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行政院衛生署 92 年度科技研究發展計畫

中醫寒與熱性體質用藥對細胞基因表現之研究

委託研究報告

計畫委託機關：中國醫藥大學

計畫主持人：陳光偉

研究人員：馮擇仁

執行期間：92 年 3 月 18 日至 92 年 12 月 31 日

** 本研究報告僅供參考，不代表本署意見 **

各機關研究計畫基本資料庫之計畫編號：

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行政院衛生署中醫藥委員會 92 年度
委託研究計畫成果報告

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執行機構：中國醫藥大學

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陳光偉

中國醫藥大學

摘要

中醫的診斷利用人體的特質與疾病狀況，將人的體質分成寒、熱、虛、實；表、裡等等類別，利用中藥將不平衡的身體調至平衡以治療疾病。數千年來，體質特性與相關疾病間的關係及其治療方法均已詳細的紀錄在中醫的書籍裡，不同中藥的藥性與其對人體的影響也有詳細記載。由於中醫的診斷方式與現今西方醫學以細胞學為主的診療方式在理論上有極大的差異，中醫的醫理與現今細胞學、分子生物學的理论也很難相容。此不相容的情形，造成兩醫學系統以對方的缺點相互攻擊，不僅造成醫學上的浪費，也耽擱許多疾病的治療時機。發展一種能將兩系統結合在一起的研究方法，是今日醫療界裡要結合兩系統，並提升兩種醫療系統的治病功能的必要條件。

近幾年，人類基因序列已公布，許多分子生物的技術與資訊提供很好的方法來分析細胞的整體表現，突破以一個基因為主的研究方式。這種以大量基因表現分佈的研究方式與中醫的診斷方式，利用個體的症狀來診斷病因有相似之處，只是細胞的基因表現太過於複雜，若無很好的研究方法，無法用來研究中醫醫理與基因表現的關係。中研院生醫所 Dr.白果能所發展的高密度多點式的生物基因晶片可提供一個很好的研究基因表現與調控的方法，它可以同時偵測一萬個或更多的基因表現。另外，在蛋白體的研究上 ICATs 與 MALDI-MS 將有效的研究蛋白的表現與修飾的差異。若能將中醫的理論結合此兩種技術，將可以利用中醫數千年的研究結晶來做各種疾病的研究，同時結合中西醫的觀點來研究複雜的基因與蛋白表現與疾病症狀的關係。

此計畫將與中研院生醫所 Dr.徐松鋨合作，利用傳統中藥中很明顯、重要並且容易感受到的「寒與熱」來研究其中有關係的基因表現。三味寒藥：黃芩、黃連、黃柏，將用來處理細胞，然後利用生物晶片來分析寒藥對細胞的相關基因表現，進而研究相關基因的藥理機制以及人體體質與基因表現的關係。其中的三味寒藥在藥理上均有抗菌去毒的特性，基因表現的分

析也同時可以瞭解不同藥間其他相同特性的相關基因。此研究方法將提供一個嶄新的領域來研究中醫的科學性，其結果更可以用來建立中西醫之間的橋樑，希望數千年中藥的人體試驗結果能利用現代分子生物的方法找出中藥特性與分子生物特性的相關性，並進而促進醫學上的進步。本研究的結果列出比較上有意義的基因。

表一、黃芩前 30 名 $>2 \times SD$ (9381.852)

HsID	Title	AVET-AVEC
Hs.20725	Homo sapiens cDNA FLJ20814 fis, clone ADSE01064	32096.918
Hs.278605	ER-associated DNAJ; ER-associated Hsp40 co-chaperone; hDj9; Erj3	20118.0647
Hs.195851	Actin, alpha 2, smooth muscle, aorta	17959.6165
Hs.136010	EST	17510.0786
Hs.179661	Tubulin, beta polypeptide	17353.0918
NA	NA	16769.9581
Hs.180920	Ribosomal protein S9	14092.2751
Hs.42644	Thioredoxin-like	13922.7309
Hs.20644	Branched chain alpha-ketoacid dehydrogenase kinase	13544.802
Hs.20580	Sterol O-acyltransferase 2	13452.1165
Hs.1706	Interferon-stimulates transcription factor 3, gamma (48kD)	12845.9393
Hs.7155	ESTS, Weakly similar to 2115357A TYKi protein [M. musculus]	12300.841
Hs.79387	Proteasome (prosome, macropain) 26S subunit, ATPase, 5	11588.2861
Hs.153436	N-acetyltransferase, homolog of S. cerevisiae ARD1	11363.1422
Hs.55781	Hypothetical protein FLJ20604	10937.262
Hs.79748	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	10884.5916
Hs.429	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3	10807.2671
Hs.77665	KIAA0102 gene product	10145.2483
Hs.1288	Actin, alpha 1, skeletal muscle	9897.98473
Hs.251064	High-mobility group (nonhistone chromosomal) protein 14	9825.59953
Hs.274416	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (14kD, B1	9764.7C
Hs.1706	Interferon-stimulated transcription factor 3, gamma (48kD)	8987.38757
Hs.69855	NRAS-related gene	8883.76167
Hs.75914	Coated vesicle membrane protein	8797.8683
Hs.55823	SMC (mouse) homolog, X chromosome	8574.9822
Hs.165843	Casein kinase 2, beta polypeptide	8546.87087
Hs.7345	MAD1 (mitotic arrest deficient, yeast, homolog)-like 1	8345.25313
Hs.177766	ADP-ribosyltransferase (NAD ⁺ ; poly (ADP-ribose) polymerase)	8323.10987
Hs.182248	Sequestosome 1	8261.9414
Hs.190703	ATPase, Na ⁺ /K ⁺ transporting, alpha 1 polypeptide	8118.31817

表二：黃芩後 30 名 < -2*SD

HsID	Title	AVET-AVEC
Hs.129953	Ewing sarcoma breakpoint region 1	-29136.4
Hs.211914	NADH dehydrogenase (ubiquinone) Fe-S protein 7 (20kD) (NADH-coenzyme Q reductase)	-29237.7
Hs.82065	interleukin 6 signal transducer (gp130, oncostatin M receptor)	-29802.9
Hs.76437	ribosomal protein L36	-30076.2
Hs.249495	heterogeneous nuclear ribonucleoprotein A1	-30472.9
Hs.182447	heterogeneous nuclear ribonucleoprotein C (C1/C2)	-30738.9
Hs.89399	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	-30957.1
Hs.157777	Homo sapiens mRNA; cDNA DKFZp434G0118 (from clone DKFZp434G0118)	-31183.9
Hs.131255	ubiquinol-cytochrome c reductase binding protein	-31733
Hs.180946	ribosomal protein L5	-32032.2
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	-32185.4
Hs.3463	ribosomal protein S23	-32239.7
Hs.111515	CGI-43 protein	-32809.6
Hs.74497	nuclease sensitive element binding protein 1	-33187.6
Hs.196177	phosphorylase kinase, gamma 2 (testis)	-33468.9
NA	NA	-33475.8
Hs.77039	ribosomal protein S3A	-33582.1
Hs.197116	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 4	-33583.6
Hs.78771	phosphoglycerate kinase 1	-33723.2
Hs.174131	ribosomal protein L6	-34103.5
Hs.6315	acetylserotonin O-methyltransferase-like	-35287.7
Hs.56	phosphoribosyl pyrophosphate synthetase 1	-35582.7
Hs.163593	ribosomal protein L18a	-35710.1
Hs.180920	ribosomal protein S9	-35910.8
Hs.179718	v-myb avian myeloblastosis viral oncogene homolog-like 2	-36646
NA	NA	-36754.3
NA	NA	-37028.3
Hs.111782	ESTs, Weakly similar to ALUI_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	-37357.5
Hs.75607	myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L)	-37644
NA	NA	-38362.9

表三、黃蓮前 30 名 >2*SD (4804.05)

