嘉南藥理科技大學專題研究計畫成果報告

長期服用龍膽瀉肝湯對肝臟酵素之影響 (二)

計畫類別：■個別型計畫　　□整合型計畫

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中文摘要

本計畫為接續前期「長期服用龍膽瀉肝湯對肝臟代謝酵素之影響」之計畫。由前期計畫之研究結果發現，經七天連續服用「龍膽瀉肝湯」後，大鼠肝臟中 P450 酵素並未產生明顯變化，然而由微陣列晶片試驗卻顯示約有 75 種的基因表現有調高(up-regulation)的現象，同時約有 30 種的基因表現有調降(down-regulation) 的現象。其中較顯著調高的基因(即治療組與對照組在晶片上的基因表現，平均值比率大於 6.0)有：Na-K-Cl cotransporter (20.026)、 peptidylarginine deiminase type IV (9.271)、 beta-globin (9.103)、phosphatidylinositol-4-phosphate 5-kinase (8.703)、lysozyme (7.85)、 hemoglobin beta subunit, major form (7.776)、 hemoglobin alpha chain (7.762)、 cyclin A (7.555)、alpha-2-globin chain (7.154)、 galectin-7 (6.906)、H. sapiens HT021 (6.803) 及 PHD-finger protein (6.162) 等。至於具較顯著調降的基因(即治療組與對照組在晶片上的基因表現，平均值比率小於 0.2)有：ribosomal phosphoprotein P0 (0.117)及 cathepsin H (0.174)。經以電腦蒐尋基因庫及文獻資料，有關上述調高與調降基因在生理上的影響將在本文中討論。

關鍵字：龍膽瀉肝湯、基因表現、生理影響

方法

利用網際網路 Pub Med 基因庫蒐尋先前計畫之晶片實驗所得結果中，具顯著調高與調降之基因對生理特性與功能之影響並加以討論。

結果與討論

A. Microarry　實驗條件

Sample type: RNA　Organism: Rat　　Tissue: Liver

A260/280: 1.842 (Control)　1.949 (Treatment)

Concentration: 2.82 μg/μl (Control)　4.26 μg/μl (Treatment)

Chip type: ABC Rat UniversoChip 8K-1

cDNA labeling: 20.02 μg (Control)　40 μg (Treatment)

Hybridization: 42℃, 15.5 hrs

B. 分析條件

Scanned by: GenePix 4000B [82719]

Image wavelengths: 635 nm (the emission wavelength of treatment)

532 nm (the emission wavelength of Control)

PMT (V): 720, 760　Laser Power (V): 5.3, 3.3

Analyzed by GenePix Pro 3.0.5.56

C. 分析結果

晶片試驗與掃描參數

	NF ratio of median (ALL)	NF ratio of mean (ALL)	635 PMT Volts	532 PMT Volts
R84,85,86	0.975499638	0.942346206	690	610

NF ratio of median = Normalization Factor of Ratio_of_Medians

NF ratio of mean = Normalization Factor of Ratio_of_Means, Base on All Genes (exclude Not found & Bad)

NF ratio of mean (HK) = Normalization Factor of Ratio_of_Means, Base on HouseKeeping Genes (exclude Bad)

試驗規劃設計

晶片使用	晶片操作	Sample Labeling
R84,85,86	百恩諾代客操作	Cy3: Reference
		Cy5: Treatment
		Ratio=(TREATMENT)/(CONTROL) Cy5/Cy3

1. 調高基因種類 （ Ratio_of_Means > 2 and (F635_Mean - B635) >= 1000）

Name	Ratio of Mean	Name	Ratio of Mean
50 kDa dynein-associated polypeptide	2.143	insulin-induced growth response protein	2.431
actin, beta, cytoplasmic	2.127	lysozyme	7.850
acyl-CoA synthetase 5	2.067	M.musculus hypothetical protein H19	2.660
alpha-2-globin chain	7.154	mannose-6-phosphate receptor	2.267
beta-globin	9.103	matrix Gla protein	2.015
C.elegans hypothetical protein F36D4.5	2.438	microsomal epoxide hydrolase	2.369
C.elegans hypothetical protein R08D7.3	2.177	Moesin	2.300
Calreticulin	2.248	Na-K-Cl cotransporter	20.026
Cathepsin A	2.332	osteonectin	2.705
cathepsin B	3.400	p41-Arc	3.325
cathepsin C	2.464	palmitoyl-protein thioesterase	2.946
Cathepsin D	2.931	peptidylarginine deiminase type IV	9.271
cathepsin K	4.260	PHD-finger protein	6.162
cathepsin L	2.300	Phosphatidylinositol 4-kinase	3.629
cell adhesion inhibitor beta ig-h3	2.810	phosphatidylinositol-4-phosphate 5-kinase	8.703

