行政院國家科學委員會專題研究計畫　成果報告

癌症病理標本微陣列基因表現核心實驗室

計畫類別：個別型計畫
計畫編號：NSC91-2323-B-039-002-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：中國醫藥大學中國藥學研究所

計畫主持人：黃國華

報告類型：完整報告

處理方式：本計畫可公開查詢

中　華　民　國 92 年 11 月 14 日

製藥與生物技術國家型計畫
全程結案成果報告

計畫編號：NSC 91－2323－B－039－002
執行期限：89 年 8 月 1 日至 92 年 8 月 1 日
主持人：黃國華　中國醫藥學院中藥研究所

一、中文摘要

我們在有限的時間裡完成了計畫書的特定目標，建立標準的微陣列核心實驗室、建立國內第一個婦科癌症微陣列基因表現資料庫、並且發展了結合微陣列數據及代謝工程模擬的方法。除此之外，我們利用自茶葉中萃取的黑色素，證明保肝作用以成為癌症化學療法的輔助藥物。

關鍵詞：微陣列、基因表現資料庫、婦科癌症、黑色素。

Abstract

We have accomplished all specific aims originally proposed within the limited period. We have established a standard microarray core laboratory; we have constructed a microarray expression data bank for gynecological cancers; we have developed utilization of microarray dataset into the simulation of metabolic engineering. In addition, we extracted melanin from tea. We also demonstrated the liver-protecting activity for the purpose of developing modulating agent for cancer chemotherapy.

Keywords: Microarray, Gene expression databank, Gynecological cancers, Melanin.

二、緣由與目的

Complimentary DNA microarray, when developed in early 90s, was anticipated as a revolution of biomedical research for the millennium, a tool that will bring researchers to the promise land in the shortest time. This high throughput, brut-force technology, with highly expectation, however, did not instantly speed up scientific research even in the United States as it promised. Ironically, the scientific literature contains more reviews about arrays than primary research papers applying them, due to an uneven distribution of microarray facility in academic society. As more and more institutes all over the world jumped into this field with sharpened instrumentation and enormous funding, researchers in Taiwan faced a tremendously competing situation that could never lose. A center providing economic, convenient and standard service of microarray is desperately in need.

We proposed to set up a microarray core laboratory providing gene-profiling services for component projects. A standard operation procedure will be developed so that gene-profiles of cancer tissues at various diseased stages can be compared. ***We proposed to establish a sequence-verified cDNA microarray containing 30,000 independent human cDNA/EST clones in the first year.*** We intended to investigate and improve the quality of cDNA and spotting ***using generally approved methodology***. ***Consistency and sensitivity will be the primary issues in this matter.*** We would optimize the tedious procedures required for probe preparation, hybridization and color development. In addition, we intended to automate key steps according to

standard operation procedure. We would build up and maintain a data bank containing raw, organized and analyzed data generated from the core. Since only handful analytical tools are available at the present time, we intended to develop novel methodology to process information derived from this project. In the year 2003, when human genome project is to be completed, the core will have the most updated cDNA collection, a standard service, a standard output and accessible data bank to serve the biomedical society in Taiwan.

The specific aims of this proposal are:

1. To establish a *quality verified*, standard microarray core laboratory, as a service core facility to analyze pathologic tissues from Cancer Research Group.
2. To establish cDNA microarray containing 30,000-sequence verified cDNA/EST clones.
3. To establish and maintain a data bank for gene expression profile of cancer tissues.
4. To develop methodology for analyzing and modeling of overall pathologic expression.

三、結果與討論

1. construction of a microarray databank containing cancer expression/microarray datasets (Welsh, 2001; Wang, 1999; Aunoble, 2000; Ono, 2000; Tonin, 2001).

A cancer tissue bank containing cancerous and normal tissues of Gynecological organs was established in the lab under the collaboration with China Medical College Hospital. The tissue bank contains sufficient quantity of cancer tissue for the purpose of constructing an Expression Databank. The result differentiated expression profiles between cervix cancer, myoma and ovarian tumor. Cluster analysis indicated that these three types of cancer exhibited substantially distinct profiles. With increasing cases adding to the analysis, we were able to isolate hundreds of genes specific for each type of cancer. At this time point, the Cancer Expression Data Bank contains datasets included 11 sets of cervix cancer, 7 sets of myoma, and 14 sets of ovarian cancer. The size of the databank is about 20 Giga bytes.