HsID	Title	AVEL-AVEC
Hs.226795	glutathione S-transferase pi	10649.72827
Hs.9280	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease2)	9340.820433
Hs.116577	prostate differentiation factor	8265.170733
Hs.129913	DNA-damage-inducible transcript 3	8066.133067
NA	NA	7636.4837
Hs.108380	Rhesus blood group, D antigen	7617.3984
Hs.117729	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)	7354.0111
Hs.99029	CCAAT/enhancer binding protein (C/EBP), beta	7317.335333
Hs.73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	6857.609667
Hs.129811	pleckstrin homology, Sec7 and coiled/coil domains 3	6157.1796
Hs.281434	sialyltransferase	6106.180433
Hs.184582	ribosomal protein L24	6034.620733
Hs.143554	ESTs, Highly similar to B45036 Pur beta-human [H.sapiens]	5974.640767
Hs.11147	KIAA0467 protein	5777.460167
Hs.154332	KIAA0212 gene product	5761.7722
Hs.77221	choline kinase	5434.816333
Hs.62813	ESTs, Moderately similar to IA1_HUMAN ZINC FINGER PROTEIN IA-1 [H.sapiens]	5319.712767
Hs.160786	argininosuccinate synthetase	4424.562367
Hs.77929	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complem	4239.60233
Hs.66718	RAD54 (S.cerevisiae)-like	4234.409267
Hs.169832	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)	4003.451967
Hs.104114	H.sapiens HCG I mRNA	3625.6482
Hs.251664	insulin-like growth factor 2 (somatomedin A)	3386.822033
Hs.7570	hypothetical protein FLJ11230	3121.7107
Hs.89436	cadherin 17, LI cadherin (liver-intestine)	2779.9825
Hs.81994	glycophorin C (Gerbich blood group)	2692.2476
Hs.240062	hypothetical protein	2507.3831
Hs.45514	v-ets avian erythroblastosis virus E26 oncogene related	2491.3494
Hs.85885	ESTs	2475.753233
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	2350.358733

表四、黃蓮後 30 名 < -2*SD

HsID	Title	AVEL-AVEC
Hs.5120	dynein, cytoplasmic, light polypeptide	-11539.1
Hs.68756	ESTs, Moderately similar to similar to smoothelin [H.sapiens]	-11642.4
Hs.166011	catenin (cadherin-associated protein), delta 1	-11709.3
Hs.216354	ring finger protein 5	-11736.2
Hs.82793	proteasome (prosome, macropain) subunit, beta type, 3	-11916.5
NA	NA	-11933
Hs.1074	surfactant, pulmonary-associated protein C	-11988.5
Hs.2934	ribonucleotide reductase M1 polypeptide	-12320.4
Hs.183800	Ran GTPase activating protein 1	-12393.7
Hs.7678	cellular retinoic acid-binding protein 1	-12399.3
Hs.251064	high-mobility group (nonhistone chromosomal) protein 14	-12454.8
Hs.2012	transcobalamin I (vitamin B12 binding protein, R binder family)	-12989.6
Hs.146354	peroxiredoxin 2	-13172
Hs.64797	amyloid beta (A4) precursor-like protein 2	-13172.3
Hs.278317	KIAA0632 protein	-13177.2
Hs.75061	MARCKS-like protein	-13294.7
Hs.2706	glutathione peroxidase 4 (phospholipid hydroperoxidase)	-13487.3
Hs.141011	calmodulin 3 (phosphorylase kinase, delta)	-13507.7
Hs.184693	transcription elongation factor B (SIII), polypeptide 1 (15kD, elongin C)	-13710.9
Hs.78996	proliferating cell nuclear antigen	-14110.4
Hs.17775	p75NTR-associated cell death executor, ovarian granulose cell protein (13kD)	-14531.2
NA	NA	-14625.6
Hs.929	myosin, heavy polypeptide 7, cardiac muscle, beta	-15243.9
Hs.1288	actin, alpha 1, skeletal muscle	-15447.1
Hs.3712	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	-15634.1
NA	NA	-1569.5
Hs.250879	ESTs	-16116.4
Hs.30738	hypothetical protein FLJ10407	-17609.8
Hs.75061	MARCKS-like protein	-17896.6
Hs.69855	NRAS-related gene	-18316.3

表五、黄柏前 30 名 >2*SD (4232.796)

HsID	Title	AVEB-AVEC
Hs.75789	N-myc downstream regulated	12579.99937
Hs.79748	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	11698.31407
Hs.226795	glutathione S-transferase pi	10579.49547
Hs.108380	rhesus blood group, D antigen	10465.83527
Hs.184582	ribosomal protein L24	9427.5359
Hs.166456	ESTs, Highly similar to HPPD_HUMAN 4-HYDROXYPHENYLPYRUVATE DIOXYGENASE [H.sapiens]	9264.160067
Hs.182248	sequestosome 1	9207.986867
Hs.80731	autocrine motility factor receptor	9060.5813
Hs.1438	gamma-aminobutyric acid (GABA) receptor, rho 1	9028.351933
NA	NA	8992.233167
Hs.15243	nucleolar protein 1 (120kD)	8838.210867
Hs.155712	follistatin-like 1	8717.892833
NA	NA	8471.4304
Hs.73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	8446.2544
Hs.270845	kinasin-like 5 (mitotic kinesin-like protein 1)	8306.606333
Hs.22559	KIAA097 protein	8158.263833
Hs.3254	ribosomal protein L23-like	8068.529
Hs.3069	heat shock 70kD protein 9B (mortalin-2)	7993.986833
Hs.16001	sema shock 70kD protein 9B (mortalin-2)	7984.466867
Hs.129811	pleckstrin homology, Sec7 and coiled/coil domains 3	7904.524967
Hs.278268	homolog of mouse MAT-1 oncogene	7857.0325
Hs.7879	interferon-related developmental regulator 1	7808.977867
Hs.697	cytochrome c-1	7573.46633
Hs.15251	Homo sapiens chromosome 14 clones RP11-111016 and RP11-61F4 containing genes for nuclear receptor coactivator NcoA-62 (nucle	7525.341933
Hs.6101	bone morphogenetic protein 6	7484.1032
Hs.76244	sermidine synthase	7393.609
Hs.146393	KIAA0025 gene product; MMS-inducible gene	7359.307367
Hs.82173	TGFB inducible early growth response	7.19.8732
Hs.116577	postate differentiation factor	7094.562333
Hs.28491	sermidine/spermine N1-acetyltransferase	6980.094033

表六、黃柏後 30 名 < -2*SD

HsID	Title	AVEB-AVEC
Hs.141011	calmodulin 3 (phosphorylase kinase, delta)	-9426.6028
Hs.78040	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	-9432.6482
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	-9455.2722
Hs.50732	protein kinase, AMP-activated, beta 2 non-catalytic subunit	-9686.0124
NA	NA	-9879.5098
Hs.167421	ESTs, Highly similar to DOC2_HUMAN DIFFERENTIALLY EXPRESSED PROTEIN 2 [H.sapiens]	-9932.4046
Hs.75807	carboxy terminal LIM domain protein 1	-10201.986
Hs.108327	damage-specific DNA binding protein 1 (127kD)	-10216.468
Hs.28914	adenine phosphoribosyltransferase	-10331.028
Hs.17775	p75NTR-associated cell death executor, ovarian granulose cell protein (13kD)	-10600.124
Hs.180714	cytochrome c oxidase Via polypeptide 1	-10697.56
Hs.184693	transcription elongation factor B (SIII), polypeptide 1 (15kD, elongin C)	-11097.213
Hs.64797	amyloid beta (A4) precursor-like protein 2	-11118.241
Hs.73980	troponin T1, skeletal, slow	-11482.268
NA	NA	-11601.061
Hs.146409	wingless-type MMTV integration site family, member 4	-11604.787
Hs.7678	cellular retinoic acid binding protein 1	-11667.039
NA	NA	-11841.165
Hs.1074	surfactant, pulmonary-associated protein C	-11918.083
Hs.17775	p75NTR-associated cell death executor; ovarian granulose cell protein (13kD)	-12094.407
Hs.2012	transcobalamin I (vitamin B12 binding protein, R binder family)	-12194.342
Hs.146354	peroxiredoxin 2	-12244.984
Hs.278317	KIAA0632 protein	-12412.009
Hs.64797	amyloid beta (A4) precursor-like protein 2	-13480.277
Hs.197116	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	-13767.997
Hs.3712	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	-13879.532
Hs.30738	hypothetical protein FLJ10407	-14705.588
Hs.929	myosin, heavy polypeptide 7, cardiac muscle, beta	-15311.565
Hs.73454	troponin T3, skeletal, fast	-15517.857
Hs.250879	ESTs	-16910.507

關鍵詞：中藥，寒熱，DNA 微陣列

Cold and hot-medicine microarray analysis in Chang liver cell

Chen Guang Wei

China Medical University, Institute of Chinese Medical Science

ABSTRACT

Traditional Chinese Medicine (TCM) system has accumulated enormous data regarding to human disease and its treatment with various natural substances, including herbs. In order to scrutinize the molecular mechanism of TCM, we aim to combine the updated techniques and TCM theory to unveil their relationship. The underlying hypothesis of this study is to find the novel genes or proteins involved in the TCM system regarding to the "hot" and "cold" characteristics. These genes can be used as a diagnostic tool for related human diseases. Three "cold" nature herbs: Rhizoma Coptidis (黃連), Cortex Phellodendri (黃柏), and Radix Scutellariae (黃芩) will be used to treat cells to generate cold effects. Gene expression, and protein profile and modification will be studied by microarray. The long-term goal of this study is to combine the vast amount of TCM data with molecular biology to investigate novel genes related to various characteristics and diseases, which will be used as powerful tool for studying human disease. We will list the gene in this study.

