Gene expression profile differences in gastric cancer, pericancerous epithelium and normal gastric mucosa by gene chip

Chuan-Ding Yu, Shen-Hua Xu, Hang-Zhou Mou, Zhi-Ming Jiang, Chi-Hong Zhu, Xiang-Lin Liu

INTRODUCTION

Differentially expressed genes from different specimens may be detected with parallel analysis by gene chips. This technique possesses many advantages, of which the greatest is to improve traditional experiments by allowing a single or several gene expression differences to be observed in a single test. More and more cDNA microarray methods are applied in the study of gene expression. In the paper, a gene chip technique was used to analyze the different gene expression patterns in gastric cancer, its pericancerous epithelium tissue and normal tissue of gastric mucosa. Exploring tumor-associated gene-clusters and their role in the process of carcinogenesis and development of gastric cancer is helpful in gaining a comprehensive understanding of the molecular mechanisms in cell transformation. This may also provide molecular markers and target genes for clinical diagnosis and prevention, and treatment of gastric cancer.

MATERIALS AND METHODS

Materials

Gastric cancer tissues and pericancerous epithelial tissues as well as normal gastric mucosa tissues were obtained from five patients who underwent gastrectomy at our hospital. For each specimen one part was immediately snap-frozen in liquid nitrogen and the other was used for histopathological examination to ensure all the pericancerous and control gastric mucosa without cancer cells but with their corresponding histological appearance. The clinical and pathological data of these patients are shown in Table 1.

Oligonucleotide microarray gene chip

Human full-length genome U133A (Affymetrix, Santa Clara, CA) contained about 18 000 full-length genes from the unigene GenBank.

Sample preparation

Total RNA was extracted by a single step method. Briefly, after T, P and C tissues were taken out from liquid nitrogen, specimens were ground completely into tiny powders while liquid nitrogen was added into a ceramic mortar. TRIzol was used to extract total RNA. A QIAGEN reagent kit was used to purify the total RNA. A spectrophotometer was used to purify the total RNA. A spectrophotometer was used to purify the total RNA.

RESULTS

When gastric cancer was compared with normal gastric mucosa, 766 genes were found, with a difference of more than four times in expression levels. Of the 766 genes, 530 were up-regulated (Signal Log Ratio [SLR]>2), and 236 were down-regulated (SLR<-2). When pericancerous epithelium was compared with normal gastric mucosa, 64 genes were found, with a difference of more than four times in expression levels. Of the 64 genes, 50 were up-regulated (SLR>2), and 14 were down-regulated (SLR<-2). Compared with normal gastric mucosa, a total of 143 genes with a difference in expression levels (more than four times, either in cancer or in pericancerous epithelium) were found in gastric cancer (T) and pericancerous epithelium (P). Of the 143 genes, 108 were up-regulated (SLR>2), and 35 were down-regulated (SLR<-2).

CONCLUSION: To apply a gene chip could find 143 genes associated with the genes of gastric cancer in pericancerous epithelium, although there were no pathological changes in the tissue slices. More interesting, six genes of pericancerous epithelium were up-regulated in comparison with genes of gastric cancer and three genes were down-regulated in comparison with genes of gastric cancer. It is suggested that these genes may be related to the carcinogenesis and development of early gastric cancer.

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Key words: Gastric cancer; Pericancerous epithelium; Gene expression profile; Gene chip
used to calculate the total RNA concentration on the basis of optical density of $A_{260}$. Total RNA was taken out from the T, P and C tissues and mixed, respectively. T7-(dT)24 was made for a primer, the first strand of cDNA was synthesized through retro-transcription on the base of the first strand as a template to synthesize the second strand. After the double stranded DNA was purified, a high yield RNA transcript labeling kit was used to transcribe synthetic cRNA directly. Then the transcribed cRNA was purified and treated at high temperature to produce fragments of 35-200 bp cRNA.

**Hybridization and washing**

The fragments of cRNA were mixed with other control agents to prepare a hybridization solution, the hybridization solution was injected into the chip. The three chips (T, P, C) were put into the hybridization oven (640 type) for 16 h to finish the hybridization procedure. The chips were taken out from the hybridization oven to recover the hybridization solution, then each composition elution and staining were automatically completed in the Fluidics Station 400.

**Fluorescence scanning and result analysis**

The chip was scanned with a GeneChip scanner. The intensity value of a fluorescent signal was obtained by expressing those genes. An internal reference gene (housekeeping gene) was chosen in advance. With regard to the primary signals, data were normalized and corrected. The acquired image was analyzed by Microarray suit software using a digital computer, and the intensity of fluorescence signals and its ratio were calculated.

