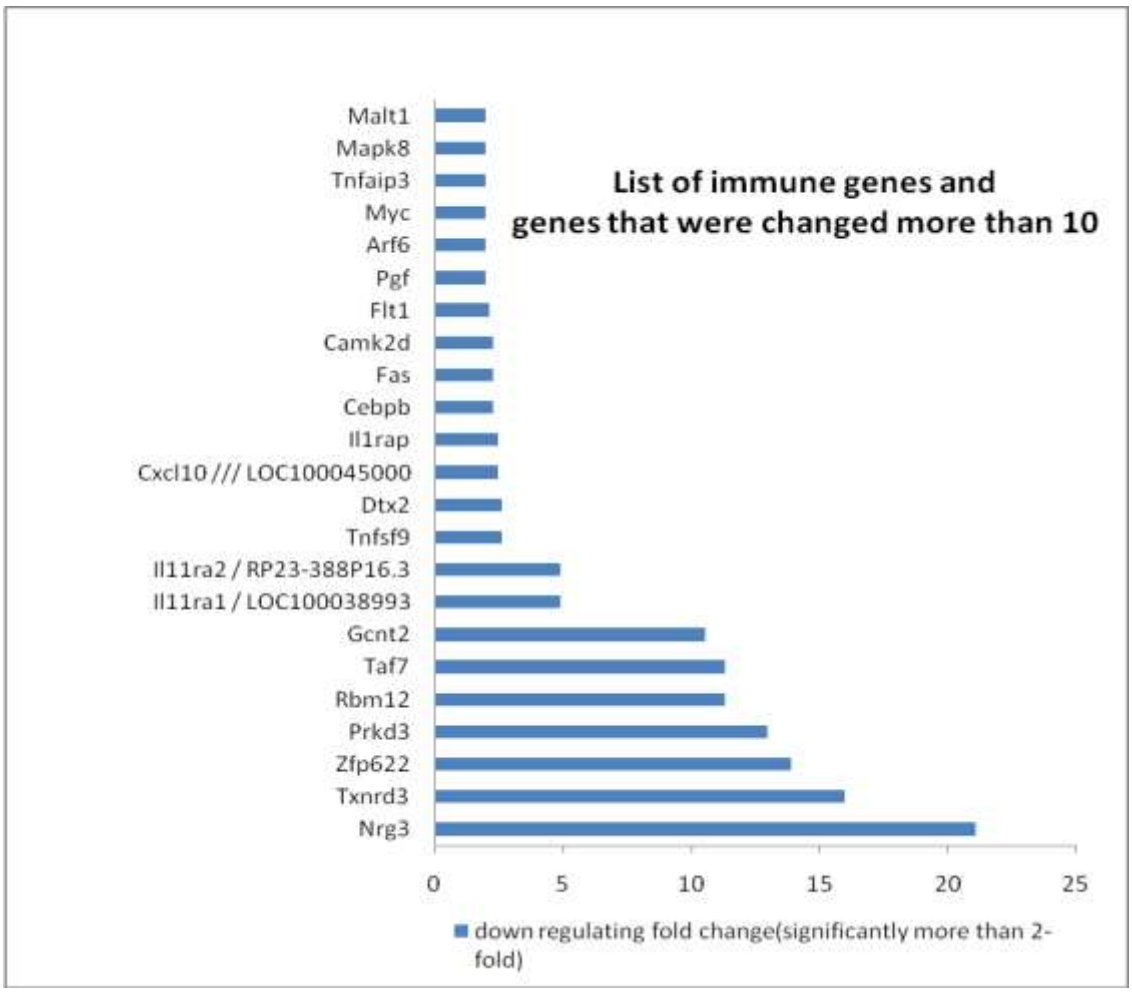


Figure11.

Figure11. We list the genes that were changed more than 10 and some genes which were related to immune system.

1. The highest is Nrg3 (neuregulin 3), a family of four structurally-related proteins that are part of the EGF family of proteins. And EGF(Epidermal growth factor) is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to its receptor EGFR. We infered extracts from Dm may block the neuregulin signal pathway. It may help to cure cancer, inflammation and so on.

2. About genes of Txnrd3(thioredoxin reductase 3), Zfp622(zinc finger protein 622), Prkd3(protein kinase D3), Rbml2(RNA binding motif protein 12), Taf7(TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor), Gcnt2(Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme) are decreased. We inferred it has function on the cell proliferations. Some cells, such as cancer cells, decreased and then apoptosis increased. Thus, extracts from Dm has the potential to cure cancers.