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HsID	Title	AVET-AVEC
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Hs.76437	ribosomal protein L36	-30076.2
Hs.249495	heterogeneous nuclear ribonucleoprotein A1	-30472.9
Hs.182447	heterogeneous nuclear ribonucleoprotein C (C1/C2)	-30738.9
Hs.89399	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	-30957.1
Hs.157777	Homo sapiens mRNA; cDNA DKFZp434G0118 (from clone DKFZp434G0118)	-31183.9
Hs.131255	ubiquinol-cytochrome c reductase binding protein	-31733
Hs.180946	ribosomal protein L5	-32032.2
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	-32185.4
Hs.3463	ribosomal protein S23	-32239.7
Hs.111515	CGI-43 protein	-32809.6
Hs.74497	nuclease sensitive element binding protein 1	-33187.6
Hs.196177	phosphorylase kinase, gamma 2 (testis)	-33468.9
NA	NA	-33475.8
Hs.77039	ribosomal protein S3A	-33582.1
Hs.197116	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 4	-33583.6
Hs.78771	phosphoglycerate kinase 1	-33723.2
Hs.174131	ribosomal protein L6	-34103.5
Hs.6315	acetylserotonin O-methyltransferase-like	-35287.7
Hs.56	phosphoribosyl pyrophosphate synthetase 1	-35582.7
Hs.163593	ribosomal protein L18a	-35710.1
Hs.180920	ribosomal protein S9	-35910.8
Hs.179718	v-myb avian myeloblastosis viral oncogene homolog-like 2	-36646
NA	NA	-36754.3
NA	NA	-37028.3
Hs.111782	ESTs, Weakly similar to ALUI_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	-37357.5
Hs.75607	myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L)	-37644
NA	NA	-38362.9

Table 3. 黃蓮前 30 名 >2*SD (4804.05)

HsID	Title	AVEL-AVEC
Hs.226795	glutathione S-transferase pi	10649.72827
Hs.9280	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease2)	9340.820433
Hs.116577	prostate differentiation factor	8265.170733
Hs.129913	DNA-damage-inducible transcript 3	8066.133067
NA	NA	7636.4837
Hs.108380	Rhesus blood group, D antigen	7617.3984
Hs.117729	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)	7354.0111
Hs.99029	CCAAT/enhancer binding protein (C/EBP), beta	7317.335333
Hs.73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	6857.609667
Hs.129811	pleckstrin homology, Sec7 and coiled/coil domains 3	6157.1796
Hs.281434	sialyltransferase	6106.180433
Hs.184582	ribosomal protein L24	6034.620733
Hs.143554	ESTs, Highly similar to B45036 Pur beta-human [H.sapiens]	5974.640767
Hs.11147	KIAA0467 protein	5777.460167
Hs.154332	KIAA0212 gene product	5761.7722
Hs.77221	choline kinase	5434.816333
Hs.62813	ESTs, Moderately similar to IA1_HUMAN ZINC FINGER PROTEIN IA-1 [H.sapiens]	5319.712767
Hs.160786	argininosuccinate synthetase	4424.562367
Hs.77929	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complem	4239.60233
Hs.66718	RAD54 (S.cerevisiae)-like	4234.409267
Hs.169832	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)	4003.451967
Hs.104114	H.sapiens HCG I mRNA	3625.6482
Hs.251664	insulin-like growth factor 2 (somatomedin A)	3386.822033
Hs.7570	hypothetical protein FLJ11230	3121.7107
Hs.89436	cadherin 17, LI cadherin (liver-intestine)	2779.9825
Hs.81994	glycophorin C (Gerbich blood group)	2692.2476
Hs.240062	hypothetical protein	2507.3831
Hs.45514	v-ets avian erythroblastosis virus E26 oncogene related	2491.3494
Hs.85885	ESTs	2475.753233
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	2350.358733

Table 4. 黃蓮後 30 名 < -2*SD

HsID	Title	AVEL-AVEC
Hs.5120	dynein, cytoplasmic, light polypeptide	-11539.1
Hs.68756	ESTs, Moderately similar to similar to smoothelin [H.sapiens]	-11642.4
Hs.166011	catenin (cadherin-associated protein), delta 1	-11709.3
Hs.216354	ring finger protein 5	-11736.2
Hs.82793	proteasome (prosome, macropain) subunit, beta type, 3	-11916.5
NA	NA	-11933
Hs.1074	surfactant, pulmonary-associated protein C	-11988.5
Hs.2934	ribonucleotide reductase M1 polypeptide	-12320.4
Hs.183800	Ran GTPase activating protein 1	-12393.7
Hs.7678	cellular retinoic acid-binding protein 1	-12399.3
Hs.251064	high-mobility group (nonhistone chromosomal) protein 14	-12454.8
Hs.2012	transcobalamin I (vitamin B12 binding protein, R binder family)	-12989.6
Hs.146354	peroxiredoxin 2	-13172
Hs.64797	amyloid beta (A4) precursor-like protein 2	-13172.3
Hs.278317	KIAA0632 protein	-13177.2
Hs.75061	MARCKS-like protein	-13294.7
Hs.2706	glutathione peroxidase 4 (phospholipid hydroperoxidase)	-13487.3
Hs.141011	calmodulin 3 (phosphorylase kinase, delta)	-13507.7
Hs.184693	transcription elongation factor B (SIII), polypeptide 1 (15kD, elongin C)	-13710.9
Hs.78996	proliferating cell nuclear antigen	-14110.4
Hs.17775	p75NTR-associated cell death executor, ovarian granulosa cell protein (13kD)	-14531.2
NA	NA	-14625.6
Hs.929	myosin, heavy polypeptide 7, cardiac muscle, beta	-15243.9
Hs.1288	actin, alpha 1, skeletal muscle	-15447.1
Hs.3712	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	-15634.1
NA	NA	-1569.5
Hs.250879	ESTs	-16116.4
Hs.30738	hypothetical protein FLJ10407	-17609.8
Hs.75061	MARCKS-like protein	-17896.6
Hs.69855	NRAS-related gene	-18316.3

Table 5. 黄柏前 30 名 > 2*SD (4232.796)

HsID	Title	AVEB-AVEC
Hs.75789	N-myc downstream regulated	12579.99937
Hs.79748	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	11698.31407
Hs.226795	glutathione S-transferase pi	10579.49547
Hs.108380	rhesus blood group, D antigen	10465.83527
Hs.184582	ribosomal protein L24	9427.5359
Hs.166456	ESTs, Highly similar to HPPD_HUMAN 4-HYDROXYPHENYLPYRUVATE DIOXYGENASE [H.sapiens]	9264.160067
Hs.182248	sequestosome 1	9207.986867
Hs.80731	autocrine motility factor receptor	9060.5813
Hs.1438	gamma-aminobutyric acid (GABA) receptor, rho 1	9028.351933
NA	NA	8992.233167
Hs.15243	nucleolar protein 1 (120kD)	8838.210867
Hs.155712	follistatin-like 1	8717.892833
NA	NA	8471.4304
Hs.73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	8446.2544
Hs.270845	kinasin-like 5 (mitotic kinesin-like protein 1)	8306.606333
Hs.22559	KIAA097 protein	8158.263833
Hs.3254	ribosomal protein L23-like	8068.529
Hs.3069	heat shock 70kD protein 9B (mortalin-2)	7993.986833
Hs.16001	sema shock 70kD protein 9B (mortalin-2)	7984.466867
Hs.129811	pleckstrin homology, Sec7 and coiled/coil domains 3	7904.524967
Hs.278268	homolog of mouse MAT-1 oncogene	7857.0325
Hs.7879	interferon-related developmental regulator 1	7808.977867
Hs.697	cytochrome c-1	7573.46633
Hs.15251	Homo sapiens chromosome 14 clones RP11-111016 and RP11-61F4 containing genes for nuclear receptor coactivator NcoA-62 (nucle	7525.341933
Hs.6101	bone morphogenetic protein 6	7484.1032
Hs.76244	sermidine synthase	7393.609
Hs.146393	KIAA0025 gene product; MMS-inducible gene	7359.307367
Hs.82173	TGFB inducible early growth response	7.19.8732
Hs.116577	postate differentiation factor	7094.562333
Hs.28491	sermidine/spermine N1-acetyltransferase	6980.094033