**RESULTS**

**Quantity judgment of the test chip**

Each scanning profile was shown after hybridization with the test chip (Figure 1). A clearly printed character “GeneChip test” was on the upper of the profile. A lot of spots and well-distributed lines were around the profile. Some spots were on the four corners and the character “+” was clear. There was a good quantity between the gene chip and the samples of RNA. So this was a complete and reliable result of the detecting gene chip. Then, the samples from three groups were hybridized with U133A gene chip and scanned, respectively.

**Hybridization result of the sample chip**

A scanning profile was obtained from the samples of three
groups after being hybridized with U133A gene chip (Figure 2). The scatter plots are shown in Figure 3.

Bioinformatics was used to detect and analyze the number of differentially expressed genes in gastric cancer (T) or pericancerous tissues (P). The results were compared to the normal gastric mucosa as shown in Table 2.

Although there were no pathological changes in the tissue slices, 143 genes were found. More interesting, six genes of pericancerous epithelium were up-regulated compared to gastric cancer and three genes were down-regulated compared to gastric cancer. Their functions were related to the activity of a number of factors such as lipid transporter factor, electron transporter, receptor of signal protein, growth factor, ATP dependent RNA helicase (Table 3).

T and P were compared with C. The gene molecular function of a simultaneous expression difference belonged to nucleic acid binding activity (including RNA, DNA), protein binding activity (calcium, growth factor, cellular skeleton), enzyme activity. The functions of 34 genes were unknown (Table 4).

Genes with a difference of more than eight times are listed in Tables 5 and 6.

**DISCUSSION**

Gastric cancer is one of the most common malignant tumors in China. At present, its treatment has been greatly improved,

**Table 2** Differentially expressed gene number in T, P and C

| Numbers of genes | Up-regulated | Down-regulated |
|------------------|--------------|----------------|
| SLR>2            | SLR>3        | SLR<-2         | SLR<-3         |
| T vs C           | 530          | 157            | 236            | 113            |
| P vs C           | 50           | 17             | 14             | 7              |
| T and P vs C     | 108          | 21             | 35             | 17             |

**Table 3** Up-regulated and down-regulated genes

| Probe SetID | T vs C SLR | P vs C SLR | Name | GO molecular function |
|-------------|------------|------------|------|-----------------------|
| 202580-x-at | 2          | 2.4        | Forkhead box M1 | GO:3702; RNA polymerase II transcription factor activity; traceable author statement |
| 203098-at   | 2.3        | 2.4        | Chromodomain protein, Y chromosome-like | GO:3682; chromatin binding; inferred from electronic annotation; GO:3824; enzyme activity; inferred from electronic annotation |
| 203334-at   | 3.1        | 3.8        | DEAD/H(Asp-Glu-Ala-Asp/His) box polypeptide 8 (RNA helicase) | GO:3723; RNA binding; inferred from electronic annotation |
| 208152-s-at | 1.9        | 2.1        | DEAD/H(Asp-Glu-Ala-Asp/His) box polypeptide 21 | GO:3723; RNA binding; inferred from electronic annotation; GO:4004; ATP dependent RNA helicase activity; traceable author statement |
| 209820-s-at | 2.2        | 2.3        | Transducin (beta)-like 3 | GO:5057; receptor signaling protein activity; predicted/computed |
| 217173-s-at | 3.4        | 3.6        | Low density lipoprotein receptor (familial hypercholesterolemia) | GO:5041; low-density lipoprotein receptor activity; traceable author statement |
| 207102-at   | -2.4       | -3.6       | Aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase) | GO:5034; ATP binding; traceable author statement; GO:5319; lipid transporter activity; inferred from electronic |
| 208500-x-at | -2.7       | -3         | H4 histone family, member E (H4FE) | GO:5489; electron transporter activity; experimental evidence |
| 218730-s-at | -2.5       | -3.3       | Osteoglycin (osteoinductive factor, mimecan) | GO:8083; growth factor activity; inferred from electronic annotation |
but it still is one of the principal diseases to seriously hurt people’s health. To explore its etiology and to seek the ideal and early molecular markers, and to develop a new method of gene therapy are a new direction against cancers. Carcinogenesis is a series of molecular changes caused by abnormal expression of tumor-associated genes or inactivation of tumor suppression genes. The gene chip has been widely used to detect gene expression differences in various specimens by parallel analysis. The largest advantage of this technique is that it changes the traditional experiment where only a single or several gene expression differences could be observed in one procedure. Therefore, more and more cDNA microarrays have been applied to the study of gene expression. For example, gene chip was used to study the relationship between Helicobacter pylori and gastric malignancy, to research the multidrug-resistance to chemotherapy, to establish carcinosgenesis models of gastric carcinoma, to find gastric cancer and its metastasis associated genes, to analyse gastric carcinoma and its prognosis and to compare gastric carcinoma with its normal gastric mucosa.

