行政院國家科學委員會專題研究計畫成果報告

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

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

計畫類別：■個別型計畫 □整合型計畫

計畫編號： NSC91-3112-B-002-007

執行期間： 91年 5月 1日至 92年4月 30日

個別型計畫：計畫主持人：林肇堂醫師

整合型計畫：總計畫主持人：
子計畫主持人：

註：整合型計畫總報告與子計畫成果報告請分開編印各成一冊，彙整一起繳送國科會

處理方式： □可立即對外提供參考
■一年後可對外提供參考
□兩年後可對外提供參考
(必要時，本會得展延發表時限)

執行單位：台大醫學院內科

中華民國 92 年 7 月 24 日

2. Progress Report

2a. 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 and the reasons for the modifications.

Gastric cancer (GC) is a major cause of cancer morbidity and mortality worldwide. Marked heterogeneity and functions exist for GC. An understanding of molecular features of GC is essential for the improvement of clinical achievement. One approach to improve diagnosis and treatment is to clarify genetic abnormalities that GC contain. However, the genetic profiles that are involved in GC development, progression and their clinical and biological significance remain poorly understood. Some of limitations were noted in previous studies. These include contamination of stromal cells in specimens and investigation focusing only on selected genes or chromosomal regions known in other cancers. With recent advent of research techniques, such limitions may be resolved. The overall goal of this project is to define the spectrum of genetic alterations of GC by laser capture microdissection (LCM) and microarray analysis. A series of specimens including various types of GC and their precancerous lesions will be tested to accomplish the following specific aims:

(1) To establish a more clear 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 moleculer basis of progression.
(3) Etiology-specific critical regulatory genes may be responsile 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.

2b. Studies and Results

Describe the studies directed toward the specific aims during the current grant period and the results obtained. Indicate the extent to which the work accomplished has successfully met the specific aims. Include negative results. If technical problems were encountered in carrying out this project, describe how your approach was modified.

In the past 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 tissue of each patient were obtained at the time of surgery and preserved separately at -70℃ until use. Their relevant demographic and clinicopathologic information was obtained from medical records. Mutator phenotype (microsatellite instability) was determined by BAT26 polymorphic marker as previous studies. Six (8%) cancers showed microsatellite instability (Fig. 1). To establish the microarray system (Agilent cDNA array), 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(DU), gastric cancer(GC), and Maltoma(GM) 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.

We have started to procure pure cancer cells for further RNA extraction and amplification by laser-assistant microdissection system (LEICA AS LMD, GERMAN) initially (Fig.2). However, we shift to use PixCell II LCM System and CapSure HS LCM caps (Acturus, Mountain View, California) later because the laser of Leica system was out of order. After procurement of more than 2000 cells, total RNA was extracted by 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 (Fig. 3). Total RNA purified from LCM samples was amplified twice by Riboamp kit (Acturus, 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). Up to

July 15,2003, we have procured cancer cells from 20 cancers by LCM. Only 9 of their RNAs qualified for further amplification. We have hybridized 12 arrays for cancer (6 cases with dye-swap duplication) and 8 arrays for nontumorous cells (4 cases with dye-swap duplication) with universal reference. Preliminary analysis of the microarray data by Prediction Analysis of Microarray (PAM) and Spotfire software showed distinct clustering of tumorous and nontumorous groups(Fig 4). We have found two novel genes distinguishingly expressed in gastric cancer. Further analysis will be performed after expanding the cases (Fig. 5).

2b. Studies and Results (Cont.)

