

行政院國家科學委員會專題研究計畫 期中進度報告

以臨床病理型態、微衛星測試和 cDNA 微陣列共同分析胃癌 之亞型特徵 (2/3)

計畫類別：個別型計畫

計畫編號：NSC92-3112-B-002-025-

執行期間：92 年 05 月 01 日至 93 年 04 月 30 日

執行單位：國立臺灣大學醫學院內科

計畫主持人：林肇堂

共同主持人：楊泮池，吳明賢

報告類型：完整報告

處理方式：本計畫可公開查詢

中 華 民 國 93 年 4 月 29 日

基因體醫學國家型科技計畫

National Research Program for Genomic Medicine
National Science Council, the Executive Yuan, ROC.

計畫名稱-中文

以臨床病理型態、微衛星測試和cDNA微陣列共同分析胃癌之亞型特徵

計畫名稱-英文

**Characterization of Subsets of Gastric Cancer by Combined
Clinicopathologic, Microsatellite, and cDNA Microarray Analyses**

報告類別：☐ 新進研究計畫 ☐ 修正後計畫書 ☒ 年度成果
報告

(New Proposal) (Revised Proposal) (Progress
Report)

計畫類別：☒ 個別型計畫 ☐ 整合型計畫
(Individual Project) (Program Project)

計畫編號：NSC91-3112-B002-007

計畫主持人 (Principle Investigator)：林肇堂

共同主持人 (Co-Principle Investigator)：楊泮池 吳明賢

執行單位 (Institution)：台大醫學院

中華民國

九十三年四月三十日

National Research Program for Genomic Medicine
National Science Council, the Executive Yuan, ROC.
Progress Report—Research Project

基因體醫學國家型科技計畫
國科會延續性計畫進度報告
個別型計畫 (Individual Project)

Program Classification:

☒ Genomic Medicine

☐ Bioinformatics

☐ Proteomics & Structural Genomics

☐ ELSI

Project Number: NSC92-3112-B002-025 (計畫編號)

NSC Funding Number: NSC93-3112-B002-017 (93 年度國科會預核編號)

Title of Project 計畫名稱	(in Chinese) 中文 以臨床病理型態、微衛星測試和 cDNA 微陣列共同分析胃癌之亞型特徵
	(in English) 英文 Characterization of Subsets of Gastric Cancer by Combined Clinicopathologic, Microsatellite, and cDNA Microarray Analyses
Institution 研究 (執行) 單位	(in Chinese) 中文 台大醫學院內科
	(in English) 英文 Department of Internal Medicine, National Taiwan University College of Medicine
Principle Investigator 計畫主持人	(in Chinese) 中文 林肇堂
	(in English) 英文 Jaw-Town Lin

FY	2002	2003	2004	Total
Budget	2,197,000	2,319,700	2,272,135	6,788,835

(in NT dollars: 1USD = 34 NTD)

Signature of the PI : _____ Date : __

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Progress Report

1. Response to previous reviewers' critiques

Please describe the previous reviewers' critiques and how based on the critiques, you made modifications to specific aims, experimental design, or resource allocation etc.

2. Specific Aims

Please state the overall goals of the project, and specific aims, as reviewed and approved by the Study Section and actually awarded. If these specific aims as actually funded did not differ in scope from those actually pursued during the grant period, and if the aims have not been modified, state this. If they have been modified, give the revised aims.

Gastric cancer (GC) is the second most frequent cause of death from cancer in both sexes in the world. It is noteworthy that GC is heterogeneous in histology, location, clinical manifestation, and course. GC can be classified into intestinal and diffuse subtypes according to histology, and can be assorted to cardia and noncardia cancer by anatomic location. Such heterogeneity suggests that GC may be a collection of related, but molecularly distinct diseases. Two genetic pathways, including chromosomal –instability pathway, also known as the microsatellite stable pathway, and microsatellite instability pathway, have been demonstrated to play a role in colonic and gastric carcinogenesis. We have demonstrated that different genetic alterations are involved in different clinicopathologic and epidemiologic features of GC with respect to its anatomic location and histologic subtypes. It is crucial to stratify GC into different histologic types and tumor stages for adequate molecular and pathogenic analysis. This project has been designed to define the spectrum of genetic alterations of GC by laser capture microdissection (LCM) and microarray analysis. Such research may further yield important information in the early detection and prognostic categorization of GC. Specific aims of this study including:

- (1) To establish a clearer picture of molecular classification of GC by analyzing gene expression profiles in different categories of GC.
- (2) To determine molecular markers for predicting progression and prognosis of GC by analyzing gene expression profiles in early, advanced, and metastatic GC.
- (3) To clarify the role *H. pylori* in gastric carcinogenesis by analyzing gene expression profiles in *H. pylori*-positive and-negative GC.
- (4) To redefine the genetic pathways leading to mutator phenotypes by analyzing gene expression profiles in microsatellite instability and microsatellite stable GC.
- (5) To identify different underlying genetic alterations in cardia and noncardia GC.

Following hypotheses will be tested:

- (1) Gastric carcinogenesis is a multistep process accompanied by accumulations of multiple genetic alterations.
- (2) The evolution of sequential histological changes from normal cells, precursor lesion, to invasive cancer is underlied by different molecular basis of progression.
- (3) Etiology-specific critical regulatory genes may be responsible for GC.
- (4) GC with microsatellite instability and microsatellite stable GC represents two different disease entities with distinct genetic pathways.

- (5) A different spectrum of genetic changes is involved in different histologic form or location.

3. Progress Summary

Summarize concisely the results obtained for **each specific aim during the past year (or reporting period)**. Negative results, if any, should also be included and approaches taken to improve the prospects of the project discussed. (Do not exceed **5 pages**, not including figures and references.)