We used the oligonucleotide of gene chip technique to analyze the gene expression profile difference in gastric cancer, pericancerous epithelium and normal mucosa. When gastric cancer was compared with normal gastric mucosa, a total of 766 genes were found with a difference of more than four times in expression levels. Of the 766 genes, 530 were up-regulated (SLR>2), and 236 were down-regulated (SLR<-2), suggesting that these genes are associated with the occurrence and development of gastric cancer. When pericancerous epithelium was compared with normal gastric mucosa, a total of 64 genes were found, with a difference of more than four times in expression levels. Of the 64 genes, 50 were up-regulated (SLR>2) and 14 were down-regulated (SLR<-2). Compared with normal gastric mucosa, a total of 143 genes with a difference in expression levels (more than four times, either in cancer or in pericancerous epithelium) were found in gastric cancer (T) and pericancerous epithelium (P). Of the 143 genes, 108 were up-regulated (SLR>2) and 35 were down-regulated (SLR<-2). Although an abnormal appearance of pericancerous epithelium was not found in pathological examination, but the 143 genes were shown to have differences in gene expression levels. A more interesting finding was that six genes of pericancerous epithelium were up-regulated compared to gastric cancer and three genes were down-regulated compared to gastric cancer. All but one gene’s function was unknown, the function of other genes was related to the transporter activity of lipid and electrons, transcription factor activity, growth factor activity and ATP dependent RNA helicase activity, suggesting that these genes are probably related to the promotion and progression of carcinogenesis at the early stage of gastric cancer. To seek for the expression products of these genes would be very useful in early diagnosis of gastric cancer.

Table 4 Comparison between gene molecular functions of T, P and C

| Molecular function                                      | Up-regulated (SLR>2) | Down-regulated (SLR<-2) |
|--------------------------------------------------------|----------------------|------------------------|
| Enzyme activity                                        | 15                   | 4                      |
| Enzyme regular activity                                | 4                    | 0                      |
| Structural molecule activity                            | 3                    | 1                      |
| Transcription factor activity                           | 3                    | 0                      |
| Nucleic acid binding (RNA, DNA)                        | 16                   | 5                      |
| Protein binding activity (Ca, GF, cell skeleton)        | 14                   | 7                      |
| Carbohydrate binding activity                          | 5                    | 0                      |
| Metal ion binding activity                              | 1                    | 0                      |
| Signal transduction activity (receptor, GF)            | 7                    | 3                      |
| Sport activity                                         | 2                    | 1                      |
| Transporter activity (electron, ion, protein)           | 7                    | 6                      |
| Tumor antigen                                          | 2                    | 0                      |
| Tumor suppressor                                        | 3                    | 0                      |
| Apoptosis suppressor                                    | 1                    | 0                      |
| Fibrinogen                                             | 0                    | 1                      |
| Unknown                                                | 27                   | 7                      |
| Total                                                  | 108                  | 35                     |

CEA is a soluble glycoprotein with a complex structure. Its molecular weight is about 200 000, CEA presents in gastrointestinal tract, pancreas and liver at embryonic development stage, and would increase in gastrointestinal malignant tumors. In other tumors such as breast and lung cancer, it was increased in the serum. Therefore, CEA is a broad-spectrum tumor marker, although it does not act as a specific marker for some malignant tumors, but it still has an important clinical value in differential diagnosis. In the present study, we found that CEA associated cell adhesion molecule 5 (CAM-5) (SLR cancer was 4.6, pericancerous epithelium was 2.1) and CAM-6 (SLR cancer was 5.6, pericancerous epithelium was 2.1) were up-regulated. Sakakura reported that CEA gene expression was up-regulated in metastatic gastric cancer. Oue reported that CEA CAM-6 gene expression was up-regulated in 50% patients with gastric cancer, suggesting that carcino-embryonic antigen associated genes are up-regulated to produce a soluble glycoprotein which could secrete into the blood, increasing the amount of CEA in serum. It could be a molecular mechanism of CEA appearance in serum of patients with gastric cancer. It is a useful tumor marker for gastric cancer.