3. Genes related to immune system are Il11ra1 /// Il11ra2 /// LOC100038993 /// RP23-388P16.3 (interleukin 11 receptor, alpha chain 1 /// interleukin 11 receptor, alpha chain 2 /// similar to interleukin 11)Tnfsf9 (tumor necrosis factor (ligand) superfamily, member 9), Dtx2 (deltex 2 homolog (Drosophila)), Cxcl10 /// LOC100045000 (chemokine (C-X-C motif) ligand 10 /// similar to Small inducible cytokine B10 precursor (CXCL10) (Interferon-gamma-induced protein CRG-2) (Gamma-IP10) (IP-10) (C7)), Il1rap(interleukin 1 receptor accessory protein), Cebpb (CCAAT/enhancer binding protein (C/EBP), beta), Fas (TNF receptor superfamily member 6), Camk2d (calcium/calmodulin-dependent protein kinase II, delta), Flt1 (FMS-like tyrosine kinase

1), Pgf (placental growth factor), Arf6 (ADP-ribosylation factor 6), Myc (myelocytomatosis oncogene), Tnfaip3 (tumor necrosis factor, alpha-induced protein 3), Mapk8 (mitogen-activated protein kinase 8), Malt1 (mucosa associated lymphoid tissue lymphoma translocation gene 1)

In the Table1, we list pathways and related molecules of immune system.

Table 1. List of pathway and molecules of immune system and others.

Pathway (immune system)	Molecules
IL-8 Signaling	CXCL1 ↓ , MAPK8 ↓ , FLT1 ↓ , GNB2L1 ↑ , PGF ↓
Chemokine Signaling	MAPK8 ↓ , PPP1CB ↑ , CAMK2D ↓
B Cell Receptor Signaling	MALT1 ↓ , MAPK8 ↓ , CAMK2D ↓ , EGR1 ↑
Acute Phase Response Signaling	IL1RAP ↑ , MAPK8 ↓ , SAA2, CEBPB ↓
Macropinocytosis	ARF6 ↓ , USP6NL ↑
NF-κB Signaling	MALT1 ↓ , TNFAIP3 ↓ , MAPK8 ↓
Apoptosis Signaling	FAS ↓ , MAPK8 ↓
IL-6 Signaling//IL-12 Signaling and Production in Macrophages	MAPK8 ↓ , CEBPB ↓
T Cell Receptor Signaling	MALT1 ↓ , MAPK8 ↓
Notch Signaling	DTX2 ↓
IL-2 Signaling// IL-10 Signaling//TGF-β Signaling	MAPK8 ↓
GM-CSF Signaling	CAMK2D ↓ , GNB2L1
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	ARF6 ↓
ERK/MAPK Signaling	MYC↓, H3F3B(↑36.75), PPP1CB↑
Pathway (other)	Molecules
Neuregulin Signaling	Nrg3 ↓

Table 2. Categories and Molecules in Network.

Molecules in Network	Score	Focus Molecules	Top Functions
ADM, C8, CFLAR, CTH, CXCL1, DTL, EGR1, ERK, ERK1/2, F3, FAS, FLT1, GCLM, hCG, LCN2, LDL, Mek, Nfat, NGF, NRG3, P38 MAPK, PGF, Pkg, PTTG1, RBL1, RCAN1, RGS3, SAA2, SAA@, SIRT1, SP4, TCR, TNFAIP3, TSC22D3, Vegf	41	22	Cellular Growth and Proliferation, Cell Cycle, Cellular Development
Adaptor protein 2, AFP, Ap1, Caspase, CD36, CXCL10, Cyclin A, Cyclin E, DLEU2, E2f, FUBP1, GCNT2, H3F3B, HDAC9, IFI202B, IFN Beta, Ige, IL12, IL1RAP, Interferon alpha, LDLRAP1, MALT1, METAP2 (includes EG:10988), MYC, NFkB, PNRC1, Ras, Rb, RGS16, Rxr, SCD2, SF3B1, Thyroid hormone receptor, TNFSF9, USP6NL	36	20	Cellular Movement, Hair and Skin Development and Function, Gene Expression
ACBD3, ALS2CR2, amino acids, ANKRD11, BOC, CCNF, CDON, DDIT4, DUSP2, DUSP8, DUSP10, EIF2S1, GCLM, glutathione, hydrogen peroxide, IL11RA, MAP3K6, MAP4K2, MOBKL1B, PAFAH1B2, platelet activating factor-C16, PLK3, PPP1CB, PRKACB, PRKAR1A, PRKD3, PROCR, PSKH1, PTGER4, SLC7A11, SRC, STAT3, STK38L, TRAF6, TXNRD3	23	15	Amino Acid Metabolism, Post-Translational Modification, Small Molecule Biochemistry