Table 6. 黃柏後 30 名 < -2*SD

HsID	Title	AVEB-AVEC
Hs.141011	calmodulin 3 (phosphorylase kinase, delta)	-9426.6028
Hs.78040	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	-9432.6482
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	-9455.2722
Hs.50732	protein kinase, AMP-activated, beta 2 non-catalytic subunit	-9686.0124
NA	NA	-9879.5098
Hs.167421	ESTs, Highly similar to DOC2_HUMAN DIFFERENTIALLY EXPRESSED PROTEIN 2 [H.sapiens]	-9932.4046
Hs.75807	carboxy terminal LIM domain protein 1	-10201.986
Hs.108327	damage-specific DNA binding protein 1 (127kD)	-10216.468
Hs.28914	adenine phosphoribosyltransferase	-10331.028
Hs.17775	p75NTR-associated cell death executor, ovarian granulose cell protein (13kD)	-10600.124
Hs.180714	cytochrome c oxidase Via polypeptide 1	-10697.56
Hs.184693	transcription elongation factor B (SIII), polypeptide 1 (15kD, elongin C)	-11097.213
Hs.64797	amyloid beta (A4) precursor-like protein 2	-11118.241
Hs.73980	troponin T1, skeletal, slow	-11482.268
NA	NA	-11601.061
Hs.146409	wingless-type MMTV integration site family, member 4	-11604.787
Hs.7678	cellular retinoic acid binding protein 1	-11667.039
NA	NA	-11841.165
Hs.1074	surfactant, pulmonary-associated protein C	-11918.083
Hs.17775	p75NTR-associated cell death executor; ovarian granulose cell protein (13kD)	-12094.407
Hs.2012	transcobalamin I (vitamin B12 binding protein, R binder family)	-12194.342
Hs.146354	peroxiredoxin 2	-12244.984
Hs.278317	KIAA0632 protein	-12412.009
Hs.64797	amyloid beta (A4) precursor-like protein 2	-13480.277
Hs.197116	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	-13767.997
Hs.3712	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	-13879.532
Hs.30738	hypothetical protein FLJ10407	-14705.588
Hs.929	myosin, heavy polypeptide 7, cardiac muscle, beta	-15311.565
Hs.73454	troponin T3, skeletal, fast	-15517.857
Hs.250879	ESTs	-16910.507

Keywords : Herb, cold, microarray

壹、前言

中醫的診斷將人體的體質分成陰、陽；表、裡；寒、熱；虛、實等類型，數千年來利用中藥方劑將不平衡的身體調回平衡以治療疾病，由於中醫與現今西方醫學的診療方式在理論上有極大的差異，中醫的醫理與現今細胞學、分子生物學的理论也很難相容，造成醫療上的浪費。發展一種能將兩系統結合在一起的研究方法，是今日醫療界裡提升醫療服務系統的必要條件。中醫關於體質概念經由對疾病的看法，以生物體整體觀念為基礎，闡明體質的形成、分類及其與發病、辨證、治療的關係，是中醫理論體系中重要組成元素。這一個宏觀的辨證體系，說明了人體對身心平衡(健康)、失衡(患病)及調節平衡的方法。

醫學心悟之「寒熱虛實表裡陰陽辨」中提到：一病之寒熱，全在渴與不渴，渴而消水與不消水，飲食喜熱與喜冷，煩躁與厥逆。此計畫將與中研院生醫所徐松鋁助研究員合作，利用中醫診斷中最明顯且最容易感受到的「寒與熱」並利用中藥藥性中之寒、熱藥來研究其中有關係的基因表現。三味寒藥：黃芩、黃連、黃柏，與三味熱藥：附子、肉桂、乾薑，將用來處理細胞，然後利用生物晶片來分析與寒或熱有相關的基因表現，進而研究相關基因的生理機制以研究人體體質與基因表現的關係。其中的三味寒藥在藥理上均有抗菌去毒的特性，基因表現的分析也同時可以瞭解不同藥間其他相同特性的相關基因。此研究方法將提供一個嶄新的領域來研究中醫的科學性，其結果更可以用來建立中西醫之間的橋樑，希望數千年中醫的人體試驗結果能利用現代分子生物的方法找出身體各症狀與分子生物特性的相關性，並進而促進醫學上的進步。中醫的許多診斷與療效已被現代科技所證實，本計畫將試探中醫與分子醫學研究結合的可行性，用所提出的方法也可以用於其他較複雜的症狀，並利用中醫數千年來所累積的龐大資訊來研究人體的特性與基因調控的關係，進而與西醫相輔相成，促成醫學上更大的進步。

貳、材料與方法

(一)細胞培養

293 細胞與 (從 ATCC 獲得) 培養於含 10% fetal bovine serum (FBS)(從 Hyclone)與 100 U/ml penicillin-streptomycin 的 Dulbecco's modified Eagle's medium (DMEM)的培養液，並置放於 37°C 充滿水氣與 5% CO₂ 的空氣中，培養液與其他細胞培養藥品將從 BRL Life Technologies 購買。為減少細胞代數不同而導致的基因表現不一致，我們已經冰存一批 HEK293 於 -80°C 中，在每次用中藥處分細胞時，會解凍一批新的細胞來使用。這樣每次所使用的細胞代數都會一樣，可以減少實驗的誤差。

(二)中藥藥物處理

所使用中藥的濃度將由中藥藥方所使用的濃度比例來決定用量。參考其他研究報告所使用的科學中藥情形 (Yano et al., 1994)，以前的實驗所使用的熱藥濃度為 1mg/ml，在此濃度下，細胞的生長情形沒有顯著的受到影響。但初步結果發現，使用黃連時在該濃度會造成部分的細胞死亡，經試驗後發現使用 0.5mg/ml 時，對細胞生長沒有影響。由中醫師臨床之常用劑量在所要使用的寒藥與熱藥的比例為附子：乾薑：肉桂=1：1：1 (常用約各一至三錢)；黃芩：黃連：黃柏=6：2：3 (常用約三錢：一錢：一錢半)，先前的實驗結果顯示，1mg/ml 的熱藥對細胞的生長沒有顯著的影響，因為各個藥物對基因調控的相似性不高，決定將處理藥物的濃度調低為 0.5mg/ml 在做比對。而寒藥的處理將採用兩個劑量：黃芩在中醫藥中教緩和，所以用較高的劑量：1mg/ml 與 0.5mg/ml；黃連：0.3mg/ml 與 0.1mg/ml；黃柏：0.5mg/ml 與 0.2mg/ml。

由順天堂所提供的科學中藥將存放於 4°C，各個中藥將溶於 DMEM 中 (1g/ml)，經 15 分鐘在室溫下攪拌後，再放入超音波震盪機中 15 分鐘以達最大的溶解效果。溶解後的溶液在過 0.22 μ 的濾紙後，立即分裝並保存於 -20°C 的冷凍庫中。293 細胞在長到 5 成

滿時，將要處理的藥物溶液加入培養液中，為了看冷藥長時間的作用，細胞將在 48 小時之後收起來抽取 RNA。

(三)全量 RNA 抽取法

HEK293 細胞株以藥物萃取後以 DPBS 清洗，於培養皿中加入 1ml 之 4M GITC homogenization buffer (5.3 M guanidine-HCL; 1.5 Triton-X100; 2.5mM Tris-HCL, pH 7.5; 0.25mM EDTA)以溶解細胞，以均質器打破細胞，再利用 25 號針頭之針筒將大分子 DNA 打斷，每 0.5 毫升細胞溶解液分裝於微量離心管，以等體積於細胞溶解液之苯酚 (phenol, 與 water saturate, pH 4.0)、0.1ml 氯仿 (chloroform) 與 0.5 μ l 醋酸鈉溶液 (2M, sodium acetate, pH 4.8)並於冰上反應 15 分鐘，經於 4°C 離心 (20,000 \times g) 20 分鐘後收集上清液，並以兩倍體積之絕對酒精沉澱之，經微量離心並去除上清液後半乾燥 RNA 並以 50 μ l 之 DEPC 處理過之去離子水溶劑之，並以分光光度計定量。

(四)純化 mRNA Dynal beads :