Na+/K+-ATPase widely distributed on cell membranes is a key enzyme for keeping Na+/K+ ion gradients of cells. Such an enzyme not only attends the ion and protein translocation and keeps the ion self-balance of stabilization, but also has an important role in embryogenesis of vertebrate, neuronal recognition, development of central nervous system, cell morphogenesis, cell adhesion, etc. Lee reported that
Na+/K+-ATPase expression levels were up-regulated in intestinal type of gastric cancer. In our study, ATPase, Na\(^+\)/K\(^+\) transporting, alpha 1 polypeptide were found (SLR cancer was 2.4, pericancerous was 1.4).

Alcohol dehydrogenase and aldehyde dehydrogenase are two key enzymes in ethanol metabolism and its metabolic products. Aldehyde dehydrogenase and its gene polymorphism (aldehyde dehydrogenase) are related to liver cancer, gastric cancer and esophageal cancer. Abe\(^{10}\) reported that aldehyde dehydrogenase expression level was obviously down-regulated in tissues of gastric cancer. In the present study, we also found that alcohol dehydrogenase IB expression levels were

### Table 5 Comparison between molecular functions of 21 genes in T, P and C

| Probe ID | T vs C SLR | P vs C SLR | Name                                                                 | GO molecular function                                                                 |
|----------|------------|------------|----------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| 201655-s-at | 3.1       | 2.6       | Heparan sulfate proteoglycan 2 (perlecan)                           | GO:5198; structural molecule activity; inferred from electronic annotation              |
|          | 201890-at  | 3.1       | Ribonucleotide reductase M2 polypeptide                            | GO:4748; ribonucleoside-diphosphate reductase activity; non-traceable author statement |
|          | 201926-s-at | 3.1    | Decay accelerating factor for complement (CD55, Cromer blood group system) | GO:16491; oxidoreductase activity; inferred from electronic annotation                   |
| 203334-at | 3.1       | 3.8       | DEAD/H (Asp-Glu-Ala-asp/His) box polypeptide 8 (RNA helicase)       | GO:3723; RNA binding; inferred from electronic annotation                               |
| 202870-s-at | 3.3      | 1.5       | CDC20 cell division cycle 20 homolog (S. cerevisiae)                | GO:3723; RNA binding; inferred from electronic annotation                               |
| 209035-at | 3.3       | 1.6       | Midkine (neutire growth-promoting factor 2)                          | GO:8201; heparin binding; not recorded                                                 |
|          | 209803-s-at | 3.4 | Tumor suppressing subtransferable candidate 3                      | GO:8201; heparin binding; not recorded                                                 |
|          | 212444-at  | 3.4       | Homo sapiens, clone IMAGE:44772, mRNA                              | GO:8083; growth factor activity; not recorded                                          |
|          | 217175-s-at | 3.4 | Low density lipoprotein receptor (familial hypercholesterolemia)     | GO:8201; heparin binding; not recorded                                                 |
|          | 217221-x-at | 3.5 | RNA binding motif protein 10                                       | Go:3723; RNA binding; inferred from electronic annotation                              |
|          | 202855-s-at | 3.6 | Solute carrier family 16 (monocarboxylic acid transporters), member 3 | Go:3723; RNA binding; inferred from electronic annotation                              |
|          | 203599-s-at | 3.6 | Amiloride binding protein 1 (amine oxidase (copper-containing))      | GO:8201; heparin binding; not recorded                                                 |
|          | 209373-at  | 3.8       | BENE protein                                                       | GO:8201; heparin binding; not recorded                                                 |
|          | 203108-at  | 4.2       | Retinoic acid induced 3                                            | GO:8201; heparin binding; not recorded                                                 |
|          | 202067-s-at | 4.5 | Low density lipoprotein receptor (familial hypercholesterolemia)     | GO:8201; heparin binding; not recorded                                                 |
|          | 218900-at  | 4.5       | Cyclin M4                                                          | Go:3723; RNA binding; inferred from electronic annotation                              |
|          | 201884-at  | 4.6       | Carcinoembryonic antigen-related cell adhesion molecule 5           | GO:8201; tumor antigen; traceable author statement                                     |
|          | 211657-at  | 5.9       | Carcinoembryonic antigen-related cell adhesion molecule 6           | GO:8201; tumor antigen; traceable author statement                                     |
|          | 212768-s-at | 4.7 | Differentially expressed in hematopoietic lineages                  | GO:8201; tumor suppressor; predicted/computed                                          |
|          | 208250-s-at | 6.3 | Deleted in malignant brain tumors 1                                 | GO:8201; tumor suppressor; predicted/computed                                          |
|          | 204855-at  | 7.7       | Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin) member 5 | GO:8201; tumor suppressor; predicted/computed                                          |
Serine protein kinase takes part in translocation and localization, as well as signal transfer of cells. Oien et al. [20] reported that serine protein kinase expression levels were obviously up-regulated in human gastric cancer tissues. In the present study, we also found that serine protein kinase inhibitor expression level was obviously up-regulated (SLR cancer was 7.7, pericancerous was 4.2).