continued on next page

Table 2,continued

AHR, Ahr-aryl hydrocarbon-RelA, ATPIF1, E2F8, EXOC1, EXOC2, EXOC3, EXOC4, EXOC5, EXOC6, FLCN, FSH, HNRNPM, HSPA8, METTL2B, MYC, NFKBIL1, NFYB, NQO1, Pka, PNN, PPP1CB, PPP1R7, PPP1R9A, PURB, RBM4B, RNPC3, RNPS1, RPL41, SERBP1, SMAD2, TMPO, TUBA8, WDR33, ZFR	22	14	Cancer, Dermatological Diseases and Conditions, Gene Expression
Actin, Akt, ALP, APLP2, Calmodulin, Cbp/p300, CEBPB, Creb, ESD, G alphai, GNB2L1, Gsk3, HABP4, Hsp70, IL1, Insulin, Jnk, Mapk, MAPK8, MARCKS (includes EG:4082), NET1, NFE2L2, NQO1, PABPC1, Pdgf, PDGF BB, PI3K, Pkc(s), PLC gamma, PP2A, Proteasome, RNA polymerase II, SFRS7, SLC7A11, Tgf beta	20	13	Gene Expression, Developmental Disorder, Neurological Disease
ARMC8, ATPase, C15ORF29, CAMK2D, CAP1, CCDC94, CFL2, CRADD, E2F4, EXOSC5, FARSB, GTF2H4, HIST1H3D, HIST2H4A, HNF4A, MYH1, MYH7, NEDD8, NFE2L1, RAD51, RIC8A, RPS18, RRM1, RSF1, RUVBL2, SENP8, SERPINB8, SFPQ, SMARCA5, SNRPA, STAM, STAU2, TYMS, UBQLN1, UXT	20	13	Cell Cycle, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair

continued on next page

Table 2,continued

ARF6, ATP5A1, ATP6V1C1, CALU, CPSF1, EEF1E1, EHMT2, FOS, GIGYF2, HGS, HIST2H2BF, LSM12, LYN, MLL, NMT1, RBBP6 (includes EG:19647), RBM12, RNASEL, SETD7, SFRS10, SPHK2, SUV39H2, TAF4, TAF5, TAF7, TASP1, TBC1D4, TP53, TRIM3, Ubiquitin, VHL, WWC2, YWHAZ, YY1, YY2 (includes EG:404281)	20	13	Gene Expression, Cell Cycle, Cellular Assembly and Organization
ACSL1, ADAMTSL4, APOA1, beta-estradiol, C9ORF80, cholesterol, Cytochrome c, DYNC1LI2, ECT2, GPX3, GREB1, ICOSLG, MAFK, MPEG1, MYBL2, NARF, NEUROG2, PARD6A, PARD6B, PARD6G, PNMA1, PTEN, RASSF2, RHOA, RHOQ, RND3, SP4, SPRY2, STK11, STK16, TARS, TRIB2, TSPO, ZNF292, ZNF622	20	13	Cell Morphology, Cellular Development, Cancer
ABI2, ADAM19 (includes EG:8728), ANGPTL4, ASB13, BCAT2, BCL9, CDC42SE1, dihydrotestosterone, DTX2, ERBB2, ESPL1, G6PD, G6PD2, GPX3, H3F3A, Histone h3, HOXA2, ID2, KIAA1609, MBD1, MGMT, MXI1, MYBL1, NET1, PAX8, PLSCR4, S100A4, SLCO3A1, SRCAP (includes EG:10847), TGFB1, TMEM2, TRIP13, TRPM7, UGDH, WNT3A	18	12	Cancer, Cellular Growth and Proliferation, Tumor Morphology
C1ORF63, CLK3	2	1	Amino Acid Metabolism, Post-Translational Modification, Small Molecule Biochemistry
KIAA1543, TUBB3	2	1	Cancer, Cell Death, Respiratory Disease
CCDC127, RNF11	2	1	Gene Expression, Post-Translational Modification, Protein Degradation

