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• *GASTRIC CANCER* •

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

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Abstract

AIM: To study the difference of gene expression in gastric cancer (T), pericancerous epithelium (P) and normal tissue of gastric mucosa (C), and to screen an associated novel gene in early gastric carcinogenesis by oligonucleotide microarray.

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

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.

Key words: Gastric cancer; Pericancerous epithelium; Gene expression profile; Gene chip

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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[1]. 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

Table 1 Clinical and pathological data of five patients with gastric cancer

Number of In-P	Name	Sex	Age (yr)	Pathological diagnosis	Lymph metastasis	Clinical stage
113702	Jiang	F	45	Lesser curvature of stomach invasion ulcer type moderate differentiation adenocarcinoma invasion serosa	1/35	III
123730	Xu	M	48	Anterior wall in body of stomach moderate differentiation adenocarcinoma invasion muscular layers and nerve	1/17	III
123673	Tang	F	57	Lesser curvature in cardia of stomach invasion ulcer type moderate differentiation adenocarcinoma invasion serosa	1/14	III
123808	Yu	F	49	Anterior wall in cardia of stomach poor-moderate differentiation adenocarcinoma, a part of mucinous adenocarcinoma and esophagus of extremities inferior	0/35	II
123733	Huang	M	60	Cardia of stomach node type moderate differentiation adenocarcinoma invasion muscular layer esophagus	5/22	III

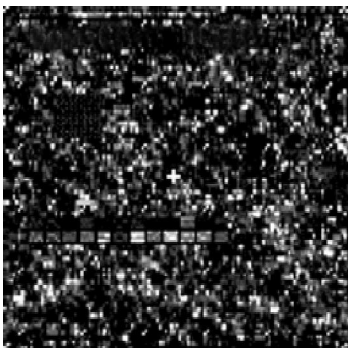

Figure 1 Control cRNA *vs* test chip post-hybridization.

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

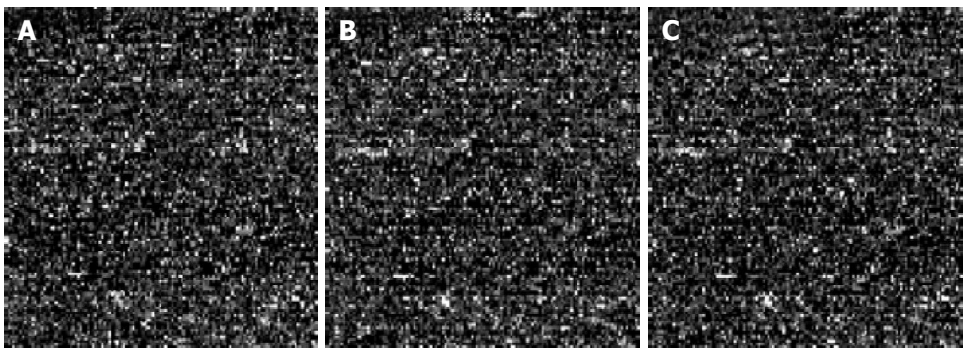

Figure 2 Hybridization results of the sample chip of scanning results. **A**: Post-hybridization of scanning results in control cRNA *vs* U133A chip; **B**: Post-hybridization of scanning results in gastric cancer group cRNA *vs* U133A chip; **C**: Post-hybridization of scanning results in pericancerous group cRNA *vs* U133A chip.

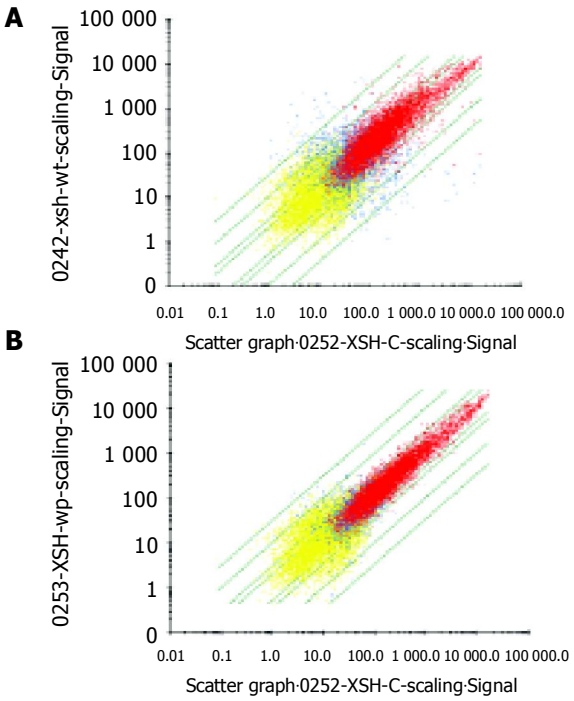

Figure 3 Scatter plots in gastric cancer and pericancerous tissues **A**: Scatter plots in gastric cancer tissue *vs* control **B**: Scatter plots in pericancerous tissue *vs* control.

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.