It has been demonstrated that the activity of topoisomerase II (Topo II) was rapidly increased in proliferating cells from S phase to G2/M phase, suggesting that this enzyme activity might be related to the malignant transformation of tumor cells. So Topo II expression levels (high or low) might be a parameters for cell proliferation. It also could be used as a marker for judging the sensitivity and tolerance of antitumor treatment.

### Table 6 Comparison between molecular functions of 17 genes in T, P and C

| Probe Set ID | T vs C SLR | P vs C SLR | Name | GO molecular function |
|--------------|------------|------------|------|-----------------------|
| 207102-at    | -2.4       | -3.6       | Aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase) | GO:5489; electron transporter activity; experimental evidence |
| 218730-s-at  | -2.5       | -3.3       | Osteoglycin (ostesinductive factor, mimecan) | GO:8083; growth factor activity; inferred from electronic annotation |
| 204940-at    | -3.3       | -1.3       | Phospholamban | GO:42030; ATPase inhibitor activity; inferred from electronic annotation |
| 208281-x-at  | -3.3       | -1.1       | Deleted in azoospermia 3 | GO:3676; nucleic acid binding; inferred from electronic annotation |
| 216351-x-at  | -3.3       | -1.6       | Deleted in azoospermia 4 | Unknown |
| 209894-at    | -3.7       | -1.5       | Leptin receptor | GO:4896; hematopoietin/interferon-class (D200-domain) cytokine receptor activity; inferred from electronic annotation |
| 214723-x-at  | -3.7       | -1.5       | KIAA1641 protein | Unknown |
| 203571-s-at  | -4.8       | -1.6       | Adipose specific 2 | Unknown |
| 218087-s-at  | -1.7       | -1.3       | Sorbin and SH3 domain containing 1 | GO:8092; cytoskeletal protein binding; experimental evidence |
| 207909-x-at  | -5.2       | -1.2       | Deleted in azoospermia | GO:3676; rm; nucleic acid binding activity; 4.3e-10; extended: inferred from electronic annotation |
| 213071-at    | -5.3       | -1.3       | Dermatopontin | GO:5315; protein binding; traceable author statement |
| 201497-x-at  | -5.4       | -1.2       | Myosin, heavy polypeptide 11, smooth muscle | GO:8094; cell adhesion molecule activity; inferred from electronic annotation |
| 207912-s-at  | -5.9       | -1.3       | Deleted in azoospermia | GO:3676; rm; nucleic acid binding activity; 4.3e-10; extended: inferred from electronic annotation |
| 220630-s-at  | -6.3       | -1.8       | Eosinophil chemotactic cytokine | GO:3725; RNA binding; predicted/computed |
| 209612-s-at  | -6.9       | -1.4       | Alcohol dehydrogenase IB (class I), beta polypeptide | GO:4024; alcohol dehydrogenase activity, zinc-dependent; traceable author statement |

Obviously down-regulated (SLR cancer was -6.9, pericancerous was -1.4).

Serine protein kinase takes part in translocation and localization, as well as signal transfer of cells. Oien et al. [20] reported that serine protein kinase expression levels were obviously up-regulated in human gastric cancer tissues. In the present study, we also found that serine protein kinase inhibitor expression level was obviously up-regulated (SLR cancer was 7.7, pericancerous was 4.2).
Cell skeleton tissues and formation of external matrix of cells play an important role in the invasion of tumors. In the present study we found a number of genes were involved in cell skeleton and matrix such as keratin protein 19 (SLR cancer was 2.5, pericancerous was 1.6), microtubule protein β 4 (SLR cancer was 2.4, pericancerous was 1.4), microtubule protein β 2 (SLR cancer was 2.1, pericancerous was 1.4), reticuloprotein 1, and interzonal fiber binding protein 500 ku (SLR cancer was 2.1, pericancerous was 1.8). Bae et al[30] reported that cell skeleton protein was up-regulated in gastric cancer. Inhibitor 3 of proteins belongs to external matrix was obviously up-regulated in human gastric cancer tissues. This kind of study models will produce a new direction for diagnosis, therapy and prevention with tumor invasion and metastasis.

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The application of gene chip technique is a revolution in life science. This kind of study models will produce a great effect on researches in life science. Gene chip might provide a new direction for diagnosis, therapy and prevention of human gastric carcinoma.

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