Continued on next page

Table 2,continued

CTSG, SERPINB1B	2	1	Cancer, Cardiovascular Disease, Cell-To-Cell Signaling and Interaction
EFTUD1, GTPase Protein-synthesizing	2	1	
ATP8B4, Mg2+-ATPase	2	1	
CA5B, Carbonic anhydrase	2	1	Genetic Disorder, Infectious Disease, Inflammatory Disease
SEC13, SEC16A, SEC16B	2	1	Cell Morphology, Cellular Assembly and Organization
KIAA1333, LST1, WDR6 (includes EG:11180)	2	1	Cellular Growth and Proliferation, Hematological System Development and Function, Immune Response
DNMT3A, DNMT3B, DNMT3L, KCNQ1OT1	2	1	DNA Replication, Recombination, and Repair, Gene Expression, Viral Function

四、結論

藉由 ELISA 分析，可以得知杭白菊水萃物對於受到 LPS 活化的巨噬細胞株具有抗發炎反應，其表現可由 cytokine IL-6 及 TNF-α 分泌量減少了解。為進一步確定其反應途徑，以 Luciferase assay 來測定並評估 NF-κB 在細胞受刺激後的表現，明顯看出細胞經樣品處理後，NF-κB 的表現量降低，推論菊花水萃物會阻礙 LPS 訊息傳導過程中對 NF-κB 的活化而造成下游 cytokine 濃度的下降。藉由 Microarray 比較加入樣品與未加入樣品的活化巨噬細胞，在大部份的 immune genes 呈現下降表現(IL-6 signal & NFκB signal 的表現，與先前的實驗得到一致性)，另外在細胞生長部分，有抑制癌細胞基因、促使細胞凋亡、延緩神經系統、加強正常細胞生長的功能。

五、應用與展望

(一) 此階段是以 RAW264.7(BALB/c 小鼠單核細胞經病毒誘發之巨噬細胞株) 做實驗，但對於人體之巨噬細胞的效用仍不確定。因此可進一步討論對於人類之巨噬細胞的效果，探討樣品對於小鼠和人類細胞發炎介質物的異同，來評估菊花水萃物抗發炎的潛力。

(二) 參考文獻顯示：酒萃菊花的有效抗發炎物質為 Flavonoid(類黃酮類)，但以水萃方式得到的有效成分為何則需再以 HPLC 或 TLC 等方法進一步探討。

(三) 關於菊花中抗發炎的有效化合物對於細胞進行抗發炎的完整反應途徑值得進一步探討。

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Supplement Data

Table 3. List of up-regulation of comparing Dm-treated LPS-activated-RAW264.7 with Dm-untreated LPS-activated-RAW264.7.(significantly changed more than 2-fold).

RefSeq Protein ID	Gene Title	Fold change
NP_032237.1	H3 histone, family 3B	36.75835
---	Transcribed locus	29.85706
---	RIKEN cDNA 4930529F21 gene	21.11213
---	Transcribed locus	18.37917
NP_083805.1	solute carrier family 35, member D3	17.14838
NP_848826.1	phospholipid scramblase 4	17.14838
NP_109613.1	interleukin 1 receptor accessory protein-like 2	16
---	RIKEN cDNA 2810427A07 gene	14.92853
---	---	12.99604
NP_598552.2	WW, C2 and coiled-coil domain containing 2	11.31371
NP_081447.1	RIKEN cDNA 2310057J16 gene	11.31371
---	---	11.31371
---	RIKEN cDNA 4930435M08 gene	9.849155
---	---	9.849155
NP_001074736.1	remodeling and spacing factor 1 /// RIKEN cDNA 4832420A03 gene	9.189587
NP_083141.2	RIKEN cDNA 1110005A03 gene	8
NP_032732.3	NAD(P)H dehydrogenase, quinone 1	5.278032
NP_742048.2	transmembrane and coiled coil domains 3	5.278032
---	---	4.924578
NP_064385.1	C-type lectin domain family 4, member n	4.287094
---	deleted in lymphocytic leukemia, 2	4.287094
NP_444424.1	resistance to inhibitors of cholinesterase 8 homolog (C. elegans)	4.287094
NP_705756.1	GRAM domain containing 1C	4.287094
NP_080244.1	leukotriene B4 12-hydroxydehydrogenase	4
NP_081686.1	RIKEN cDNA 2210010N04 gene	4