In the past two year, we have collected 75 cancer tissues and their respective non-cancerous tissues from patients with GC who underwent surgery at the National Taiwan University Hospital. There were totally 54 males and 21 females with a ratio of 2.57:1. Both the tumor and the neighboring nontumorous tissues of each patient were obtained at the time of surgery and preserved separately at -70 C until use. Their relevant demographic and clinicopathologic information was obtained from medical records. Mutator phenotype (microsatellite instability) was determined by 5 microsatellite loci defined by the National Cancer Institute, including BAT 25, BAT26, D5S346, D2S123, and D17S250. To establish the microarray system for further analysis of GC tissues in vitro, we have used the RNAs extracted from the human gastric cancer (AGS) and macrophage (THP-1) cell lines, which were co-cultured with 3 different clinical isolates of *H. pylori* from duodenal ulcer, gastric cancer, and Maltoma patients, respectively. Three different *H. pylori* strains induced distinct gene expression patterns in GC and macrophage cell lines. The results, in addition to shed light on the interaction of bacteria and host and possible mechanism of *H. pylori*-related gastric carcinogenesis, confirm the accountability of the microarray system we used (Agilent cDNA array).

Till the April 30, 2004, we have procured pure cancer cells from 45 cancerous tissues from GC for further RNA extraction and amplification by PixCell II LCM System and CapSure HS LCM caps (Acturus, Mountain View, California). After procurement of more than 2,000 cells, total RNA was extracted using PicoPure RNA

Isolation Kit (Arcturus, Mountain View, California, USA). The quality of RNA extracted from LCM procured cells was determined by Bioanalyzer before further amplification and hybridization. Only 21 of their RNAs qualified for further amplification. Total RNA purified from LCM samples was amplified twice by Riboamp kit (Arcturus, Mountain View, California) to antisense RNA (aRNA). Then, 1 µg of aRNA are converted to cDNA using a 3DNA™ Array 50 Expression Array Detection Kit (Genisphere, USA). The labeled cDNA is hybridized to Human 1 cDNA microarray (Agilent Technologies, USA). After first hybridizing with cDNA, microarrays are then hybridized with Cy3 and Cy5 dendrimers in formamide-based buffer. After washing and drying by centrifugation, microarrays are scanned with a Virtek fluorescence reader (Virtek, CA) at 535 nm for Cy3 and 625 nm for Cy5. Scanned images are analyzed by Array-Pro image acquisition software (Media Cybernetics, USA), an image analysis algorithm is used to quantify signal and background intensity for each target element. Data normalization is performed by Lowess method using R package (written by Terry Speeds Microarray Data Analysis Group, University of Berkeley). Dye Swap duplication was performed in each sample. We have hybridized total 62 arrays, including 42 arrays for cancer (21 cases with dye-swap duplication) and 20 arrays for nontumorous cells (10 cases with dye-swap duplication) with universal reference. Preliminary analysis of the microarray data by unsupervised hierarchical clustering algorithm yields no meaningful classification of cancerous and noncancerous groups. We used a software developed by researcher in Stanford University- Prediction Analysis of Microarray (PAM) and Spotfire software to analyze the data and found that there were 504 genes which can distinguish the cancerous and noncancerous groups with more than 99% accuracy. Analysis the tumor group with these 504 genes showed that there were 3 subgroups of gastric cancer did not

respond to any classification system used today, including Lauren' classification (Figure 1). Of the 504 genes, Trefoil factors 1, 2, and 3 are in the list of genes and their expression pattern are similar to previous study. The results confirmed our microarray data indirectly and implied there existed a new molecular classification of gastric cancer. The clinical significance of the new classification needs further studies. In addition, one aim of this study is elucidate the different genetic alternations of MSI and MS stable gastric cancer. We found there were 11 genes, including MutL (involved in DNA repair), interleukin 13, annexin A1, Down syndrome critical region gene 1, and Human (JER47) MUC5AC mRNA for mucin (partial) et al, differentially expressed between these two groups of gastric cancer (figure 2&3). In addition to compare the differential gene expression profile of MSI and MS stable gastric cancer, we also analyzed the differential gene expression profiles between cardiac and noncardiac gastric cancers (figure 4&5), and between intestinal and diffuse type gastric cancers (figure 6&7). Further studies to elucidate the role of these genes in the pathogenesis of MSI and MS gastric cancer and different subtypes of gastric cancer are under going.

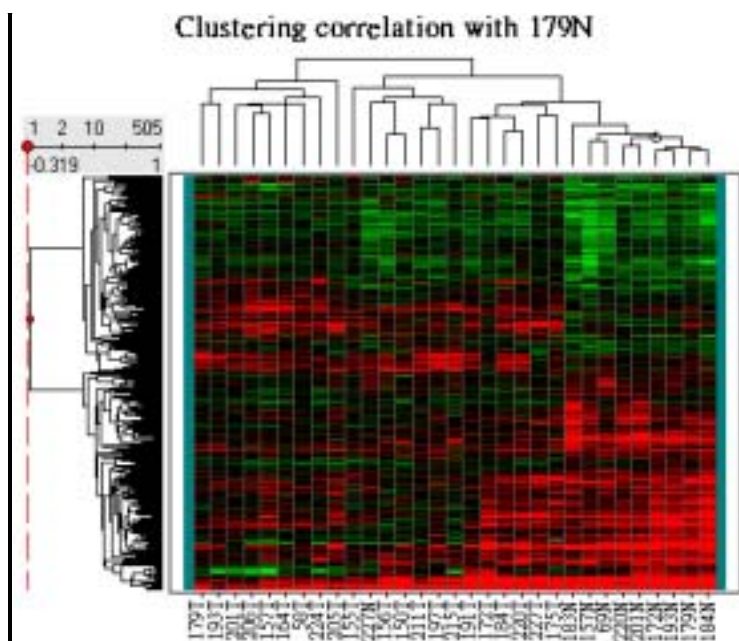


Figure 1

2b. Studies and Results (Cont.)