1. 取含有 75 μ g 的 total RNA 溶液，將此溶液體積以 DEPC water 調整至 100 μ l。
2. 取 100 μ l Binding beffer 加入此 RNA 溶液，混合均勻後置於 65 °C 2 分鐘，再迅速置於冰上。
3. 此時取 200 μ l (約含有 1mg)的 Dynabeads Oligo (dT) 25 移至一新的離心管，將此管置於 Dynal MPC 磁座上。
4. 30 秒後，待 Dynabeads 均被磁附在管底，移除上清液，取下離心管並加入 100 μ l Binding buffer，混合均勻，將此管置於 Dynal MPC 上。
5. 重複步驟 4，移除上清液，取下離心管置再加入 100 μ l Binding beffer，取步驟 2 之 RNA 溶液混合均勻，並將此管置於 roller machine 上，在室溫下轉動 5 分鐘，使 mRNA 吸附於 Dynabeads

上。

6. 將離心管置於 Dynal MPC 上，待 Dynabeads 均被磁附在管底，移除上清液，加以 200 μ l Washing Buffer B，混合均勻，將此管置於 Dynal MPC 上。
7. 重複步驟 6，移除上清液，加入 20 μ l DEPC water，與 Dynabeads 混合均合，將離心管置於 75°C 水浴中 2 分鐘，迅速置於 Dynal MPC 上，待 Dynabeads 均被磁附在管底，即可移出純化之 mRNA 溶液 (估計約 2 μ g)。

(五)標示互補 DNA 與微陣列晶片雜交反應

將 50 μ l 之 RNA 以反轉錄標示試劑組標示藥物反應組與對照組之樣品，將 RNA 與 oligo (dT) 引子於 70°C 反應 10 分鐘後，加入含螢光或酵素標定之核苷酸、反應緩衝液與反轉錄酶 (4 μ l MgCl₂, 25mM; 2 μ l 10 \times RT-buffer; 2 μ l dNTP, 10mM each; 0.5 μ l Rnasin, 50 U/ μ l; and 0.5 μ l AMV-RT, 30 U/ μ l) 後於 42°C 反應 1 小時，反應結束後將樣品以 72°C 加熱 5 分鐘終止反轉錄反應必保存於 -20°C 備用。將以反轉錄探針，經加熱 90°C 兩分鐘後迅速加入 cDNA 微陣列晶片中，於 65°C 恆溫水槽反應隔夜，經雜交反應後以沖洗緩衝液 (0.5 \times SSC, 0.01% SDS) 清洗晶片，再經由序列的高專一性沖洗緩衝液 (0.5 \times SSC, 0.01% SDS; 0.06 \times SSC) 清洗非專一性雜交樣品後，以晶片掃瞄器 (Genepix 4000B Array scanner) 與晶片分析軟體 (Genepix pro 4.0 Array Analysis Software) 分析之。

微陣列初步數據的分析是微陣列影像之定量，CDNA arrays 之定量我們使用 GenePix 軟體。經過定量之數據要進行標準化 (正常化，均一化)，經過標準化之數據可以進一步使用 Gene Spring 等相關的實驗條件，樣品等來分析。

參、結果

表一、黃芩前 30 名 $> 2 \times SD$ (9381.852)

HsID	Title	AVET-AVEC
Hs.20725	Homo sapiens cDNA FLJ20814 fis, clone ADSE01064	32096.918
Hs.278605	ER-associated DNAJ; ER-associated Hsp40 co-chaperone; hDj9; Erj3	20118.0647
Hs.195851	Actin, alpha 2, smooth muscle, aorta	17959.6165
Hs.136010	EST	17510.0786
Hs.179661	Tubulin, beta polypeptide	17353.0918
NA	NA	16769.9581
Hs.180920	Ribosomal protein S9	14092.2751
Hs.42644	Thioredoxin-like	13922.7309
Hs.20644	Branched chain alpha-ketoacid dehydrogenase kinase	13544.802
Hs.20580	Sterol O-acyltransferase 2	13452.1165
Hs.1706	Interferon-stimulates transcription factor 3, gamma (48kD)	12845.9393
Hs.7155	ESTS, Weakly similar to 2115357A TYKi protein (M. musculus)	12300.841
Hs.79387	Proteasome (prosome, macropain) 26S subunit, ATPase, 5	11588.2861
Hs.153436	N-acetyltransferase, homolog of S. cerevisiae ARD1	11363.1422
Hs.55781	Hypothetical protein FLJ20604	10937.262
Hs.79748	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	10884.5916
Hs.429	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3	10807.2671
Hs.77665	KIAA0102 gene product	10145.2483
Hs.1288	Actin, alpha 1, skeletal muscle	9897.98473
Hs.251064	High-mobility group (nonhistone chromosomal) protein 14	9825.59953
Hs.274416	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (14kD, B1	9764.70
Hs.1706	Interferon-stimulated transcription factor 3, gamma (48kD)	8987.38757
Hs.69855	NRAS-related gene	8883.76167
Hs.75914	Coated vesicle membrane protein	8797.8683
Hs.55823	SMC (mouse) homolog, X chromosome	8574.9822
Hs.165843	Casein kinase 2, beta polypeptide	8546.87087
Hs.7345	MAD1 (mitotic arrest deficient, yeast, homolog)-like 1	8345.25313
Hs.177766	ADP-ribosyltransferase (NAD ⁺ ; poly (ADP-ribose) polymerase)	8323.10987
Hs.182248	Sequestosome 1	8261.9414
Hs.190703	ATPase, Na ⁺ /K ⁺ transporting, alpha 1 polypeptide	8118.31817

二：黄芩後 30 名 < -2*SD

HsID	Title	AVET-AVEC
Hs.129953	Ewing sarcoma breakpoint region 1	-29136.4
Hs.211914	NADH dehydrogenase (ubiquinone) Fe-S protein 7 (20kD) (NADH-coenzyme Q reductase)	-29237.7
Hs.82065	interleukin 6 signal transducer (gp130, oncostatin M receptor)	-29802.9
Hs.76437	ribosomal protein L36	-30076.2
Hs.249495	heterogeneous nuclear ribonucleoprotein A1	-30472.9
Hs.182447	heterogeneous nuclear ribonucleoprotein C (C1/C2)	-30738.9
Hs.89399	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	-30957.1
Hs.157777	Homo sapiens mRNA; cDNA DKFZp434G0118 (from clone DKFZp434G0118)	-31183.9
Hs.131255	ubiquinol-cytochrome c reductase binding protein	-31733
Hs.180946	ribosomal protein L5	-32032.2
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	-32185.4
Hs.3463	ribosomal protein S23	-32239.7
Hs.111515	CGI-43 protein	-32809.6
Hs.74497	nuclease sensitive element binding protein 1	-33187.6
Hs.196177	phosphorylase kinase, gamma 2 (testis)	-33468.9
NA	NA	-33475.8
Hs.77039	ribosomal protein S3A	-33582.1
Hs.197116	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 4	-33583.6
Hs.78771	phosphoglycerate kinase 1	-33723.2
Hs.174131	ribosomal protein L6	-34103.5
Hs.6315	acetylserotonin O-methyltransferase-like	-35287.7
Hs.56	phosphoribosyl pyrophosphate synthetase 1	-35582.7
Hs.163593	ribosomal protein L18a	-35710.1
Hs.180920	ribosomal protein S9	-35910.8
Hs.179718	v-myb avian myeloblastosis viral oncogene homolog-like 2	-36646
NA	NA	-36754.3
NA	NA	-37028.3
Hs.111782	ESTs, Weakly similar to ALUI_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	-37357.5
Hs.75607	myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L)	-37644
NA	NA	-38362.9

表三、黃蓮前 30 名 >2*SD (4804.05)

HsID	Title	AVEL-AVEC
Hs.226795	glutathione S-transferase pi	10649.72827
Hs.9280	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease2)	9340.820433
Hs.116577	prostate differentiation factor	8265.170733
Hs.129913	DNA-damage-inducible transcript 3	8066.133067
NA	NA	7636.4837
Hs.108380	Rhesus blood group, D antigen	7617.3984
Hs.117729	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)	7354.0111
Hs.99029	CCAAT/enhancer binding protein (C/EBP), beta	7317.335333
Hs.73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	6857.609667
Hs.129811	pleckstrin homology, Sec7 and coiled/coil domains 3	6157.1796
Hs.281434	sialyltransferase	6106.180433
Hs.184582	ribosomal protein L24	6034.620733
Hs.143554	ESTs, Highly similar to B45036 Pur beta-human [H.sapiens]	5974.640767
Hs.11147	KIAA0467 protein	5777.460167
Hs.154332	KIAA0212 gene product	5761.7722
Hs.77221	choline kinase	5434.816333
Hs.62813	ESTs, Moderately similar to IA1_HUMAN ZINC FINGER PROTEIN IA-1 [H.sapiens]	5319.712767
Hs.160786	argininosuccinate synthetase	4424.562367
Hs.77929	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complem	4239.60233
Hs.66718	RAD54 (S.cerevisiae)-like	4234.409267
Hs.169832	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)	4003.451967
Hs.104114	H.sapiens HCG I mRNA	3625.6482
Hs.251664	insulin-like growth factor 2 (somatomedin A)	3386.822033
Hs.7570	hypothetical protein FLJ11230	3121.7107
Hs.89436	cadherin 17, LI cadherin (liver-intestine)	2779.9825
Hs.81994	glycophorin C (Gerbich blood group)	2692.2476
Hs.240062	hypothetical protein	2507.3831
Hs.45514	v-ets avian erythroblastosis virus E26 oncogene related	2491.3494
Hs.85885	ESTs	2475.753233
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	2350.358733