continued on next page

Table 3,continued

NP_663755.2	oocyte specific homeobox 5	4
---	---	4
---	---	4
---	KCNQ1 overlapping transcript 1	3.732132
NP_064385.1	C-type lectin domain family 4, member n	3.482202
NP_766640.1	serine (or cysteine) peptidase inhibitor, clade B, member 1b	3.031433
NP_038917.2 /// XP_620009.2 /// XP_914840.3	zinc finger protein 292	3.031433
NP_001095925.1 /// NP_001095926.1 /// NP_033821.1	amyloid beta (A4) precursor-like protein 2	2.828427
---	RIKEN cDNA 5730405O12 gene	2.828427
---	---	2.828427
NP_036120.1	solute carrier family 7 (cationic amino acid transporter, y+ system), member 11	2.639016
NP_076092.1	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	2.639016
NP_058599.1	esterase D/formylglutathione hydrolase	2.639016
---	RIKEN cDNA 4632427E13 gene	2.639016
NP_766155.2	Methyltransferase like 2	2.639016
---	Transcribed locus	2.639016
NP_082114.1	SUMO/sentrin specific peptidase 8	2.639016
NP_032155.1	glutamate-cysteine ligase , modifier subunit	2.462289
NP_077163.1	coiled-coil domain containing 127	2.462289
XP_620381.2 /// XP_913145.2	RIKEN cDNA B230216G23 gene	2.462289
NP_001074017.1 /// NP_852064.2	USP6 N-terminal like	2.462289
---	---	2.462289
---	DNA segment, Chr 3, ERATO Doi 162, expressed	2.462289

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Table 3,continued

NP_001104741.1 /// NP_001104742.1 /// NP_079579.1	staufen (RNA binding protein) homolog 2 (Drosophila)	2.297397
NP_783597.2	histone cluster 2, H2bb	2.297397
---	deleted in lymphocytic leukemia, 2	2.297397
NP_598438.1	RIKEN cDNA 2310061J03 gene	2.297397
NP_033166.1	chemokine (C-X-C motif) ligand 2	2.297397
NP_032088.1 /// NP_062341.1	glucose-6-phosphate dehydrogenase 2 /// glucose-6-phosphate dehydrogenase X-linked	2.143547
NP_038576.1	histone cluster 1, H3f	2.143547
NP_766295.2	protein phosphatase 1, catalytic subunit, beta isoform	2.143547
NP_083216.1	retinaldehyde binding protein 1-like 1	2.143547
NP_573488.1	acyl-Coenzyme A binding domain containing 3	2.143547
---	RIKEN cDNA 4930592A05 gene	2.143547
NP_775608.1	protein serine kinase H1	2.143547
---	RIKEN cDNA 2310009A05 gene	2.143547
---	Transcribed locus	2.143547
NP_031620.1 /// NP_908942.1	calumenin	2.143547
---	Transcribed locus	2.143547
NP_084209.3	B-cell CLL/lymphoma 9	2.143547
NP_950200.1	asparagine-linked glycosylation 8 homolog (yeast, alpha-1,3-glucosyltransferase)	2.143547
NP_001074848.1	Ankyrin repeat domain 11	2.143547
NP_033154.1	stearoyl-Coenzyme A desaturase 2	2
NP_031449.3	alpha fetoprotein	2
NP_031939.1	early growth response 1	2
NP_031531.1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	2
NP_035301.1	protein C receptor, endothelial	2
NP_034951.1	macrophage expressed gene 1	2
XP_895327.1 /// XP_913324.1	leucine rich repeat containing 51	2

continued on next page

Table 3,continued

NP_035941.2	phenylalanyl-tRNA synthetase, beta subunit	2
NP_780562.2	exocyst complex component 6	2
NP_031669.2	CD36 antigen	2
NP_780562.2	exocyst complex component 6	2
---	RIKEN cDNA 2210403K04 gene	2
---	RIKEN cDNA 2310009A05 gene	2
NP_849256.1	NOL1/NOP2/Sun domain family 3	2
NP_032169.1	Guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1	2
---	RIKEN cDNA D330040H18 gene	2
---	---	2
NP_082278.1	dihydrouridine synthase 4-like (S. cerevisiae)	2
	WD repeat domain 89	2

Table 4. List of down-regulation of comparing Dm-treated LPS-activated-RAW264.7 with Dm-untreated LPS-activated-RAW264.7.(significantly changed more than 2-fold).