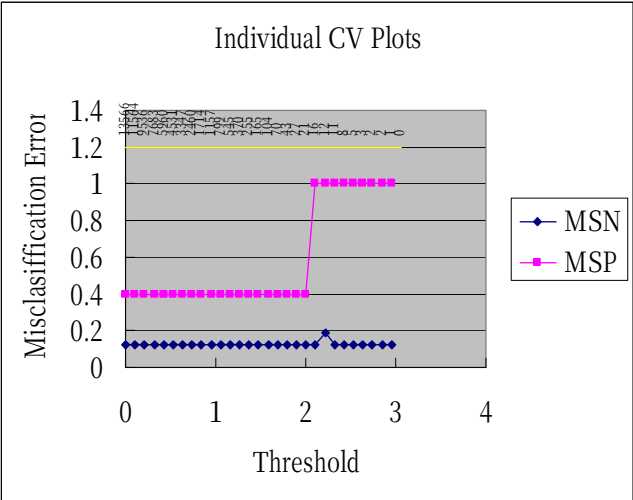


Figure 2

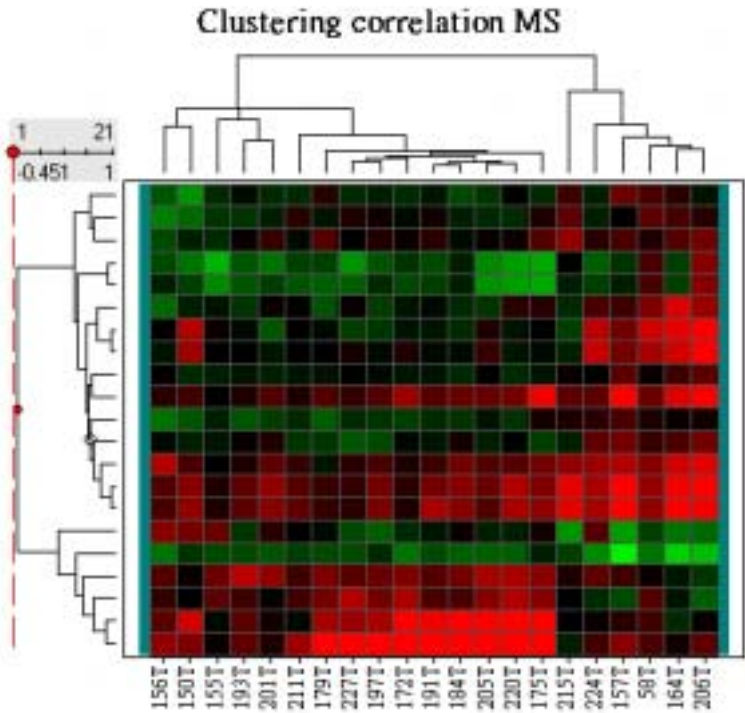


Figure 3

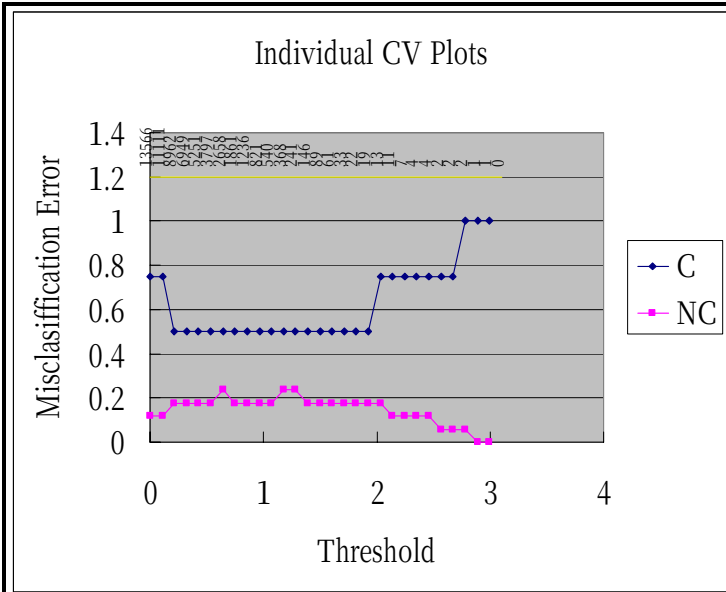


Figure 4

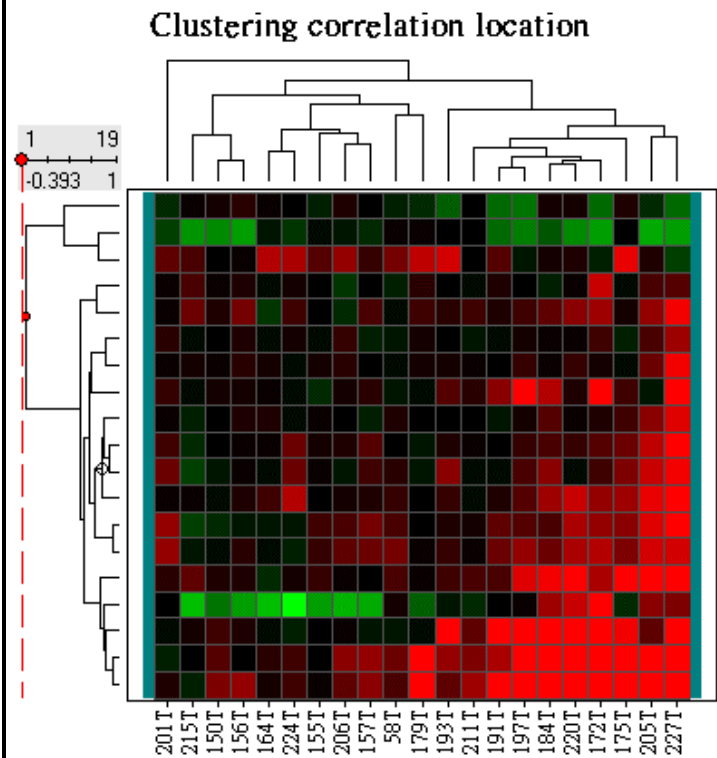


Figure 5

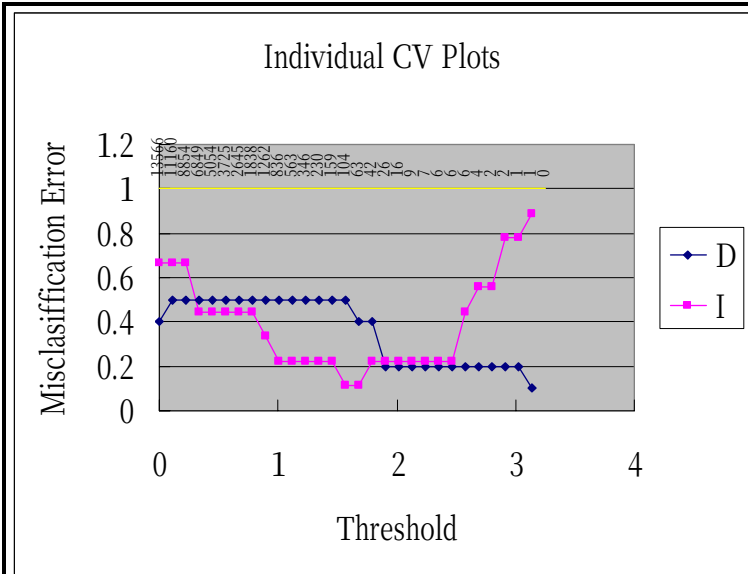


Figure 6

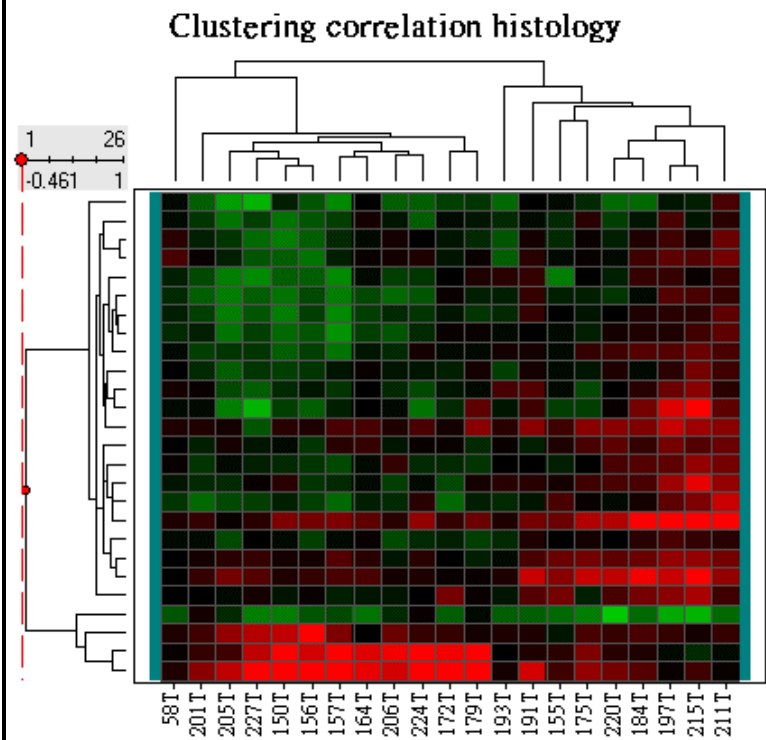


Figure 7

Provide a short paragraph to describe the plans for next year including a realistic timetable and appropriate milestones for the project, based on the progress reached so far. (Do not exceed **1 page**.)

In the next year, we are going to confirm the new classification of different subtypes of gastric cancers by our microarray results. The interesting genes, including IL13 and annexin A1, are under evaluation by immunohistochemical staining on gastric cancer tissues. The result will be published in the near future.

[illegible]

5. Personnel

Summarize the **personnel involved in the project during the grant period**. List the personnel in accordance to the following categories: (1) senior investigators, including visitors; (2) postdoctoral fellows; (3) graduate students; (4) technicians or research assistants. Specify for each individual the period of involvement and the percentage commitment of effort.

Name		Position Title	Education Degree	% of personal effort on this project	Job Description or Responsibilities
In Chinese	In English				
林肇堂	Lin Jaw-Town	PI	MD. PhD.	100	Study design, administration, data collection, interpretation, report writing
楊泮池	Yang Pan-Chyr	Co-PI	MD. PhD.	30	Director and monitor of microarray techniques