表四、黃蓮後 30 名 < -2*SD

HsID	Title	AVEL-AVEC
Hs.5120	dynein, cytoplasmic, light polypeptide	-11539.1
Hs.68756	ESTs, Moderately similar to similar to smoothelin [H.sapiens]	-11642.4
Hs.166011	catenin (cadherin-associated protein), delta 1	-11709.3
Hs.216354	ring finger protein 5	-11736.2
Hs.82793	proteasome (prosome, macropain) subunit, beta type, 3	-11916.5
NA	NA	-11933
Hs.1074	surfactant, pulmonary-associated protein C	-11988.5
Hs.2934	ribonucleotide reductase M1 polypeptide	-12320.4
Hs.183800	Ran GTPase activating protein 1	-12393.7
Hs.7678	cellular retinoic acid-binding protein 1	-12399.3
Hs.251064	high-mobility group (nonhistone chromosomal) protein 14	-12454.8
Hs.2012	transcobalamin I (vitamin B12 binding protein, R binder family)	-12989.6
Hs.146354	peroxiredoxin 2	-13172
Hs.64797	amyloid beta (A4) precursor-like protein 2	-13172.3
Hs.278317	KIAA0632 protein	-13177.2
Hs.75061	MARCKS-like protein	-13294.7
Hs.2706	glutathione peroxidase 4 (phospholipid hydroperoxidase)	-13487.3
Hs.141011	calmodulin 3 (phosphorylase kinase, delta)	-13507.7
Hs.184693	transcription elongation factor B (SIII), polypeptide 1 (15kD, elongin C)	-13710.9
Hs.78996	proliferating cell nuclear antigen	-14110.4
Hs.17775	p75NTR-associated cell death executor, ovarian granulosa cell protein (13kD)	-14531.2
NA	NA	-14625.6
Hs.929	myosin, heavy polypeptide 7, cardiac muscle, beta	-15243.9
Hs.1288	actin, alpha 1, skeletal muscle	-15447.1
Hs.3712	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	-15634.1
NA	NA	-1569.5
Hs.250879	ESTs	-16116.4
Hs.30738	hypothetical protein FLJ10407	-17609.8
Hs.75061	MARCKS-like protein	-17896.6
Hs.69855	NRAS-related gene	-18316.3

表五、黃柏前 30 名 >2*SD (4232.796)

HsID	Title	AVEB-AVEC
Hs.75789	N-myc downstream regulated	12579.99937
Hs.79748	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	11698.31407
Hs.226795	glutathione S-transferase pi	10579.49547
Hs.108380	rhesus blood group, D antigen	10465.83527
Hs.184582	ribosomal protein L24	9427.5359
Hs.166456	ESTs, Highly similar to HPPD_HUMAN [H.sapiens] 4-HYDROXYPHENYLPYRUVATE DIOXYGENASE	9264.160067
Hs.182248	sequestosome 1	9207.986867
Hs.80731	autocrine motility factor receptor	9060.5813
Hs.1438	gamma-aminobutyric acid (GABA) receptor, rho 1	9028.351933
NA	NA	8992.233167
Hs.15243	nucleolar protein 1 (120kD)	8838.210867
Hs.155712	follistatin-like 1	8717.892833
NA	NA	8471.4304
Hs.73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	8446.2544
Hs.270845	kinasin-like 5 (mitotic kinesin-like protein 1)	8306.606333
Hs.22559	KIAA097 protein	8158.263833
Hs.3254	ribosomal protein L23-like	8068.529
Hs.3069	heat shock 70kD protein 9B (mortalin-2)	7993.986833
Hs.16001	sema shock 70kD protein 9B (mortalin-2)	7984.466867
Hs.129811	pleckstrin homology, Sec7 and coiled/coil domains 3	7904.524967
Hs.278268	homolog of mouse MAT-1 oncogene	7857.0325
Hs.7879	interferon-related developmental regulator 1	7808.977867
Hs.697	cytochrome c-1	7573.46633
Hs.15251	Homo sapiens chromosome 14 clones RP11-111016 and RP11-61F4 containing genes for nuclear receptor coactivator NcoA-62 (nucle	7525.341933
Hs.6101	bone morphogenetic protein 6	7484.1032
Hs.76244	sermidine synthase	7393.609
Hs.146393	KIAA0025 gene product; MMS-inducible gene	7359.307367
Hs.82173	TGFB inducible early growth response	7.19.8732
Hs.116577	postate differentiation factor	7094.562333
Hs.28491	sermidine/spermine N1-acetyltransferase	6980.094033

表六、黃柏後 30 名 < -2*SD

HsID	Title	AVEB-AVEC
Hs.141011	calmodulin 3 (phosphorylase kinase, delta)	-9426.6028
Hs.78040	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	-9432.6482
Hs.84981	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80kD)	-9455.2722
Hs.50732	protein kinase, AMP-activated, beta 2 non-catalytic subunit	-9686.0124
NA	NA	-9879.5098
Hs.167421	ESTs, Highly similar to DOC2_HUMAN DIFFERENTIALLY EXPRESSED PROTEIN 2 [H.sapiens]	-9932.4046
Hs.75807	carboxy terminal LIM domain protein 1	-10201.986
Hs.108327	damage-specific DNA binding protein 1 (127kD)	-10216.468
Hs.28914	adenine phosphoribosyltransferase	-10331.028
Hs.17775	p75NTR-associated cell death executor, ovarian granulose cell protein (13kD)	-10600.124
Hs.180714	cytochrome c oxidase Via polypeptide 1	-10697.56
Hs.184693	transcription elongation factor B (SIII), polypeptide 1 (15kD, elongin C)	-11097.213
Hs.64797	amyloid beta (A4) precursor-like protein 2	-11118.241
Hs.73980	troponin T1, skeletal, slow	-11482.268
NA	NA	-11601.061
Hs.146409	wingless-type MMTV integration site family, member 4	-11604.787
Hs.7678	cellular retinoic acid binding protein 1	-11667.039
NA	NA	-11841.165
Hs.1074	surfactant, pulmonary-associated protein C	-11918.083
Hs.17775	p75NTR-associated cell death executor; ovarian granulose cell protein (13kD)	-12094.407
Hs.2012	transcobalamin I (vitamin B12 binding protein, R binder family)	-12194.342
Hs.146354	peroxiredoxin 2	-12244.984
Hs.278317	KIAA0632 protein	-12412.009
Hs.64797	amyloid beta (A4) precursor-like protein 2	-13480.277
Hs.197116	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	-13767.997
Hs.3712	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	-13879.532
Hs.30738	hypothetical protein FLJ10407	-14705.588
Hs.929	myosin, heavy polypeptide 7, cardiac muscle, beta	-15311.565
Hs.73454	troponin T3, skeletal, fast	-15517.857
Hs.250879	ESTs	-16910.507

肆、討論

在小於負 2 倍標準差方面黃芩、魂連、黃柏三組中藥均下調的基因有 cytochrome c oxidase, subunit Via, polypeptide 1, ubiquinol-cytochrome c reductase-binding pretein, calmodulin 3, interferon-induced transmembrane protein 1, chromosome-associated polypeptide C, high mobility group nucleosomal binding protein 1, endoplasmic reticulum KDEL receptor, GTPase-activating protein RNA, 1-amyloid beta (A4) precursor-like protein 2, peroxiredoxin 2, solute carrier family 7 member4, thyroid hormone receptor interaction 13, X-ray repair, complementing defective, in Chinese hamster 5, metallothionein 1L, dystrophic myotonia-containing WD repeat motif, hepatocyte growth factor receptor, macrophage migration inhibitor factor, MHC classI, A 共 18 個基因。

其中 ubiquinol-cytochrome c reductase-binding protein 及 cytochrome c oxidase, subunit Via, polypeptide 1 為粒腺體內膜上，電子傳遞鏈的成員，電子傳遞鏈有四個複合體，ubiquinol-cytochrome c reductase 是第三個複合體，cytochrome c oxidase 是第四個複合體，由於電子傳遞煉是藉由質子梯度來驅動 ATP 的合成，一旦此過程中任何一個複合體被抑制則 ATP 將無法產生，而 ATP 是人體能量的來源，ATP 的製造一旦減少則機體的能量便下降。