RefSeq Protein ID	Gene Title	Fold change
---	Transcribed locus	24.25147
NP_032760.1	neuregulin 3	21.11213
NP_694802.2	thioredoxin reductase 3	16
NP_653106.1	zinc finger protein 622	13.92881
NP_083515.2	protein kinase D3	12.99604
NP_083673.3 /// NP_733486.2	RNA binding motif protein 12	11.31371
NP_036031.1 /// NP_786964.1	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor	11.31371
NP_032131.2 /// NP_076376.3 /// NP_573482.1	Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	10.55606
NP_062622.1	methionine aminopeptidase 2	9.849155

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Table 4,continued

---	RIKEN cDNA 4930432O09 gene	9.189587
NP_079993.2	RNA binding motif protein 4B	8
---	---	8
NP_032564.1	myristoylated alanine rich protein kinase C substrate	7.464264
NP_081608.1 /// NP_081647.2	brix domain containing 5	6.498019
NP_084461.1	zinc finger, CCHC domain containing 4	6.498019
NP_001078984.1 /// XP_001476989.1 /// XP_283952.1	expressed sequence AI464131	6.062866
NP_056579.2	gene regulated by estrogen in breast cancer protein	5.656854
NP_898935.1	RIKEN cDNA 1700029I15 gene	5.278032
---	---	4.924578
NP_001092818.1 /// NP_001094066.1 /// NP_034679.1 /// NP_034680.2 /// XP_001472104.1 /// XP_001478667.1	interleukin 11 receptor, alpha chain 1 /// interleukin 11 receptor, alpha chain 2 /// similar to interleukin 11 receptor, alpha chain 2 /// interleukin 11 receptor, alpha chain 2-like	4.924578
NP_067384.1	par-6 (partitioning defective 6) homolog beta (C. elegans)	4
NP_780526.1	elongation factor Tu GTP binding domain containing 1	4
NP_940816.1	ORAI calcium release-activated calcium modulator 3	4
---	Transcribed locus	3.732132
---	---	3.482202
---	Transcribed locus	3.482202
---	---	3.482202
NP_149066.1	DNA segment, Chr 17, human D6S56E 5	3.24901
NP_067384.1	par-6 (partitioning defective 6) homolog beta (C. elegans)	3.24901

continued on next page

Table 4,continued

NP_663529.2	low density lipoprotein receptor adaptor protein 1	3.24901
NP_001013386.2	E2F transcription factor 8	3.24901
NP_766282.1	multiple EGF-like-domains 9	3.24901
NP_666130.1	folliculin	3.031433
---	Transcribed locus	3.031433
NP_149066.1	DNA segment, Chr 17, human D6S56E 5	2.828427
NP_001070832.1 /// NP_034416.3	TSC22 domain family 3	2.828427
NP_766094.1	biregional cell adhesion molecule-related/down-regulated by oncogenes (Cdon) binding protein	2.828427
NP_032517.1	lipocalin 2	2.828427
NP_038622.2	methyl-CpG binding domain protein 1	2.828427
NP_076154.3	DNA segment, Chr 4, Wayne State University 53, expressed	2.828427
NP_203505.2	SEC16 homolog B (S. cerevisiae)	2.828427
NP_031660.3	cyclin F	2.828427
---	---	2.828427
NP_035032.1	nuclear factor, erythroid derived 2, like 2	2.828427
NP_032564.1	myristoylated alanine rich protein kinase C substrate	2.639016
NP_034301.3	coagulation factor III	2.639016
NP_001092818.1 /// NP_001094066.1 /// NP_034679.1 /// NP_034680.2 /// XP_001472104.1 /// XP_001478667.1	interleukin 11 receptor, alpha chain 1 /// interleukin 11 receptor, alpha chain 2 /// similar to interleukin 11 receptor, alpha chain 2 /// interleukin 11 receptor, alpha chain 2-like	2.639016
NP_031714.1	cofilin 2, muscle	2.639016
NP_033430.1	tumor necrosis factor (ligand) superfamily, member 9	2.639016
NP_035379.1	retinoblastoma-like 1 (p107)	2.639016
---	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)	2.639016