吳明賢	Wu Ming-Shiang	Co-PI	MD. PhD.	30	Conduct experiments in genetic alterations other than those from microarray
張毓廷	Chang Yu-Ting	Ph.D. Student	MD. MS.	100	Full time experiment including genetic alteration, LCM, and microarray
王珮琳	Wang Pai-Lin	Research Assistant	Bachelor	100	Full time experiment including genetic alteration, LCM, and microarray

6. Publications and/or Patents

6a. Publications

List the title and complete references (author(s), journal or book, year, page number) of all publications directly resulting from studies supported by the project (i.e., with citation of this grant in the acknowledgement section). List the publications for the project in accordance to the following categories: (1) manuscripts published and accepted for publications; (2) manuscripts submitted; and (3) conference proceedings. Provide one copy of each publication not previously reported to the National Science Council in the Appendix.

Distinct Gene Expression Profiles in Gastric Epithelial Cells Induced by Different Clinical Isolates of *Helicobacter pylori*---- Implication of Bacteria and Host Interaction in Gastric Carcinogenesis (manuscript submitted) (See appendix)

Distinct Gene Expression Profiles in Microsatellite Instable and Stable Gastric Cancers Analyzed by Microarray (manuscript in preparation)

6b. Patents

List all inventions disclosed, patents filed, and patents granted. Please note the inventors, assignee, title of patent, country or area where patent applied for, filing or issued number and date.