就中醫理論來看，八綱辨證中以陰陽為手，寒證與熱證分屬其下，寒證的定義為感受寒邪或機體陽虛陰盛，機體活動衰減所表現的證候，臨床包括口不渴或渴而不喜飲，喜熱引，手足冷，面色蒼白，小便透明而長，大便下痢而稀，舌苔白滑，脈遲等；而熱證的定義為感受熱邪或陽盛陰虛，機體活動亢進時表現的證候，臨床包括口渴引飲，喜冷飲，潮熱煩燥，面紅木赤，小便短赤，小便短赤，大便秘結，舌苔黃而糙，脈數等；若機體的產生不足，便會使機體的活動能力衰減，以寒熱來看是屬於寒症，而此三種藥均出現抑制電子傳遞鏈中第三及第四個複合體，阻斷 ATP 的產生，故使機體能量的產生降低，頗符合寒藥導致寒證的中醫理論。

而鈣調素 (calmodulin, CaM) 是細胞內另一個重要的信號傳導分子，介

導 Ca^{2+} -CaM-CaMK (Calcium/Calmodulin dependent protein kinases, CaMK) 的信號傳導。CaM 和 Ca^{2+} 有很高的親合力，一個 CaM 分子可與四個 Ca^{2+} 結合。CaM 本身沒有活性，但和 Ca^{2+} 結合後，再與酶結合，會形成酶- Ca^{2+} -CaM 複合體，出現活性。酶- Ca^{2+} -CaM 複合體可與細胞中的 Ca^{2+} -ATP 酶、NAD 激酶、磷酸酶、蛋白質激酶和 β -葡聚糖合成酶結合，使這些酶活化，從而調節細胞的許多代謝過程。

我們發現黃芩、黃連、黃柏共同下調的 Calmodulin 是 Calmodulin 3，Calmodulin 分成 Calmodulin 1、Calmodulin 2 及 Calmodulin 3，但三者間其 nucleotide 約有 80% 是相同的，因目前單獨對 Calmodulin 3 的了解並不多，且大多以 Calmodulin 來統稱，故我們試著藉由當今西方醫學對 Calmodulin 這個基因的了解，嘗試來解釋這三種寒藥的可能機轉。

Ca;modulin 在科學的驗證上有以下功能：一、調節鈣離子濃度。二、協調神經系統。三、激活酵素系統。四、能量的啟動器。五、活化巨噬細胞。

危北海在體外實驗中發現，由黃連、黃芩、木通、大黃、甘草組成之方藥，對內毒素誘導之小鼠腹腔巨噬細胞分泌的 TNF、IL-1、IL-6，有明顯的抑制效應；另外，對內毒素誘導而產生的人外周血單核細胞分泌上述的 cytokines 亦呈顯著抑制作用。

本研究發現三種寒藥均可下調 Calmodulin，而 Ca^{2+} /CaM 可活化 phosphodiesterase 1，是否藉由此一機制來抑制內原性熱原的分泌而對抗內毒素所致機體發熱等多種熱象，可能尚需進一步研究。

由結果得知，大於 2 倍標準差上調的基因中並無三種中藥均出現上調者，不過在兩兩比較之後卻發現在大於 2 倍標準差上調的基因中黃連和黃柏有 12 個上調基因相同，黃芩和黃柏有 3 個上調基因相同，而黃芩和黃連則沒有交集；在小於負 2 倍標準差下調的基因中兩兩比較之下發現下黃連和黃柏有 34 個下調基因相同；黃芩和黃柏有 14 個下調基因相同，而黃芩和黃連則有 12 個下調基因相同。可知黃連與黃柏在基因的表達方面似乎較

接近。

在中醫的藥理上，這三種藥均有清熱燥濕、瀉火解毒的作用，可療瘡瘍腫毒，並可治濕熱痢及黃疸；但黃芩則較偏於上焦，即機體的上半部位，黃連則偏於中焦，即機體的中半部位，黃柏則偏於下焦，即機體的下半部位，不論在中醫古籍的歸經或主治項目，可以發現黃芩和黃連比較接近，但在我們的結果中卻發現在基因晶片的表現上反而是黃連和黃柏有較多共同上調或下調的基因，這或許是因為黃連與黃柏在現代的藥理研究中發現二者之主要成分均為小檗鹼(黃連素)，黃連另含黃連鹼、甲基黃連鹼棕櫚鹼及非洲防己鹼等；而黃柏則另含黃柏鹼、木蘭花鹼、掌葉防己鹼、黃柏內酯等，但主要的有效成分是一樣的，只是黃連(5-8%)其小檗鹼的含量較黃柏(1.6-4%)為高，黃芩不含小檗鹼，但含黃芩甘、黃芩素、漢黃芩甘、漢黃芩素與黃芩新素，其中黃芩甘及黃芩素為黃芩之有效成分，如此或許可以解釋為何黃連與黃柏的上調、下調基因較接近。

中醫古籍《本草綱目》中記載黃芩「治風熱，濕熱，頭痛，奔豚熱痛，火咳肺萎，喉腥，諸失血。」、「得酒上行，得豬膽汁除肝膽火，得柴胡退寒熱，得芍藥治下痢，得桑白皮瀉肺火，得白朮安胎。」；其中亦提到黃連「黃連治目及痢為要藥，……治消渴，用酒蒸黃連；治伏暑，用酒煮黃連；治下血，用黃連、大蒜；治肝火，用黃連、茱萸；治口瘡，用黃連、細辛，……。」在《珍珠囊》中亦記載黃連「其用有六：瀉心臟火，一也；去中焦濕熱，二也；諸瘡必用，三也；去風濕，四也；治赤眼暴發，五也；止中部見血，六也。」而在《本經》中記載黃柏「主五臟腸胃中結熱，黃疸，腸痔，止泄痢，婦女漏下赤白，陰陽傷蝕瘡。」，《本經逢源》亦提到「黃柏苦寒迅利，疏肝脾而泄濕熱，清膀胱而排瘀濁，殊有捷效。」可知黃柏較偏中下焦，黃芩及黃連較偏上中焦；雖然古籍所載與本研究及現代藥理研究有不盡符合之處，不過這樣的結果也提供了我們另外一個角度的思考空間。

在黃芩、黃連、黃柏組個別的基因中，我們做了以下的探討：

就黃芩而言，對 parathyroid hormon-like hormone (PTHrP) 這個基因有上調的作用，此基因為一內生性抗增生因子 (endogenous antiproliferative factor)，可調節表皮及毛髮的生長，所以可以抑制此基因的藥物有治療乾癬的作用，中醫認為「肺主皮毛」，皮膚的疾病是歸類於「肺」這個臟象，黃芩在中醫古籍的記載之一是歸於肺經，即歸類於「肺」這個臟象系統，中西醫學的鴻溝在此已被拉近；再者，erythropoietin receptor 這個基因是促進造血細胞的成長和分化，對紅血球的增生有幫助，而黃芩對「胎熱不安」有療效，在中醫而言所謂的「胎熱不安」是指懷孕時新陳代謝上升，因產婦須聚血以養胎，以致於身體的血液、體液在其它部位相對的灌流不足，為求血循的充足，身體消耗能量上升，心臟做功增加，而導致所謂的「火氣」(hyperfunction)便上來了，在懷孕期中醫稱之為「胎熱不安」，黃芩對 erythropoietin receptor 這低基因有上調的作用，可促進造血細胞的成長、分化和紅血球的增生，剛好解釋了黃芩為何能治療胎熱不安的原因；另外黃芩對 major histocompatibility complex class I, B(HLA-B)有下調的作用，HLA-B 可與 cytotoxic T cell 上的 CD8 結合，以進行細胞毒殺，黃芩可下調此免疫反應，而中醫認為其有清熱瀉火解毒的作用，可能與此抑制發炎及免疫反應有密切的相關。