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Table 4,continued

NP_083359.1	DNA-damage-inducible transcript 4	2.639016
NP_001028397.2	proline-rich nuclear receptor coactivator 1	2.639016
NP_076231.1	deltex 2 homolog (Drosophila)	2.639016
NP_032800.2	poly A binding protein, cytoplasmic 1	2.639016
NP_766535.1	LSM12 homolog (S. cerevisiae)	2.639016
NP_001075426.1	predicted gene, OTTMUSG00000000971	2.639016
---	Transcribed locus	2.639016
---	---	2.639016
---	---	2.639016
NP_067249.1 /// XP_001473379.1	chemokine (C-X-C motif) ligand 10 /// similar to Small inducible cytokine B10 precursor (CXCL10) (Interferon-gamma-induced protein CRG-2) (Gamma-IP10) (IP-10) (C7)	2.462289
NP_056605.1	icos ligand	2.462289
NP_034626.1	inhibitor of DNA binding 2	2.462289
NP_032390.1 /// NP_598864.1	interleukin 1 receptor accessory protein	2.462289
NP_659148.2	ADAMTS-like 4	2.462289
NP_001073598.1 /// NP_001073599.1 /// NP_001073600.1 /// NP_001073601.1 /// NP_001073603.1 /// NP_035735.2	thymopoietin	2.462289
NP_077038.2	histone deacetylase 9	2.462289
---	Transcribed locus	2.462289
---	Transcribed locus	2.462289
---	RIKEN cDNA C130057M05 gene	2.462289
NP_033757.1	adrenomedullin	2.297397
NP_038945.1	pituitary tumor-transforming 1	2.297397
NP_666065.1	cystathionase (cystathionine gamma-lyase)	2.297397
NP_036012.1	ribonuclease L (2', 5'-oligoisoadenylate synthetase-dependent)	2.297397
NP_034013.1	CCAAT/enhancer binding protein (C/EBP), beta	2.297397

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Table 4,continued

NP_849200.1	tubulin tyrosine ligase-like 1	2.297397
NP_035445.1	serum amyloid A 3	2.297397
NP_038622.2	methyl-CpG binding domain protein 1	2.297397
NP_083142.2	WD repeat domain 33	2.297397
NP_032013.1	Fas (TNF receptor superfamily member 6)	2.297397
NP_001074549.1	PHD finger protein 3	2.297397
---	melanoma antigen family F, 1	2.297397
NP_001038978.2 /// XP_001475048.1 /// XP_001479527.1	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	2.297397
---	---	2.297397
NP_064370.2	hyaluronic acid binding protein 4	2.297397
NP_001074413.2	ATPase, class I, type 8B, member 4	2.297397
---	Transcribed locus	2.297397
---	---	2.297397
NP_082251.2	RIKEN cDNA 2310007D09 gene	2.297397
NP_705778.1	disrupted in renal carcinoma 2 (human)	2.297397
NP_001015099.1	RIKEN cDNA 6030408C04 gene	2.297397
NP_084042.1	denticleless homolog (Drosophila)	2.297397
---	Transcribed locus	2.297397
NP_001020609.1 /// NP_001020610.1 /// NP_076302.1	calcium/calmodulin-dependent protein kinase II, delta	2.297397
---	Transcribed locus	2.297397
NP_937760.1	abl-interactor 2	2.297397
---	---	2.297397
NP_001074848.1 /// XP_899618.1	ankyrin repeat domain 11 /// hypothetical protein LOC629623	2.297397
NP_076244.2	transcription factor CP2-like 1	2.143547
NP_112456.2	splicing factor 3b, subunit 1	2.143547
NP_062786.1	sirtuin 1 (silent mating type information regulation 2, homolog) 1 (S. cerevisiae)	2.143547
NP_032353.2 /// NP_036070.2	interferon activated gene 202B	2.143547

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Table 4,continued

NP_033848.1	neurogenin 2	2.143547
NP_032741.2	integrator complex subunit 6	2.143547
NP_001028931.1 /// NP_114386.3	transmembrane protein 2	2.143547
NP_780434.1	taspase, threonine aspartase 1	2.143547
NP_035377.2 /// NP_778188.1	retinoblastoma binding protein 6	2.143547
NP_035397.1	regulator of G-protein signaling 16	2.143547
NP_780654.1	Ras association (RalGDS/AF-6) domain family member 2	2.143547
NP_112442.2 /// XP_923629.1 /// XP_930845.1	heat shock protein 8 /// hypothetical LOC624853 /// similar to heat shock protein 8 /// similar to heat shock protein 70 cognate	2.143547
NP_034626.1	inhibitor of DNA binding 2	2.143547
NP_543133.1 /// NP_840068.1	ankyrin repeat and SOCS box-containing protein 13	2.143547
NP_035397.1	regulator of G-protein signaling 16	2.143547
NP_001013595.1	RIKEN cDNA 1110054O05 gene	2.143547
NP_001093579.1 /// XP_001479109.1 /// XP_981485.1	signal recognition particle 54b	2.143547
NP_001038978.2 /// XP_001475048.1 /// XP_001479527.1	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	2.143547
---	RIKEN cDNA C330006P03 gene	2.143547
---	Transcribed locus	2.143547
---	Transcribed locus	2.143547
NP_034358.2	FMS-like tyrosine kinase 1	2.143547
---	Transcribed locus	2.143547
NP_001074286.1	PR domain containing 10	2.143547
---	Transcribed locus	2.143547
---	Transcribed locus	2.143547
NP_062386.1 /// NP_851832.1	carbonic anhydrase 5b, mitochondrial	2.143547