Appendix

Distinct Gene Expression Profiles in Gastric Epithelial Cells Induced by Different Clinical Isolates of *Helicobacter pylori*---- Implication of Bacteria and Host Interaction in Gastric Carcinogenesis

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Running title: Gene Expression in Gastric Epithelial Cells Induced by *Helicobacter pylori*

Key words: *Helicobacter pylori* (*H. pylori*), gastric cancer, microarray, C/EBP β

Abstract

Helicobacter pylori (*H. pylori*) infection is a major risk factor of peptic ulcer, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The interplay between *H. pylori* and host determines the various outcomes after infection and is an important issue for further elucidation of pathogenesis of *H. pylori*-related diseases. The availability of cDNA microarray creates the unprecedented opportunity to examine simultaneously dynamic changes of multiple molecular pathways affected by different *H. pylori* strains infection. To elucidate the cross-talk of *H. pylori* and gastric epithelial cells, three different clinical isolated *H. pylori* strains, GC, DU and MA strain, isolated from patients of gastric cancer(GC), duodenal ulcer(DU), and gastric MALT lymphoma (MA), were cocultured with AGS cells for 6 hours. Total RNA was extracted and used for detection of genes represented in the Human 1 cDNA Microarray (Agilent). Validation of microarray data were done by Western blot analysis of selected genes. Of the 12,814 clones on the Human 1 cDNA microarray, there were 522 genes expressed differently in the three groups. Of the 522 genes, there were 4 genes, 4 genes and 13 genes, either up- or down-regulated more than twofold change, in AGS cells induced specifically by GC, MA and DU strain respectively. The GC and DU strains induced more genes involving in carcinogenesis, such as pim-1, jun B, and VEGF. In the study, we provide evidence of bacterial factors may determine the outcomes of *H. pylori* infection. Different bacterial virulent factors, other than *cagA* and *vacA*, are critical in the development of various gastroduodenal diseases. The expression profiles of cDNA microarray provide clues for diagnosis, treatment and prevention of *H. pylori*-related gastroduodenal diseases.

Introduction

Helicobacter pylori (*H. pylori*) is a prevalent gram-negative bacterium. The infection of *H. pylori* has been recognized as a major risk factor in the development of chronic active gastritis, peptic ulcer disease, and gastric cancer[1]. The prevalence of *H. pylori*-related diseases varies in different geographic regions and patient populations[2]. However, the majority of *H. pylori* colonized individuals remain asymptomatic; approximately 20% of infected patients develop clinically significant diseases such as peptic ulcer, gastric adenocarcinoma, or gastric mucosa-associated lymphoid tissue (MALT) lymphoma[1, 3]. This variability in clinical outcomes may result from differences in inflammatory responses governed by host genetics, and specific interactions between host and microbial determinants[3]. Bacterial virulence, host genetic, and environmental factors are among the critical determinants that predispose to the clinical manifestation of *H. pylori* infection.

H. pylori populations are extremely diverse[4, 5] because *H. pylori* has a very plastic genome, reflecting its high rate of recombination and point mutation. Different *H. pylori* strains have different effects on cellular turnover and level of apoptosis of gastric mucosal epithelial cells [6-10]. Previous studies have shown that the presence of *cag* pathogenicity island (PAI) and specific genotype of *vacA* or *iceA* gene are more frequently associated with *H. pylori*-related diseases [11, 12]. It implied that differences in virulence factors that characterize different strains of *H. pylori* could influence the clinical outcome of *H. pylori* infection. However, the virulent markers for *H. pylori* strains that affect Western populations have little or no predicative power in East Asian populations[13, 14]. The molecular pathways of gastric carcinogenesis and *H.*

pylori-related diseases are multiple, producing complex pattern of molecular changes in gastric epithelial cells. It is of fundamental importance to understand the main molecular pathways affected by *H. pylori* in epithelial cells in order to identify the critical steps in induction of *H. pylori*-related diseases, such as duodenal ulcer, gastric cancer, and gastric MALT lymphoma. The availability of cDNA microarray reference creates the unprecedented opportunity to examine simultaneously dynamic changes of multiple pathways affected by different *H. pylori* strains infection. In this study, we apply the microarray technology to examine the broad patterns of gene expression of gastric epithelial cell lines which co-culture with three different clinical isolates of *H. pylori*. Understanding the molecular pathway affected by different *H. pylori* in the gastric epithelium may provide novel approaches to screening the high risk group and identification of molecular targets for further prevention of peptic ulcer disease, gastric cancer, and gastric MALT lymphoma.

Materials and Methods

Bacteria and growth conditions

The *H. pylori* strains employed in this study included gastric cancer (GC) strains, duodenal ulcer (DU) strains, and gastric MALT lymphoma (MA) strains originally isolated from patients with gastric adenocarcinoma, duodenal ulcer, and gastric MALT lymphoma, respectively. *H. pylori* strains were grown on Columbia agar plates containing 5% (vol/vol) sheep blood and antibiotics supplement (GIBCO BRL, Rockville, Md.) at 37°C in a microaerophilic chamber (Don Whitley, West Yorkshire, England) containing 5% O₂, 85% N₂, and 10% CO₂ for 48 hours. Bacterial cells were collected and washed with PBS buffer (pH 7.4) and pelleted. Cell pellets were then resuspended in PBS buffer (pH7.4) and used for infection experiment immediately. Bacterial numbers were measured by spectrophotometry at 600 nm.

Cell culture

The gastric cancer cell line 1739-CRL (AGS cell) was used to perform in vitro co-culture experiment with *H. pylori*. The AGS cells were maintained in RPMI 1640 (Sigma Co.) supplemented with 10% fetal bovine serum (FBS) (GIBCO BRL, Rockville, MD., USA), 100U/ml of penicillin, and 100µg/ml of streptomycin. Co-culture with bacteria was prepared by seeding 1x10⁶ AGS cells in 10cm plates in RPMI 1640 without antibiotics. The cell lines were treated with three different clinically isolated *H. pylori* strains for 6 hours with MOI of 300 or a similar volume of PBS as control. After washing the cell lines with PBS and removing the bacteria, total RNA were extracted from AGS cells by TRIzol reagent (Gibco BRL) and quantified by OD 260nm and qualified by Bioanalyzer (Agilent Technology, USA).