In addition to the individual differentially expressed gene, there is a systematic change for the gene expression from normal tissue, ovarian tumor, borderline ovarian malignancy, to ovarian cancer. In fact, differential gene expression of our survey revealed the down regulation of several systems including protein synthesis, stress response, apoptosis, and cell-cell signaling. Our data also exhibited up-regulation of transmembrane receptors, nucleic acid metabolism.

2. development of a microarray core with standard operating procedure.

成功結合 CARD 及 Cy3-Cy5 玻璃螢光技術，已經可利用<1μg total RNA 進行 microarray 實驗。**比較 membrane microarray 敏感度提高 500 倍以上。**

3. Correlating microarray datasets with physiological systems.

We have explored possible incorporation of microarray data to the simulation of metabolic pathways. We incorporated gene expression information from cDNA microarray into flux analysis to simulate yeast diauxic growth. Expression ratios of both growth phases were applied to assign the split ratio at glyoxylate shunt during simulation, in which the equation was mathematically unsolvable due to the singularity and artificial split ratios, which were traditionally introduced without biological evidence. In addition, the

directionality of microarray dataset was used as a further constraint during simulation. Metabolic fluxes obtained by this modified approach are in general consistent with microarray analysis. However, discrepancies occurred when the quantity of fluxes was compared, probably due to the substantial reduction of substrates at phase II in which the increase in the enzymatic levels was not proportional to the increase of substrate flow, as would be predicted from microarray dataset. The modified flux analysis might have brought a new approach to investigate other cellular pathways (Huang, in press.

4. Tea melanin for modulator of cancer chemotherapy

Possible supportive therapy for chemotherapy was explored using melanin extracted from tea.

Antioxidants are recognized as a universal defense against toxic and carcinogenic effects of free radicals. The antioxidants presented in food are of particular interest (Stavric, 1994). For example, the antioxidants of green tea have been recognized as promising natural protectors for many kinds of tumor (Katiyar et al. 1993; Wang, 1989). The protective activity of melanin derived from tea (MDFT) was studied using hydrazine as a DNA-reactive chemical agent. Intra-peritoneal administration of MDFT at the doses of 5 or 20 mg/kg dose-dependently prevented liver toxicity induced by hydrazine in rats. It normalized rises in serum alanine transferase activity and a decrease in the glutathione level in the liver. It also reduced the hepatic malondialdehyde concentration. Monitoring the intensity of chemiluminescence showed that MDFT could prevent the production of free radicals that are generated owing to metabolic transformation of hydrazine. It also prevented the formation 8-hydroxy-deoxyguanosine (8-OH-dG) DNA adducts. The results obtained *in vivo* and *in vitro* suggest that MDFT confers marked protection of the liver against hydrazine-induced oxidative toxicity (Hung, 2003).

四、計畫成果自評

a. We have dramatically improved the sensitivity for membrane-type microarray. Although 30,000-format membrane is not ready for service due to its sensitivity to the background fluctuation, the 9,600-format membrane was well characterized and is currently in service.
b. We provided free membrane to the academic research in Taiwan. We also collaborated with laboratories due to specialties required to complete the mission.
c. We have completed the construction of Gynecological Cancer Expression Databank. Sufficient datasets have been performed and indicated that we were capable of isolating candidate genes with confidence.
d. The membrane was also applied for the complete profiling of umbilical cord blood, a stem cell that possessed unprecedented potential as applied in the tissue engineering.
e. The glass chip with fluorescence detection was also developed and ready for service.

All our publications in this period indicated that the proposal was executed well and progression is much more than expectation.