cell-binding bone sialoprotein	2.875	pro-alpha-2(I) collagen	2.045
Chloride channel protein (p64)	2.614	profilin	2.233
chloride intracellular channel protein 1	2.058	Protein disulfide isomerase	2.010
Clusterin	2.800	protein tyrosine phosphatase-like protei	2.542
collagen alpha 3(VI) chain	2.283	putative steroid dehydrogenase KIK-I	3.621
cyclin A	7.555	Ran/TC4 GTP-binding nuclear protein	2.046
cystatin beta	2.127	RGICP19	2.502
cytokeratin 5	2.021	Ribosomal phosphoprotein P0	2.091
dihydropteridine reductase	2.000	squalene epoxidase	4.684
DIM1 protein homolog	5.301	syndecan	3.032
erb B-2	2.031	thymosin beta-10	2.038
far upstream element-binding protein 2	3.061	TIMP-2 (AI058866)	2.133
Farnesyl pyrophosphate synthetase	3.173	Topoisomerase II	2.837
fibronectin	3.872	TPA	3.198
galectin-7	6.906	transcription factor B-ATF	2.086
glucose-6-phosphate dehydrogenase	2.058	Tubulin, beta-15	2.277
glutathione peroxidase	2.067	tubulin, gamma	2.215
glutathione S-transferase	3.785	Ubiquitin	2.079
GTP-binding nuclear protein Ran/TC4	2.239	ubiquitin-homology domain protein	2.487
guanine nucleotide-binding protein, G(i)	2.118	UDP-glucose dehydrogenase	3.987
H.sapiens HT021	6.803	UDP-glucuronosyltransferase UGT1A7	2.950
hemoglobin alpha chain	7.762		
hemoglobin beta subunit, major form	7.776		
hypoxia-inducible factor-1 alpha	3.004		

2. 跳降基因種類 （ Ratio_of_Means < 0.5 and (F532_Mean - B532) >= 1000）

Name	Ratio of Mean	Name	Ratio of Mean
3-hydroxy 3-methylglutaryl coenzyme A sy	0.466	MAP kinase kinase	0.317
Adenine phosphoribosyltransferase	0.347	mitochondrial	0.486
cardiac triadin	0.336	myosin light chain 2	0.270
cathepsin H	0.174	myosin light chain kinase	0.421
CCAAT-binding transcription factor I, su	0.355	NADH-ubiquinone oxidoreductase 18 kDa su	0.381
Chromogranin A	0.327	NADH-ubiquinone oxidoreductase NDUFS2 su	0.361

Collagenase	0.349	protein disulfide isomerase-related prot	0.369
creatine kinase	0.464	Ras protein, c-Ha	0.289
cystatin C	0.428	rat leukemia virus polymerase	0.118
cytochrome C oxidase polypeptide VIIb	0.478	ribosomal phosphoprotein P0	0.117
golgi stacking protein homolog GRASP55	0.331	Ribosomal protein S3	0.348
Heparin-binding growth associated protei	0.428	secreted apoptosis related protein 1	0.253
heparin-binding neurotrophic factor	0.370	Stat3	0.489
homeobox containing nuclear transcriptio	0.336	Syntaxin 2	0.211
immunoglobulin superfamily-like protein	0.294	transcobalmin II	0.486

經由基因庫蒐尋及文獻查詢得知，增加 Na-K-Cl cotransporter 基因之表現，可經由過渡增加 Na+ 及 Cl-進入神經細胞而與中樞神經細胞之興奮有關，同時也增強 ADH 的抗利尿作用，因此可能引起水腫與神經之傷害（is involved in the acute excitotoxicity as a result of excessive Na+ and Cl- entry and disruption of ion homeostasis.）；peptidylarginine deiminase type IV 基因之過渡表現與增加關節炎感受性有關；beta-globin 及 alpha-2-globin chain 基因表現之增加則可改善骨髓及脾臟造血細胞的功能，避免引起貧血發生；phosphatidylinositol-4-phosphate 5-kinase 基因表現的增強可能經由促進 actin remodeling 的功能而增強巨噬細胞的吞噬功能；lysozyme 基因表現的增強牽涉消炎的增強，可促進殺菌作用；cycline A 基因表現的增加則與腫瘤細胞（如 acute lymphoblastic leukemia）之增殖有正關係；galectin-7 基因的過度表現與某些腫瘤的增殖(如 aggressive lymphoma)有關，但也有報告與細胞之凋亡也有相關；PHD-finger protein 的過度表現也與腫瘤的形成有關；hemoglobib alpha chain 與 hemoglobin beta subunit, major form 兩者基因表現接高度增強，有利於血色素之形成。至於調降之基因經蒐尋文獻則未見有意義的影響。

由結果顯示，長期投予「龍膽瀉肝湯」雖然對 cytochrome 450 酵素系統無顯著影響，但仍然會影響許多具有生理活性基因。雖然有些基因的表現增強具有提高免疫及殺菌、消炎與造血的效果，但是也有某些基因卻顯現有可能導致腫瘤細胞增殖。以上所呈現基因變化之複雜性可能與此方劑所含成分之多樣複雜性有關，因此，長期使用中藥製劑仍須小心評估。

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