Probe Labeling and microarray hybridization and scanning

Total 15µg of purified RNA are converted to cDNA using a 3DNA™ Array 50 Expression Array Detection Kit (Genisphere, U. S. A.). Correspondingly synthesized cDNA products are combined and concentrated by ethanol precipitation and suspended in hybridization buffer. Hybridization of labeled cDNA is hybridized to Human 1 cDNA microarray (Agilent Technologies, USA) at 65°C for 17 hours. After hybridization, slides were washed by 0.5X SSC/0.01% SDS at room temperature for 5 minutes, and 0.06X SSC at room temperature for 2 minutes. Washed microarrays are then hybridized with Cy3 and Cy5 dendrimers in formamide-based buffer at 53°C for 3 hours. After hybridization with dendrimers, slides were washed by 2X SSC/0.01% SDS at 42°C for 15 minutes, 2X SSC at room temperature for 10 minutes, and 0.2X SSC at room temperature for 10 minutes. After washing and drying by centrifugation, microarrays are scanned with a Virtek fluorescence reader (Virtek, CA) at 535 nm for Cy3 and 625 nm for Cy5. Scanned images are analyzed by Array-Pro image acquisition software (Media Cybernetics, USA). An image analysis algorithm is used to quantify signal and background intensity for each target element. The targets which signal to background intensity ratio more than 2.5 were selected for further analysis. Data normalization is performed by Lowess method using R package (written by Terry Speeds Microarray Data Analysis Group, University of Berkeley, USA). The microarray data were analyzed by Spotfire software (Someville, MA., USA).

Preparation of Cell Extracts and Western Blot Analysis

Rabbit polyclonal antibodies specific for CCAAT enhancer-binding protein (C/EBP β) and goat polyclonal antibody specific for actin were purchased from

Santa Cruz Biotechnology (Santa Cruz, CA., USA). After incubation with various *Helicobacter pylori* strains for 3, 6, 12, 18, and 24 hours, AGS cells were rapidly washed with PBS to remove bacteria, and then lysed with ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4, 1 mM EGTA, 1 mM NaF, 150 mM NaCl, 1 mM PMSF, 5 µg/ml of leupeptin, 20 µg/ml of aprotinin, 1 mM Na₃VO₄, 10mM β-glycerophosphate, 5mM Na-pyrophosphate, 1% Triton X-100). The cell lysate (100 µg total protein) was subjected to SDS-PAGE using 10 % running gels. The proteins were transferred to nitrocellulose paper. The membranes were blocked for 1 h at 25°C with 0.1% milk in Tris-buffered saline/Tween 20 (TTBS)) and then incubated for 1 h at 25°C with rabbit antibodies specific for C/EBPβ or goat antibodies specific for actin. Then, the membrane was incubated for 30 min at 25°C with horseradish peroxidase-labeled secondary antibody against rabbit or goat. After each incubation, the membrane was washed extensively with TTBS. The immunoreactive band was detected using ECL detection reagents (Amersham) and visualized using Hyperfilm-ECL. Quantitative data were obtained using a computing densitometer and ImageQuant software (Molecular Dynamics, Sunnyvale, CA., USA).

Genotyping and sequencing of *vacA*

DNAs from three clinically isolated *H. pylori* were extracted for *vacA* analysis as previously described[15, 16].

Statistical Analysis

The average of the two replicate experiments was determined and used for fold change determination by comparing the gene expression fold change relative to the non-infected control. Pearson correlation method was used to analyze the similarity of gene expression between each two groups.

Discriminate genes and differences between the three groups were analyzed using two-tailed ANOVA test. After ANOVA test, those genes shown to undergo more than twofold up- or down-regulation induced by *H. pylori* were selected for further analysis. $P < 0.05$ was considered statistically significant.

Results

Comparative analysis of gene expression of AGS cells induced by different clinical isolated *H. pylori* infection

The intensity of 99% targets of the 12,814 clones on the six Human 1 cDNA microarray were more than or equal to 2.5 times of background. We found the gene expression profile was more similar between the GC strain and DU strain ($r=0.552$) than that between GC strain and MA strain ($r=0.393$), and that between DU strain and MA strain ($r=0.355$). ANOVA analysis showed that there were 522 genes expressed differently in the three groups. There were 4 genes up- or down-regulated expression in AGS cells induced specifically by GC strain (Table 1), 4 genes up- or down-regulated expression in AGS cells induced specifically by DU strain (Table 2), and 13 genes up- or down-regulated expression in AGS cells induced specifically by MA strain (Table 3). Those genes shown to undergo more than twofold up- or down-regulation induced by *H. pylori* were further analyzed. We found that there were 32 genes, including *pim-1* oncogene, vascular endothelial growth factor (VEGF), *jun B*, CCAAT/enhancer binding protein β (C/EBP β), and cyclin G2, etc. up- or down-regulated expression in AGS cells induced both by GC and DU strains but not by MA strain (Table 4).

Validation of microarray data by Western blot analysis

For verifying the results of microarray, the protein expression of C/EBP β in AGS cells induced by three *H. pylori* strains at 3, 6, 12, 18, and 24 hours were quantified by Western blot analysis. We found that the GC and DU strain induced C/EBP β expression in AGS cells at 6, 12, 18 and 24hours (Figure 1). The MA strain did not induced C/EBP β expression in AGS cells at different time

point in our experiments. The result was consistent with our microarray data.

Genotypes of *Helicobacter pylori*

All of the three strains of *Helicobacter pylori* express *cagA*. The genotype of *vacA* of the three strains is s1m2.