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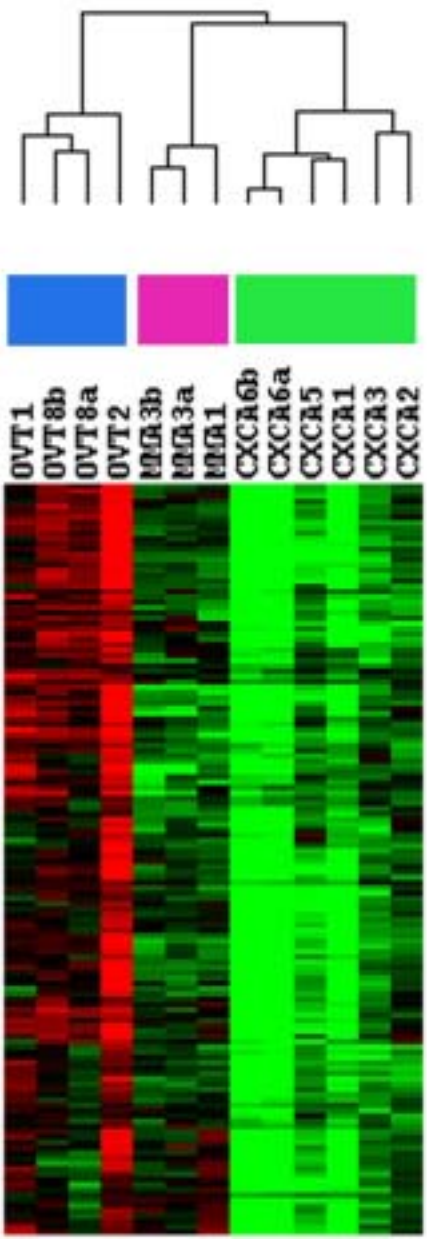
Figure caption:

Fig.1. Cluster analysis of cervix cancer, myoma and ovarian cancer. The calculation of cluster involved 4 cases of ovarian cancer, 3 cases of myoma and 6 cases of cervix cancer. Left: shows myoma-specific genes. Center: shows ovarian cancer specific genes. Right: shows cervix cancer specific genes. Each column contains approximately 200 to 300 gene clusters.

Fig. 2. Gene expression analysis of ovarian tumor/cancers by functional categories. Logarithmic expression ratios (based on 2) for all genes in the same category were averaged. The averaged values were plotted by category for OVT (blank bar), OVTT (shadowed), and OVCA (solid).