Fig. 1

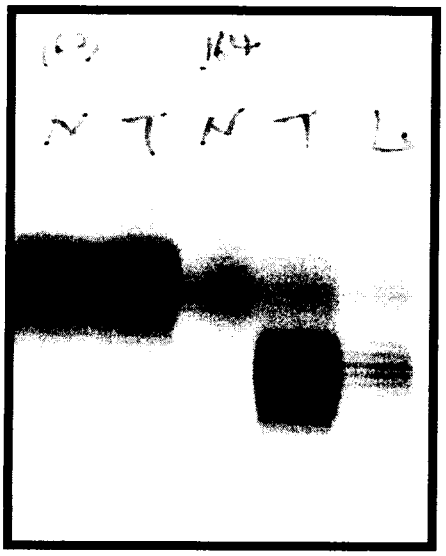

Fig. 2

Fig. 3

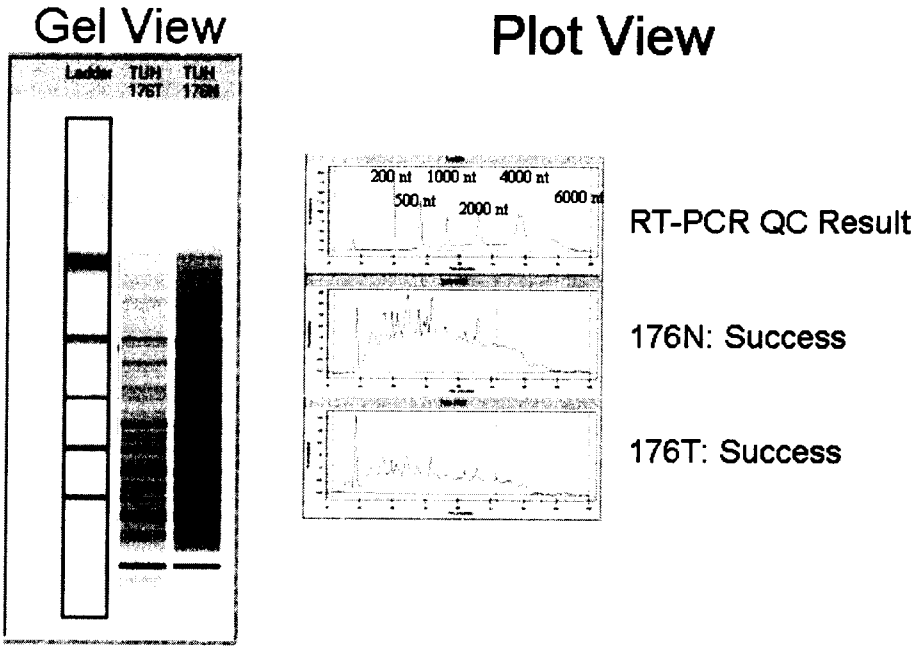

Fig. 4

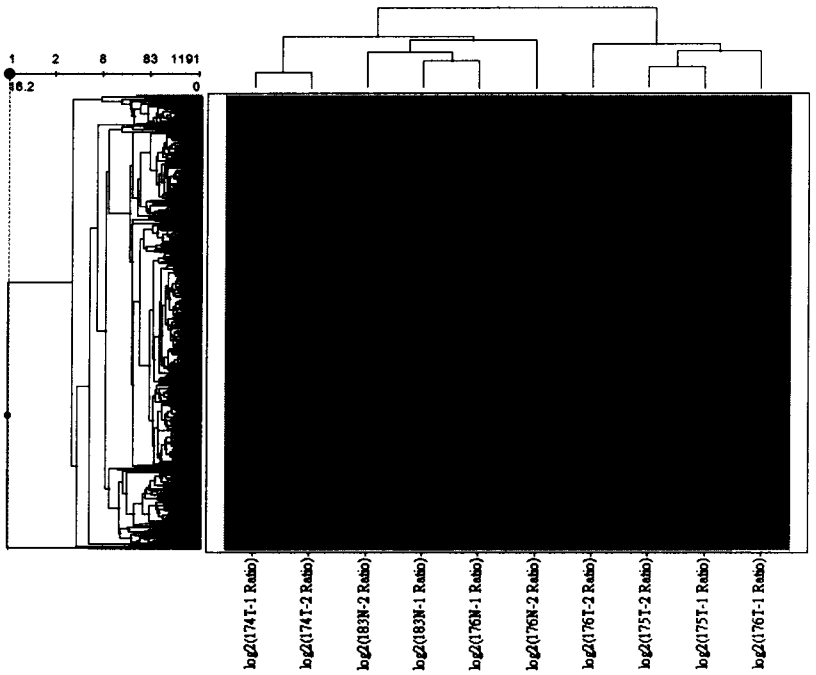
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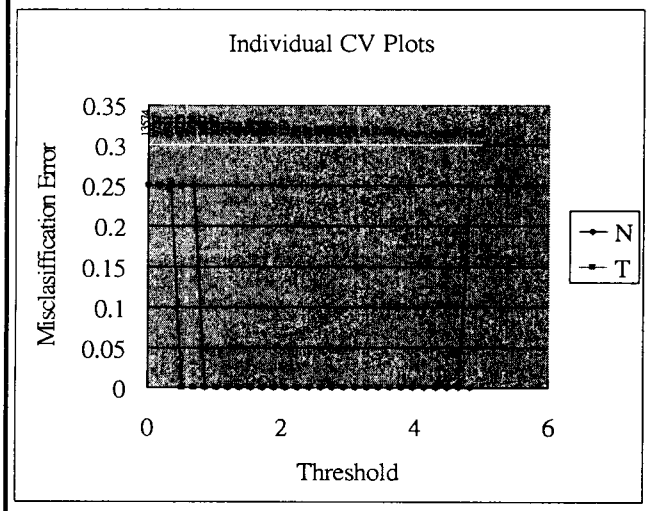
Fig. 5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