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以基因體研究平台為基礎進行防治肝病相關中藥之研究

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摘要

研究目的

　　中醫利用方劑及單味藥治療肝病已有長久的經驗,例如小柴胡湯、龍膽瀉肝湯、三黃瀉心湯等,都是常見的治肝病方劑。雖然這些中草藥療法已有長久的臨床使用經驗,不過許多中草藥的作用機制並不清楚,而且中草藥於新藥開發的應用仍有很大的發展空間。

研究方法

　　本計畫主要利用 DNA 微陣列為工具,分析中草藥對細胞基因的生物活性反應,以建立一套完整的中藥生物活性基因圖譜資料庫,並將資料庫應用於中草藥之作用機轉、傳統中醫藥之現代藥性、新藥的開發及臨床前藥物安全性之分析。

結果與討論:在中藥生物活性基因圖譜資料庫方面,我們已建立一套標準操作流程來執行,標準操作流程包括細胞加藥或是動物給予藥物的流程管控、萃取 RNA 時的細胞狀況管控、萃取 RNA 的質和量的管控、DNA 微陣列實驗以及數據分析過程的品質管控等。以利用基因表現圖譜分析天然化合物之作用機轉為例,藉助 Gene Ontology 的程式運算,我們發現許多中藥組成份所影響的基因,大多坐落於細胞週期及細胞凋亡等兩種群組。利用基因表現圖譜分析天然化合物對肝臟代謝藥物的影響的分析呈現,發現某些蒽醌類化合物會降低 phase II 藥物代謝相關基因(例如 UDP glycosyltransferase 等)的表現,代表這類化合物可能會減緩藥物的排泄,增加藥物於體內存留的時間。在利用基因表現圖譜分析中醫方劑治療肝病之機轉及其組方概念方面,以三黃瀉心湯為例,在中藥方劑及單味藥的現代藥性方面,我們分析三黃瀉心湯及其單味藥的基因表現圖譜,發現這些藥物主要藉助 p53, p53 activated 及 DNA damage 等訊息傳導路徑,展現很獨特的抗細胞增殖模式,而利用 network analysis,發現這些藥物影響的基因都與核心分子 p53 有關,進一步利用 hierarchical clustering 分析,發現三黃瀉心湯之組成份—黃連所影響的基因波動圖譜非常類似於三黃瀉心湯所影響的基因波動圖譜,代表傳統醫學認為黃連為三黃瀉心湯之君藥的合理性,而這個結果也是首次利用現代生物技術,揭露中藥方劑及單味藥的相關性。在中藥生物活性基因圖譜資料庫的建立及分析方面,我們已經進行健保常用方藥及肝病常用方藥之轉錄體學分析。將這些方藥給予小鼠,萃取肝臟和腎臟的 RNA 進行 DNA 微陣列分析,再藉由 Pathway 分析後,發現除了代謝相關的 pathways 以外,antigen processing and presentation pathways 及 IGF signaling pathways 也受到這些方藥的調控與影響。而在疾病相關性的分析方面,發現與方藥相關的前 30 名 MeSH disease terms 主要是與 diabetes-associated、cardiovascular 以及 hepatic 相關的疾病。另外,連結方藥與 Connectivity Map 的基因表現圖譜,發現方藥與抗癌、抗發炎以及抗氧化方面的西藥或化合物有較高的相似性。最後,我們分析毒理相關基因的表現,我們藉助 223 個化學藥物所構建的 toxicology-related cDNA 微陣列的資料庫,比對方藥處理至動物腎臟毒理代謝相關基因的表現情形,發現兩者趨勢不同且圖譜相距甚遠。因此,我們認為中醫方藥在 toxicology-related genes 的基因圖譜表現上與化學藥物是有所區別的,也表示,中醫方藥處理至實驗動物後,其藥物在腎臟毒理方面是無顯著急性顧慮的。其他常用肝病方藥之生物活性基因圖譜正在持續擴充及分析中,未來預計開發新的生物資訊程式用以進行中藥方劑與疾病和西藥相關性的分析,進一步則期望能將中藥生物活性基因圖譜資

料庫所含的大量資訊和既有的傳統中醫知識做整合與對應。

關鍵詞：肝病、中草藥、DNA 微陣列

Research and Development of Chinese Medicinal Herbs for Anti-Liver Diseases by the Genomic-Based Platform

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ABSTRACT

Aim

Although several herbal formulae and herbal components have been used in patients with liver diseases, their applications in new drug development remain to be discovered. Method: We applied microarray technology to analyze and explain the modern biological activities of medicinal herbs. The gene expression databases established in this project further provided the basis for the herb-activity relationship, modern definition of Traditional Chinese Medicine (TCM), new drug development, and preclinical drug safety analysis.

Results and Discussion:

The gene expression databases were established according to the standard operation protocols, including quality controls of drug treatment, cells, RNA extraction, microarray, *etc*. Microarray data have been used for the prediction of therapeutic potentiation. For examples, in the aspect of new drug development, we treated human hepatocytes with herbal compounds at the dosages of TC_0 and TC_{50}, and analyzed microarray data by Cluster and Gene Ontology Tree Machine. Our results indicated that most herbal compounds affected clusters of genes involved in cell cycle and apoptosis. Genes down-regulated by herbal compounds were grouped into three gene ontology categories, including regulation of cellular process, cell cycle, and death. Furthermore, *k*-means clustering analysis showed that a cluster of nuclear factor-κB-regulated genes was down-regulated by herbal compounds. In the aspect of drug safety evaluation, we found that the expressions of genes (DP glycosyltransferases) in phase II drug metabolism were reduced during anthraquinones treatments. These findings suggested that anthraquinones may slow down the excretion of drugs, leading to the increased half-life of drugs. In the aspect of modern definition of TCM, San-Huang-Xie-Xin-Tang (SHXXT) has been used for the treatment of liver diseases for years. Gene set enrichment analysis indicated that SHXXT and its herbal components displayed a unique anti-proliferation pattern via p53, p53 activated, and DNA damage signaling pathways in HepG2 cells. Network analysis showed that most SHXXT-affected genes were regulated by a central molecular, p53. Additionally, hierarchical clustering analysis showed that Rhizoma Coptis shared a similar gene expression profile with SHXXT. These findings may explain why Rhizoma Coptis is the principal herb that exerts the major and leading effect in the herbal combination SHXXT. Moreover, this is the first time to reveal the relationship between formulae and their herbal components in TCM by microarray analysis and bioinformatics tools. So far, we have established and analyzed the gene expression profiles of herbal formulae that are regularly used in the treatment of liver diseases in TCM. Mice were orally administered with herbal extracts, and the gene expression signatures from livers and kidneys were analyzed by DNA microarray. By Pathway analysis, we found that herbal formulae affected metabolism-related pathways as well as antigen processing and presentation pathways and IGF signaling pathways. Connectivity Map analysis showed that top 30 MeSH disease terms related to the gene expression signatures of herbal formulae were diabetes-associated diseases, cardiovascular diseases, and hepatic diseases. Moreover, the gene expression profiles of herbal formulae were similar to the ones of drugs or compounds with anti-cancer, anti-inflammatory, and anti-oxidative abilities. Finally, clustering analysis of gene expression signatures from herbal formulae and toxicology-related database from 223 chemicals showed that herbal formulae and 223 chemicals belonged to two different clusters.

These findings indicated that administration of herbal formulae did not induce acute nephro-toxicological effects in mice. In conclusion, the microarray-based gene expression database of herbs can provide a translation platform for TCM and scientific knowledge.