Discussion

Although *H. pylori* is associated with chronic gastritis, duodenal ulcer, gastric cancer, and gastric MALT lymphoma, only a small portion of infected population developed these diseases while the majority of infected people remained asymptomatic. Therefore it is crucial to investigate the distinct response of gastric epithelial cells to different *H. pylori* strains. Examination of the effects of *H. pylori* on gene expression in the gastric epithelial cells or mucosa has been reported in literature [17-19]. However, only a single strain or isogenic mutant strains of *H. pylori* were used in the previous studies. With the involvement of the bacterial factors, host susceptibility, and interaction of bacteria, environment and host immune response in vitro experiments using gastric cancer cell lines in co-cultured with a single strain of *H. pylori* can not fully elucidate the pathogenesis of *H. pylori*-related gastric diseases. Microarray has been used to identify differences in gene content between *H. pylori* strains that induce distinct pathological outcomes in an animal model [20]. In the present study, we report the different clinically isolated *H. pylori* strains would induce distinct gene expression patterns in gastric epithelial cells. Understanding the interaction between *H. pylori* and gastric epithelial cells is an essential prerequisite in elucidating the underlying mechanisms of different *H. pylori*-related diseases. The conventional method is to analyze the defined signal pathway in co-culture model. This approach provides a reliable but limited clue. Applying the technology of microarray to study host-microbe interaction can generate a holistic view of signal alterations in a single experiment. This is the first study to show that the mRNA expression of gastric epithelial cells after infection by different strains of *H. pylori* is not general but

rather strain-specific. Of the three clinical isolates, the DU strain from the patient with duodenal ulcer and the GC strain from the patient of gastric cancer induced more oncogenes, protooncogenes, or genes involved in carcinogenesis, such as *fos*, *jun B*, *pim-1* and *VEGF*, etc. The expressions of these genes in AGS cells induced by *H. pylori* were similar to the result of previous study [17]. However, the MA strain from the patient of gastric MALT lymphoma did not induce those genes. The result implied that the strain-specific factors play an important role in gastric carcinogenesis and the role of *H. pylori* strain in the pathogenesis of gastric MALT lymphoma is quite different to that of duodenal ulcer and gastric cancer. In addition to the difference of *H. pylori* strains, the genes up- or down-regulated in AGS cells induced by *H. pylori* are related to the MOI of bacteria, duration of coculture, and the type of microarray we used. This is why there are some differences in the genes induced by *H. pylori* between our report and previous studies[17-19].

C/EBP β is one of the family of transcription factor, CAAT box enhancer binding proteins (C/EBPs). C/EBP β is strongly upregulated at the transcription level by inflammatory stimuli, participates in both inflammatory and metabolic regulations, and controls cell cycle progression[21]. The increase in C/EBP β results in accelerated entrance of liver cells to S phase for replication [22]. Our experiment showed that the GC and DU strains induced higher C/EBP β transcription and translation in AGS cells than MA strains did. The result implied that the *H. pylori* may induce the signal pathway related to C/EBP β in gastric carcinogenesis. Previous study had shown that the COX-2 expression was regulated by the expression of the C/EBP β [23]. The production of COX-2 has been linked to different gastroduodenal diseases including ulcer and cancer. In

addition, increased activation of C/EBP β has been reported to promote tumor invasiveness and render a malignant phenotype of renal cell carcinoma [24] and C/EBP β may be a target for tumor inhibition[25]. The role of C/EBP β in the pathogenesis of *H. pylori*-related diseases has not been studied yet. Collectively, these results suggest C/EBP β may play a distinct role in the pathogenesis of *H. pylori*-related gastric carcinogenesis and ulcer formation and indicate microarray is a rapid and reliable way to identify targets involved in pathogenesis of *H. pylori*-related diseases. Further study for the role of C/EBP β in gastric carcinogenesis is needed.

It is of fundamental importance to understand the main molecular pathways affected by *H. pylori* in epithelial cells in order to identify the critical steps of gastric carcinogenesis. In this study, we have identified distinct genes involved in different clinically isolated *H. pylori* infection. The results provide evidence of bacterial factors and molecular cross-talk between bacteria and the epithelial cells may determine the outcomes of *H. pylori* infection. Further studies in this line including the identification of the bacterial virulent factors, and elucidation of the different pathways involved, are critical in understanding gastric carcinogenesis and provide novel clues for diagnosis, treatment, and prevention of gastric cancer.

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Table 1. Selected genes up- or down-regulated by GC strain in AGS cells

Up regulated genes			
Unigene ID	Gene Name	Average of log2 fold change in treatment with GC strain	Average of log2 fold change in treatment with DU strain
Hs.1395	early growth response 2 (Krox-20 (Drosophila) homolog)	1.26	0.25
Hs.622	v-raf murine sarcoma viral oncogene homolog B1	1.08	-0.23
Hs.121499	solute carrier family 6 (neurotransmitter transporter, glycine), member 9	1.02	-0.23
Down regulated genes			
Unigene ID	Gene Name	Average of log2 fold change in treatment with GC strain	Average of log2 fold change in treatment with DU strain
Hs.1440	gamma-aminobutyric acid (GABA) A receptor, beta 3	-1.12	0.19

GC: gastric cancer.

Table 2. Selected genes up- or down-regulated by DU strain in AGS cells

Up regulated genes				
Unigene ID	Gene Name		Average of log2 fold change in treatment with DU strain	Average of log2 fold change in treatment with GC strain
Hs.75812	phosphoenolpyruvate (mitochondrial)	carboxykinase	2 1.46	0.72
Hs.13131	lymphocyte adaptor protein		1.30	0.58
Hs.102865	interleukin 1 receptor-like 2		1.33	0.40
Down regulated genes				
Unigene ID	Gene Name		Average of log2 fold change in treatment with DU strain	Average of log2 fold change in treatment with GC strain
Hs.18141	ladinin 1		-1.01	-0.23

DU: duodenal ulcer.