Vascular cell adhesion molecule 1 為一種 cell surface glycoprotein，可藉由 cytokine 活化 endothelium 來表達，並吸引 monocyte 及 lymphocyte 靠近，黃柏可下調此基因，以對抗發炎反應；黃柏對 major histocompatibility complex, class II, DR beta-5(HLA-DRB5)亦有抑制作用，使帶有 CD4 的 helper T cell 無法辨識以活化 B cell、macrophage、T cell 等來啟動免疫反應，所以黃柏其清熱瀉火，可療癰瘡腫毒在此已提出實證；在黃柏上調的基因方面，phosphoenolpyruvate carboxykinase 為肝糖合成 (gluconeogenesis) 的 key enzyme，黃柏可上調 phosphoenol pyruvate carboxykinase 2, mitochondrial (PEPCK2)，故對機體醣類的合成有上升的作用，中醫認為黃柏雖為寒藥但作用不強，且稍具補性，或許在此可得到一些啟發。另外，ADP-ribosylation factor-like 4 (ARL4) 這個基因在青春期的 germ cell 與成人的 testis 有表達，其與精子的產生也有一定的相關，而黃柏對比基因有上調的作用，中醫認

為黃柏的歸經之一是入腎經，屬下焦，與人體的泌尿生殖系統有關，不知可否以此基因的作用來解讀黃柏一部分的中醫藥理。

在黃連上調或下調的基因中發現 MARCKS-like protein (MLP)、solute carrier family 1, member3 (SLC1A3), potassium channel, voltage-gated, delayed-rectifier, subfamily s, member1(KCNS1) 與神經系統的傳導、動作電位的控制、neurotransmitter 的調節有關，即與人體中樞神經系統、大腦皮質層的活動有關，這或許與黃連在中醫的歸經之一是入心經可相印證；此外，黃連對 S100 calcium-binding protein A13 (S100A13), calcium-modulating cyclophilin ligand (CAMLG)等與 Ca²⁺有相關的基因分別有下調與上調的作用，顯示與肌肉的收縮或訊息的傳遞有一定的關聯；desmocollin 2 是屬於 calcium-dependent glycoproteins 這個 superfamily 中的一員，為一種 cell adhesion molecules，對促進傷口組織的癒合有一定的作用，而黃連可上調此基因，故可能對療瘡口癰瘍有幫助。

另外，本研究之所以採單色酵素呈色法，乃因在實驗過程中雙色呈色系統會出現紅、藍色度差異太大(偏紅或偏藍)，而造成整個基因表現上的上調或下調，容易因酵素呈色反應的過度與不足而影響基因表現量的值，故為改善上述問題，本研究便以單色酵素呈色法來進行。

貳、結論

本研究嘗試將中藥傳統的臨床療效和現代分子生物科技相結合，從基因的層次來探討中藥的作用。從黃芩、黃連、黃柏三種中藥寒藥共同下調的基因像 cytochrome c oxidase, subunit VIa, polypeptide 1 (COX6A1), ubiquinol-cytochrome c reductase-binding protein (UQCRB)來看，可知中藥寒藥確實會藉由抑制呼吸鏈的第三及第四個複合體，使電子傳遞鏈無法進行，ATP 無法產生，而造成機體能量降低。

而 Calmodulin 則會調節體內鈣離子的分布，並具有五大功能。一、調節鈣離子濃度。二、協調神經系統。三、激活酵素系統。四、能量的啟動器。五、活化巨噬細胞。此三種中藥寒藥亦可下調此基因。

另外，macrophage migration inhibitor factor, MHC class I, A, interferon-induced transmembrane protein 1 等此三種中藥亦有抑制的作用，顯示抑制發炎及免疫反應亦為寒藥共同之特性。

本研究亦發現在基因晶片的表現上反而是黃連和黃柏有較多共同上調或下調的基因，剛好與現代藥理研究相吻合，因黃連與黃柏之主要有效成分均為小檗鹼(黃連素)，只是黃連中小檗鹼的含量較黃柏為高;黃芩則不含小檗鹼，主要有效成分為黃芩苷及黃芩素。如此或許便可解釋為何黃連與黃柏的上調、下調基因較接近，此結果雖與中醫古籍之記載有所出入，但也讓我們了解到中醫現代化與中西醫結合之必要，因此，中醫藥的基礎研究已是刻不容緩的課題。

就個別的基因而言，黃芩尚有抑制 IL-6 及促進 interferon-stimulated transcription factor 3, gamma 的作用；黃連則可抑制 interferon, alpha-induced protein (IFI-6-16)及上調 macrophage migration inhibitor factor 的作用；黃柏會下調 T cell receptor delta locus 而上調 macrophage migration inhibitor factor, interferon-related development regulator 1, interleukin enhancer binding factor 2, 45KD 等，但黃芩、黃連、黃柏這三種中藥對 macrophage migration inhibitor factor 亦有共同下調的作用，可知這三種中藥在一些特定的基因上(例如：macrophagemigration inhibitor factor) 似乎同時具有上調及下調的作用，或許可以此來解釋中藥的雙向調節的作用，而中藥在臨床的應用上也會將某一特定的藥物或方劑，利用其與不同藥物的配伍或其本身劑量的調節，以使其針對不同機體的生理狀況而有不同的藥理作用;從本研究中可發現同一種藥物可同時上調或下調的一些特定的基因，這或許可以用來說明中藥為何能利用君臣佐使的配伍及引經藥而使同樣的藥物產生不同的藥理作用。

黃芩、黃連、黃柏這三種寒藥中有些共同之功效，如治療濕熱瀉痢、黃疸及其共同之歸經，本研究並未能找出可能相關對應之基因；其他如附表所示，三種寒藥個別之主治所列舉之可能相關基因名稱(表 M-P)，乃筆者就本研究三種寒藥取 log2 後，分別就其上調及下調之前、後 30 名基因，

上網至 Pubmed 及 NCBI 作搜群所呈現之結果，希望能提供一些相關的參考，有些功效亦無法找出相關之基因名稱，至於正確的基因名稱為何？尚待後續相關的研究來做詳盡的歸類。

在中醫的藥理上，黃芩、黃連、黃柏均是屬於清熱燥濕、瀉火解毒之苦寒藥，適用於熱性病，且脾胃虛寒者皆忌之三者不同之處在於黃芩有清肺火及止血安胎的作用，黃連有清心火及胃火的作用，而黃柏則有清退虛熱及清腎火的作用；故有"黃芩治上焦，黃連治中焦，黃柏治下焦"之說。對照於基因層面而言，此三藥有相似的，也有不同，可知三者啟動的寒性基因之不同，亦即未來再配合臨床研究，寒性基因仍可再進一步歸類，作更精準的分類。

本研究嘗試從基因的層面來探討中藥的藥理作用，而結果也使古人的觀察有了可以用現代醫學基因層次來解釋的空間。不僅發現了作用在 HEK293 腎細胞上之可能的寒藥相關作用基因，同時也發現中藥的確是多靶向、多基因的作用，此次找出了許多可能與寒藥相關的基因，未來更需再重複做幾次實驗，已確定其再現性的高低；Northern blot 加以分析定量；且進一步的研究其基因之作間交互作用的影響；結合熱藥、甚至結合 Western blot 蛋白質體學及動物實驗和人體實驗，以徹底了解其意義之所在。

參、參考文獻

1. Chen JJ, Wu R, Yang PC, Huang JY, Sher YP, Han MH, Kao WC, Lee PJ, Chiu TF, Chang F, Chu YW, Wu CW, Peck K. Related Articles Profiling expression patterns and isolating differentially expressed genes by cDNA microarray system with colorimetry detection. *Genomics*. 1998 Aug 1; 51(3):313-24.
2. Gening LV, Klincheva SA, Gusev AS, Surovoy AY, Potapov VK. SSCP screening of individual aptamers. *Biotechniques* 2001 Oct; 31(4):828, 830, 832, 834.
3. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Related Articles Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotechnol*. 1999 Oct; 17(10):994-9.

4. Izumoto S, Ohnishi T, Kanemura H, Arita N, Maruno M, Moriuchi T, Suzuki S, Yoshimine T. PTEN mutations in malignant gliomas and their relation with meningeal gliomatosis. *J Neurooncol* 2001 May; 53(1):21-6.
5. Oda Y, Nagasu T, Chait BT Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome. *Nat Biotechnol.* 2001 Apr; 19(4):379-82.
6. The International SNP Map Working Group. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001; 409:928-933.
7. Zhou H, Watts JD, Aebersold R. Related Articles A systematic approach to the analysis of protein phosphorylation. *Nat Biotechnol.* 2001 Apr; 19(4):375-8.
8. Bains W, Smith GC: A Novel method for Nucleic Acid Determination. *J theor Biol.* 1998; 135:303-307.
9. Hacia JG: Resequencing and mutational analysis using oligonucleotide microarrays. *Nature genet (suppl)* 1999; 231:42-47.
10. Fodor SPA: Massively parallel genomics, *Science* 1997; 277:393-394.
11. Marshall A, Hodgson J: DNA chips: An array of possibilities. *Nat Biotechnol.* 1998; 16(1):27-31.