Gene Expression Profile Differences in Gastric Cancer and Normal Gastric Mucosa by Oligonucleotide Microarrays

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OBJECTIVE To study the difference of gene expression in gastric cancer (T) and normal tissue of gastric mucosa (C), and to screen for associated novel genes in gastric cancers by oligonucleotide microarrays.

METHODS U133A (Affymetrix, Santa Clara, CA) gene chip was used to detect the gene expression profile difference in T and C. Bioinformatics was used to analyze the detected results.

RESULTS When gastric cancers were compared with normal gastric mucosa, a total of 270 genes were found with a difference of more than 9 times in expression levels. Of the 270 genes, 157 were up-regulated (Signal Log Ratio [SLR] >3), and 113 were down-regulated (SLR ≤-3). Using a classification of function, the highest number of gene expression differences related to enzymes and their regulatory genes (67, 24.8%), followed by signal-transduction genes (43, 15.9%). The third were nucleic acid binding genes (17, 6.3%), fourth were transporter genes (15, 5.5%) and fifth were protein binding genes (12, 4.4%). In addition there were 50 genes of unknown function, accounting for 18.5%. The five above mentioned groups made up 56.9% of the total gene number.

CONCLUSION The 5 gene groups (enzymes and their regulatory proteins, signal transduction proteins, nucleic acid binding proteins, transporter and protein binding) were abnormally expressed and are important genes for further study in gastric cancers.

KEYWORDS: gastric cancer, normal gastric mucosa, gene-expression profile.

Differentially expressed genes in diverse tissue specimens may be detected with parallel analysis using gene chips, which have greatly improved the traditional experiments in which only a single, or several gene expressions can be observed for each test. More and more cDNA microarray methods are now being applied in the study of gene expression. In the present paper, the gene chip technique was used to analyze different gene expression patterns between gastric carcinomas and normal tissue of the gastric mucosa. In addition we have explored the tumor-associated gene-cluster and their functions involved in the process of formation and development of the gastric carcinomas. These studies will be helpful to comprehensively understand the mechanism of carcinogenesis at the molecular level with the hope that this research will provide molecular markers and target genes for clinical diagnosis, prevention, prognosis and treatment of gastric cancer.

MATERIALS AND METHODS
Materials
All the tissue specimens including gastric carcinomas (T) and gastric mucosa, which were from the distant cutting margin (C), were taken from 5 patients being operated in our hospital. For each specimen one part was frozen immediately in liquid nitrogen after surgical resection, and another part was used for histopathological examination to ensure that all normal gastric mucosa was devoid of cancer cells and that it had maintained the corresponding histological appearance. The clinical and pathological data from these patients are shown in Table 1.

Methods
Oligonucleotide microarray gene chips
Human full-length genome U133A chips (Affymetrix, Santa Clara, CA) were used. This array contains about 18,000 full-length genes from the Unigene GenBank.

Sample preparation
RNA was extracted from the tissues by a single-step method. Briefly, after removing the T and C tissues from the liquid nitrogen, the specimens were ground completely into a tiny powder in a ceramic mortar while adding liquid nitrogen. TRIZOL was used to extract total RNA followed by use of a QIAGEN’S reagent kit for its purification. Spectrophotometric analysis was employed (one optical density unit at A 260 nm equals 40 µg/ml of RNA) to calculate the total RNA concentration. An equivalent of a total RNA, from the T and C samples, then was mixed. Using the T7- (dT) 24 (oligonucleotide) for a primer, the first strand of cDNA was synthesized through retro-transcription, then the first strand was used as a template to synthesize the second strand. After the double stranded DNA was purified, a BioAssay High Yield RNA Transcript Labeling kit was employed to transcribe the synthesis of cRNA directly, and at the same time to biotin-label the cRNA. Then a certain quantity of cRNA product was taken to make a 35–200 bp fragment of cRNA which was produced under high temperature and high salt conditions.

Hybridization and washing
The fragmentated cRNA was mixed with its control solution to prepare a hybridization solution. The hybridization solution was placed on the chips, and the 2 chips (T, C) placed into the hybridization 640 oven for 16 h to finish the hybridization procedure. The chips were removed from the hybridization oven, washed and stained, and eluted automatically in the Fluidics Station 400.

Fluorescence scanning and analysis of results
The chip was scanned with a GeneChip Scanner and the intensity value of the fluorescent signals obtained from the expressed genes. Using an internal reference gene (housekeeping gene) the primary signal data were normalized and corrected. The images produced were analyzed by Microarray Suite Software using digital computation, and the intensity of the fluorescence signals and their ratios calculated.

RESULTS
Quantity judgment of the test chip
For each cRNA sample a scanning profile was produced after hybridization with the test chip (Fig.1). A clearly printed character "GeneChip TEST3" was on the upper portion of the profile. Many spots and well-distributed lines were around the profile. Some spots

Table 1. Clinical features and pathological diagnosis of 5 cases with gastric carcinoma

<table>
<thead>
<tr>
<th>No. of In-P</th>
<th>Sex</th>
<th>Age</th>
<th>Pathological diagnosis</th>
<th>Lymph metastasis</th>
<th>Clinical stage</th>
</tr>
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<tbody>
<tr>
<td>113702</td>
<td>F</td>
<td>45</td>
<td>Lesser curvature of stomach invasion ulcer type middle differentiation adenocarcinoma invasion of serosa</td>
<td>1/35</td>
<td>III</td>
</tr>
<tr>
<td>123730</td>
<td>M</td>
<td>48</td>
<td>Anterior wall in body of stomach middle differentiation adenocarcinoma invasion of muscular layers and nerve</td>
<td>1/17</td>
<td>III</td>
</tr>
<tr>
<td>123673</td>
<td>F</td>
<td>57</td>
<td>Lesser curvature in cardia of stomach invasion ulcer type middle differentiation adenocarcinoma invasion of serosa</td>
<td>1/14</td>
<td>III</td>
</tr>
<tr>
<td>123808</td>
<td>F</td>
<td>49</td>
<td>Anterior wall in cardia of stomach low-middle differentiation adenocarcinoma, a part of mucinous adenocarcinoma and esophagus of extremities inferior</td>
<td>0/35</td>
<td>II</td>
</tr>
<tr>
<td>123733</td>
<td>M</td>
<td>60</td>
<td>Cardia of stomach node type middle differentiation adenocarcinoma invasion of esophagus muscular layer</td>
<td>5/22</td>
<td>III</td>
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