Table 3. Selected genes up- or down-regulated by MA strain in AGS cells

Up regulated genes			
Unigene ID	Gene Name	Average of log2 fold change in treatment with MA strain	Average of log2 fold change in treatment with GC strain
Hs.99236	regulator of Gz-selective protein signaling	1.47	-0.31
Hs.430	plastin 1 (I isoform)	1.12	0.13
Hs.147996	protein kinase, X-linked	1.12	-0.19
Hs.66141	prostate derived STE20-like kinase PSK	1.11	0.29
Hs.174044	dishevelled 3 (homologous to Drosophila dsh)	1.07	0.18
Hs.275924	dystrophia myotonica-containing WD repeat motif	1.02	0.19
Down regulated genes			
Unigene ID	Gene Name	Average of log2 fold change in treatment with MA strain	Average of log2 fold change in treatment with GC strain
-	Human DiGeorge syndrome critical region, centromeric end.	-1.03	0.10
-	unnamed protein product	-1.06	-0.57
Hs.165986	testin	-1.09	0.71
Hs.74552	caspase 3, apoptosis-related cysteine protease	-1.13	-0.14
Hs.124186	ring finger protein 2	-1.17	-0.27
Hs.181125	immunoglobulin lambda locus	-1.17	0.04
Hs.84673	troponin I, skeletal, slow	-1.37	0.11

MA: MALT lymphoma.

Table 4. Selected genes up- or down-regulated by both GC and DU strain in AGS cells

Up regulated genes			
Unigene ID	Gene Name	Average of log2 fold change in treatment with GC strain	Average of log2 fold change in treatment with DU strain
Hs.127799	baculoviral IAP repeat-containing 3	2.39	2.00
Hs.171695	dual specificity phosphatase 1	2.30	2.76
Hs.25647	v-fos FBJ murine osteosarcoma viral oncogene homolog	2.24	2.06
Hs.460	activating transcription factor 3	2.11	2.53
Hs.211600	tumor necrosis factor, alpha-induced protein 3	2.11	2.33
Hs.96	phorbol-12-myristate-13-acetate-induced protein 1	2.06	1.99
Hs.75692	asparagine synthetase	1.89	2.63
Hs.73793	vascular endothelial growth factor	1.89	2.17
Hs.77054	B-cell translocation gene 1, anti-proliferative	1.83	1.93
Hs.285313	core promoter element binding protein	1.76	1.38
Hs.82173	TGFB inducible early growth response	1.70	1.90
Hs.198951	jun B proto-oncogene	1.67	2.32
Hs.79069	cyclin G2	1.67	2.02
Hs.298275	amino acid transporter 2	1.67	2.06
Hs.10706	epithelial protein lost in neoplasm beta	1.62	2.06
Hs.81170	pim-1 oncogene	1.57	1.83
Hs.99029	CCAAT/enhancer binding protein (C/EBP), beta	1.55	1.77
Hs.78465	v-jun avian sarcoma virus 17 oncogene homolog	1.50	2.09
Hs.12289	Cdc42 effector protein 2	1.48	2.02
-	Human leptin receptor (LEPR) gene, exon 3.	1.30	1.76
Hs.75716	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	1.25	1.84
-	Human low density lipoprotein receptor gene, exon 18.	1.24	1.60
Hs.54468	tubby (mouse) homolog	1.20	1.00
Hs.283565	FOS-like antigen-1	1.14	1.80
Hs.154276	BTB and CNC homology 1, basic leucine zipper transcription factor 1	1.12	1.11
Hs.3337	transmembrane 4 superfamily member 1	1.12	1.41
-	Human low density lipoprotein receptor gene, exon 18.	1.08	1.38
Hs.19904	cystathionase (cystathionine gamma-lyase)	1.08	1.76
Down regulated genes			
Unigene ID	Gene Name	Average of log2 fold change in treatment with	Average of log2 fold change in treatment with

		GC strain	DU strain
Hs.150601	chymotrypsin-like	-1.22	-1.23
Hs.268016	solute carrier family 5 (inositol transporters), member 3	-1.47	-1.59
Hs.70669	HMP19 protein	-1.49	-1.90
Hs.8997	heat shock 70kD protein 1A	-1.86	-1.79

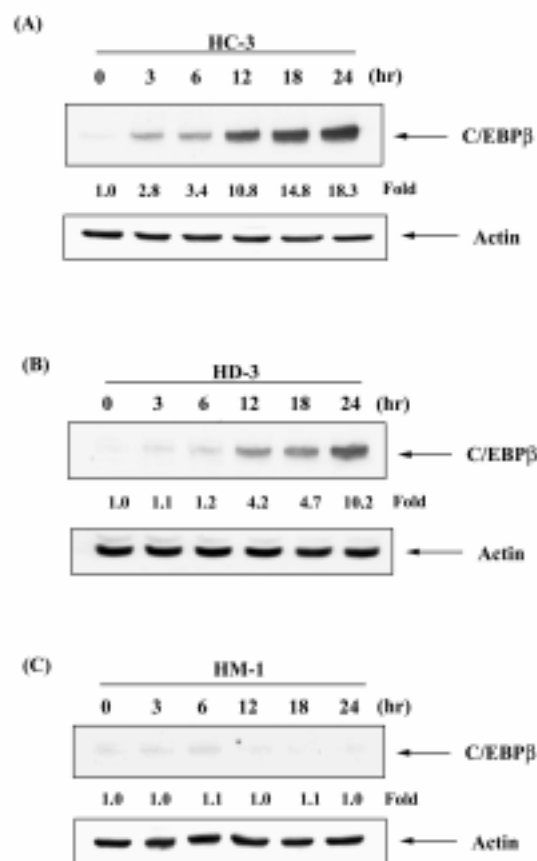


Figure 1 Western blot analysis of C/EBPβ expression in AGS cells. HC-3: GC strain; HM-1: MA strain.