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Table 4,continued

XP_622877.4 /// XP_917591.3 /// XP_996307.2	OTU domain containing 1	2.143547
---	cDNA sequence BC062258	2.143547
---	Transcribed locus	2.143547
---	Transcribed locus	2.143547
NP_001075018.1 /// NP_062339.2	regulator of calcineurin 1	2
NP_083673.3 /// NP_733486.2	RNA binding motif protein 12	2
NP_032853.1	placental growth factor	2
NP_031507.1	ADP-ribosylation factor 6	2
NP_001040624.1 /// NP_062645.2	neuroepithelial cell transforming gene 1	2
NP_032801.2	platelet-activating factor acetylhydrolase, isoform 1b, alpha2 subunit	2
NP_073561.2	suppressor of variegation 3-9 homolog 2 (Drosophila)	2
NP_666195.1	splicing factor, arginine/serine-rich 7	2
NP_034979.3	myelocytomatosis oncogene	2
NP_001075119.1 /// NP_062365.2 /// NP_599018.3	regulator of G-protein signaling 3	2
NP_080548.3	nuclear prelamin A recognition factor	2
NP_666110.1	H6 homeo box 2	2
NP_476513.2	far upstream element (FUSE) binding protein 1	2
NP_663457.2	SET domain containing 4	2
NP_033265.2	trans-acting transcription factor 4	2
NP_033935.2 /// NP_997536.1 /// XP_001475700.1 /// XP_001476803.1	CASP8 and FADD-like apoptosis regulator /// CASP8 and FADD-like apoptosis regulator pseudogene	2
NP_033423.2	tumor necrosis factor, alpha-induced protein 3	2
NP_032917.2	pinin	2

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Table 4,continued

NP_061368.1	tripartite motif-containing 3	2
NP_109622.1	KN motif and ankyrin repeat domains 3	2
---	DNA segment, Chr 13, ERATO Doi 787, expressed	2
---	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)	2
---	RIKEN cDNA 1110018F16 gene	2
NP_001033785.1 /// NP_080319.2	RNA-binding region (RNP1, RRM) containing 3	2
NP_083159.1	RIKEN cDNA 4632415K11 gene	2
NP_067425.1	transient receptor potential cation channel, subfamily M, member 7	2
NP_077216.1	RIKEN cDNA 2410042D21 gene	2
NP_001013398.1	dynein, cytoplasmic 1 light intermediate chain 2	2
NP_001074869.1 /// XP_001480188.1 /// XP_912757.2	RIKEN cDNA 1810013L24 gene	2
NP_083044.1	armadillo repeat containing 8	2
NP_766322.1	serine/threonine kinase 38 like	2
NP_084455.2 /// XP_001480284.1 /// XP_001480288.1	CAP-GLY domain containing linker protein family, member 4 /// similar to CAP-GLY domain containing linker protein family, member 4	2
NP_057909.1	mitogen-activated protein kinase 8	2
NP_082657.1	coiled-coil domain containing 94	2
---	---	2
---	Transcribed locus	2
NP_035897.2	zinc finger RNA binding protein	2
XP_001472628.1 /// XP_001480524.1	RIKEN cDNA 2810055G20 gene	2
NP_001087219.1 /// XP_142262.3 /// XP_916412.2	patched domain containing 1	2
---	expressed sequence AL023008	2
---	Transcribed locus	2

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Table 4,continued

NP_766421.1	mucosa associated lymphoid tissue lymphoma translocation gene 1	2
NP_780591.1	RIKEN cDNA 2700049P18 gene	2
NP_032353.2 /// NP_036070.2	interferon activated gene 202B	2
---	Transcribed locus	2
NP_035589.1	serine (or cysteine) peptdiase inhibitor, clade B, member 8	2
---	Transcribed locus	2
---	Transcribed locus	2

評語

能設計詳盡的實驗步驟，測得明確的變化並進行分析。

對於發炎反應的訊號傳遞機制應深入瞭解。

應予表達清楚，條理分明。