Fig. 3. Schematic representation of gene expression ratios (growth phase II/ growth phase I) derived from (A) cDNA microarray analysis and (B) from MBT analysis. Arrows show directionality of pathways. Relative scale for flux ratios is shown as thickness of lines.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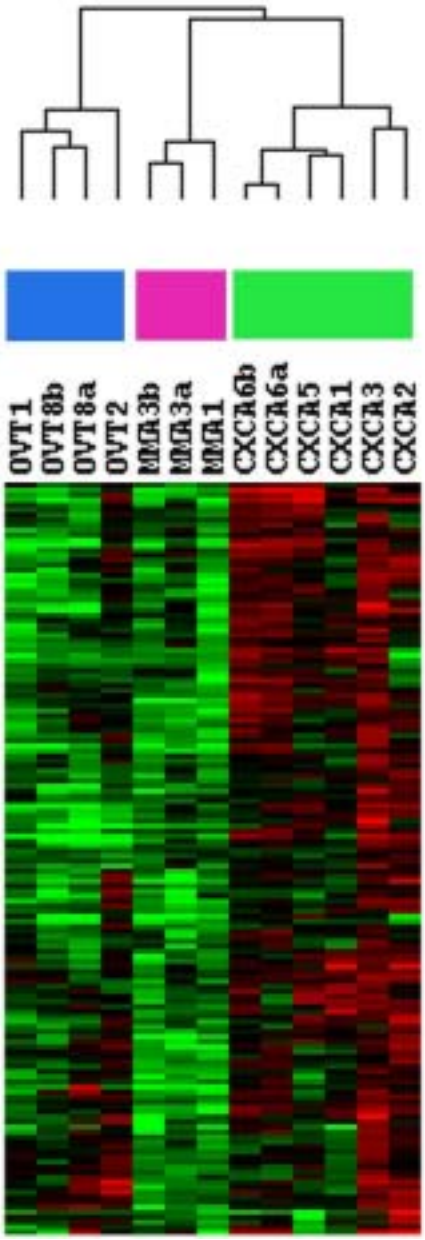
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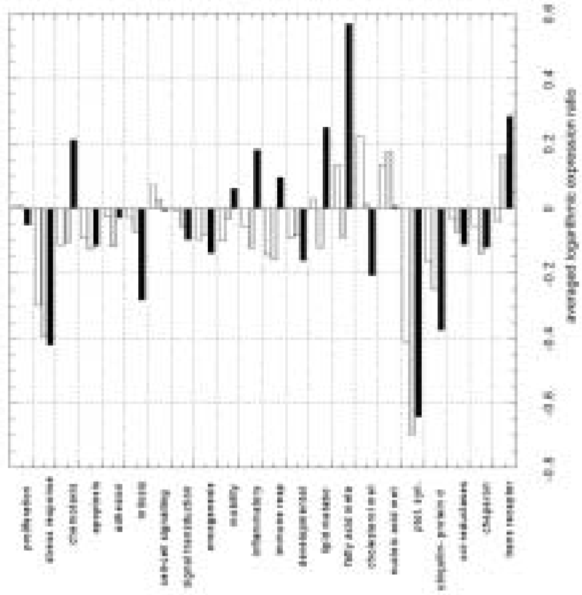
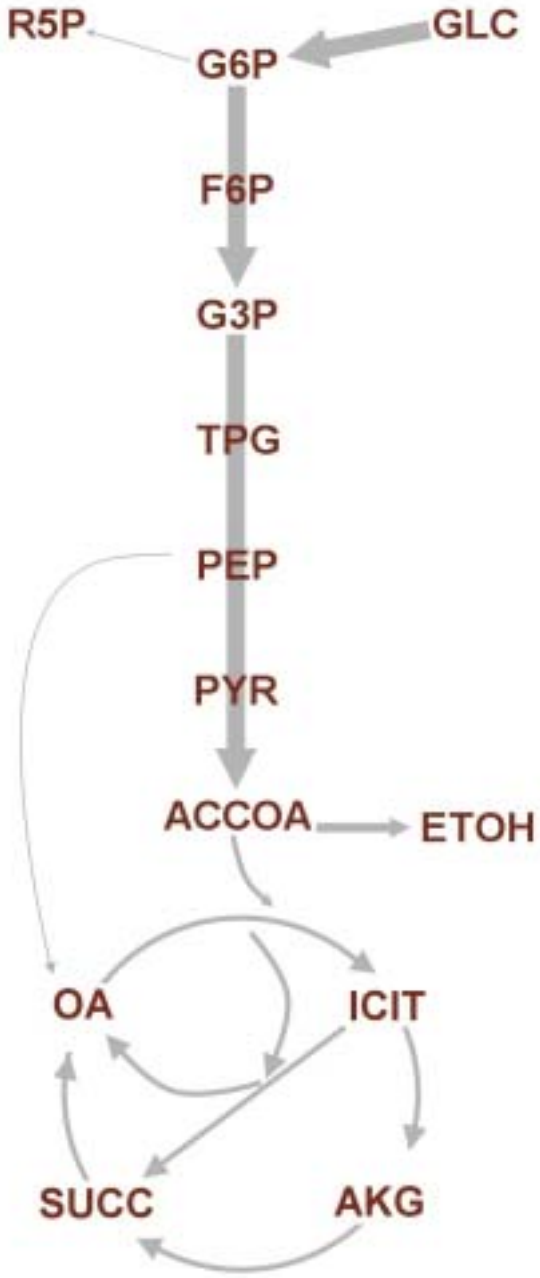

averaged logarithmic expression ratio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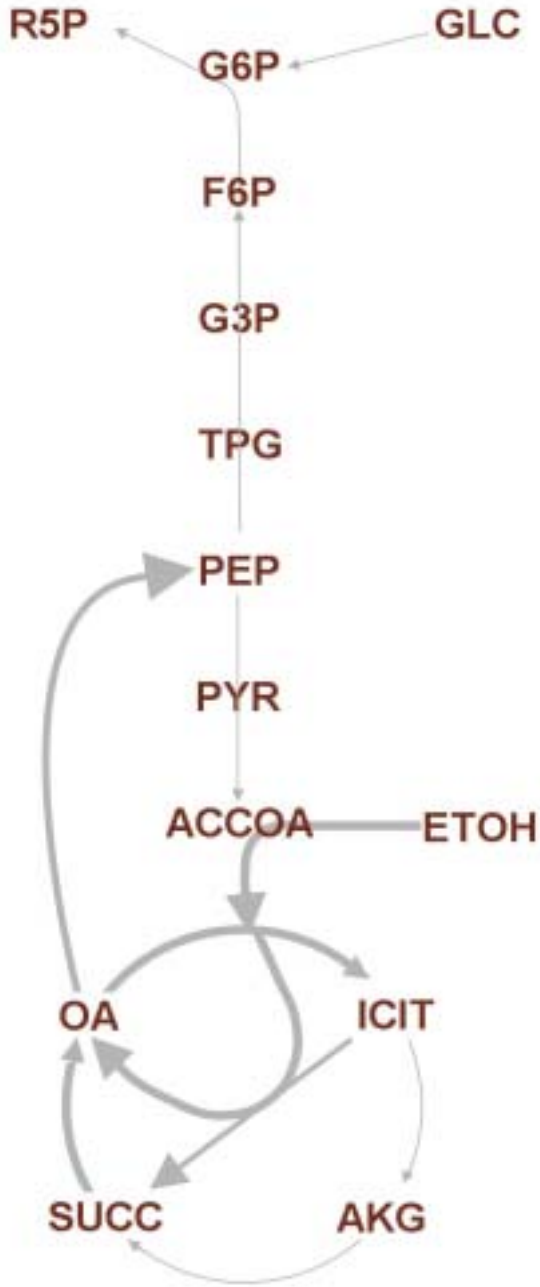
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全程結案成果報告

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※ 計畫中文名稱 癌症病理標本微陣列基因表現核心實驗室 ※
※ ※
※ 計畫英文名稱 Microarray core laboratory for gene expression ※
※ profiling of cancer tissue ※
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計畫類別：■個別型計畫 □整合型計畫

計畫編號：NSC 91ー 2323 ー B ー 039 ー 002 ー

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計畫主持人：黃國華

共同主持人：

計畫參與人員：

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執行單位：中國醫藥學院中國藥學研究所

中 華 民 國 91 年 12 月 30 日

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序號	計畫產出名稱	產出型式	SCI*	致謝與否
1.	Sava VM, Yang SM, Hong MY, Yang PC and **Huang GS*** (2001) Isolation and characterization of melanic pigments derived from tea and tea polyphenols. ***Food Chemistry*** **73**(2): 177-184 (SCI 1749).	國外期刊	0.6	是
2.	Liu, YC, Cheng KY, and **Huang GS**, (2001) *Singularity analysis on the metabolic network equations.* ***J Chin Inst Chem Engrs*** **32**: 69-73.	國內期刊	是	是
3.	Sava, VM, Galkin BN, Hong MY, Yang PC, and **Huang GS*** (2001) A novel melanin-like pigment derived from black tea leaves with Immuno-stimulating activity. ***Food Research International*** 34(4): 337-343 (SCI 1755).	國外期刊	0.615	是
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6.	Hung YC, Sava, VM, Galkin BN, Hong MY and **Huang GS*** (2002) Mechanism of antioxidant activity of melanin derived from tea: comparison between the different oxidative states ***Food Chemistry 78:***233-240 (SCI 1749)	國外期刊	0.6	是
7.	Hung YC, Sava VM, Blagodarsky VA, Hong MY and **Huang GS***, (2003) Protection of tea melanin on hydrazine induced liver injury. ***Life Sciences*** 72: 1061-71 (SCI 3388)	國外期刊	2.38	是
8.	**Huang G. S.***, Hong M.Y. and Liu Y. C. Incorporation of microarray datasets to the simulation and validation of flux analysis in yeast diauxic growth ***Life Sciences*** (in press, SCI 3388).	國外期刊	2.38	是
9.	Sava, VM, Hung YC, Blagodarsky VA, Hong MY and **Huang GS***, The liver-protecting activity of melanin-like pigment derived from black tea ***Food Research International*** (in press, SCI 1755)	國外期刊	0.615	是
10.	Hsieh MT, Hsieh CL, Lin LW, Wu CR and Huang GS, Differential gene expression of scopolamine-treated rat hippocampus- application of cDNA microarray technology. ***Life Sciences*** 73:1007-16	國外期刊	2.38	是
11.	Yao-Ching Hung, Vasyl Sava, Bing-Hsien Wu, Meng-Yen Hong, and **G. Steven Huang***, Inhibitory effects on phospholipase A2 and antivenom activity of melanin derived from *Thea sinensis* Linn. ***Life Sciences*** (in press)	國外期刊	2.38	是
12.	**Huang GS* and Hung YC**, Microarray-nuts and bolts (invited paper, Bio/Pharma Quarterly Journal).	國外期刊		是

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