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

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 3 Up-regulated and down-regulated genes

Probe Set ID	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 GO:3700; 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 GO:8248; pre-mRNA splicing factor activity; traceable author statement GO:5524; ATP binding; inferred from electronic annotation GO:4004; ATP dependent RNA helicase activity; traceable author statement
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:5524; ATP binding; inferred from electronic annotation GO:4004; ATP dependent RNA helicase activity; traceable author statemen
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 GO:5509; calcium ion binding; inferred from electronic annotation GO:8034; lipoprotein binding; traceable author statement GO:5319; lipid transporter activity; inferred from electronic
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
208580-x-at	-2.7	-3	H4 histone family, member E (H4FE)	Unknown
218730-s-at	-2.5	-3.3	Osteoglycin (osteoinductive factor, mimecan)	GO:8083; growth factor activity; inferred from electronic annotation

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	3	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

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[2-6], to research the multidrug-resistance to chemotherapy[7-9], to establish carcinogensis models of gastric carcinoma[10], to find gastric cancer and its metastasis associated genes[11-14], to analyse gastric carcinoma and its prognosis and to compare gastric carcinoma with its normal gastric mucosa[15-29].

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 carcinogensis 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.

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[12] 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[24] reported that

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

Probe Set 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 GO:5206; heparin sulfate proteoglycan; traceable author statement
201890-at	3.1	1.6	Ribonucleotide reductase M2 polypeptide	GO:4748; ribonucleoside-diphosphate reductase activity;non-traceable author statement GO:16491; oxidoreductase activity; inferred from electronic annotation
201926-s-at	3.1	1	Decay accelerating factor for complement (CD55, Cromer blood group system)	Unknown
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 GO:8248; pre-mRNA splicing factor activity; traceable author statement GO:5524; ATP binding;inferred from electronic annotation GO:4004; ATP dependent RNA helicase activity; traceable author statement
202870-s-at	3.3	1.5	CDC20 cell division cycle 20 homolog (S. cerevisiae)	Unknown
209035-at	3.3	1.6	Midkine (neurite growth-promoting factor 2)	GO:8201; heparin binding; not recorded GO:8083; growth factor activity; not recorded GO:5125; cytokine activity; not recorded
209803-s-at	3.4	1.8	Tumor suppressing subtransferable candidate 3	Unknown
212444-at	3.4	1.2	*Homo sapiens*, clone IMAGE:4471726, mRNA	Unknown
217173-s-at	3.4	3.6	Low density lipoprotein receptor (familial hypercholesterolemia)	GO:5041; low-density lipoprotein receptor activity; traceable author statement GO:5509; calcium ion binding; inferred from electronic annotation GO:8034; lipoprotein binding; traceable author statement GO:5319; lipid transporter activity; inferred from electronic
217221-x-at	3.5	2.2	RNA binding motif protein 10	GO:3676; rrm; nucleic acid binding activity; 8.4e-05; extended: inferred from electronic annotation GO:3676; rrm; nucleic acid binding activity; 0.76; extended: inferred from electronic annotation
202855-s-at	3.6	1.2	Solute carrier family 16 (monocarboxylic acid transporters), member 3	GO:8028; monocarboxylic acid transporter activity; traceable author statement GO:15355; monocarboxylate porter activity; inferred from electronic annotation GO:15293; symporter activity; inferred from electronic annotation
203559-s-at	3.6	1.2	Amiloride binding protein 1(amine oxidase (copper-containing))	GO:8144; drug binding;not recorded GO:8201; heparin binding; inferred from electronic annotation GO:8122; amine oxidase (copper-containing) activity; inferred from electronic annotation GO:16491; oxidoreductase activity; inferred from electronic annotation
209373-at	3.8	1.6	BENE protein	Unknown
203108-at	4.2	1.5	Retinoic acid induced 3	GO:8067; metabotropic glutamate, GABA-B-like receptor activity; inferred from electronic annotation GO:4872; receptor activity; inferred from electronic annotation
202067-s-at	4.5	3.8	Low density lipoprotein receptor (familial hypercholesterolemia)	GO:5041; low-density lipoprotein receptor activity; traceable author statement GO:5509; calcium ion binding; inferred from electronic annotation GO:8034; lipoprotein binding; traceable author statement GO:5319; lipid transporter activity; inferred from electronic
218900-at	4.5	3.7	Cyclin M4	Unknown
201884-at	4.6	2.1	Carcinoembryonic antigen-related cell adhesion molecule 5	GO:8222; tumor antigen; traceable author statement
211657-at	5.9	2.1	Carcinoembryonic antigen-related cell adhesion molecule 6	GO:8222; tumor antigen; traceable author statement
212768-s-at	4.7	1.3	Differentially expressed in hematopoietic lineages	Unknown
208250-s-at	6.3	1.7	Deleted in malignant brain tumors 1	GO:8181; tumor suppressor; predicted/computed GO:5044; SRCR; scavenger receptor activity; 3.7e-42;extended: unknown
204855-at	7.7	4.2	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin) member 5	GO:4868; serpin; not recorded GO:4867; serine protease nhibitor activity; inferred from electronic annotation GO:8181; tumor suppressor; traceable author statement serp

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 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 (osteoinductive 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 GO:5246; calcium channel regulator activity; not recorded
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
208282-x-at	-4.3	-1.2	Deleted in azoospermia 2	GO:3676; nucleic acid binding; inferred from electronic annotation
219564-at	-4.3	-1.2	Potassium inwardly-rectifying channel, subfamily J, member 16	GO:5242; inward rectifier potassium channel activity; inferred from electronic annotation GO:5244; voltage-gated ion channel activity; inferred from electronic annotation GO:5267; potassium channel activity; inferred from electronic annotation
203571-s-at	-4.8	-1.6	Adipose specific 2	Unknown
218087-s-at	-5	-1.3	Sorbin and SH3 domain containing 1	GO:8092; cytoskeletal protein binding; experimental evidence GO:3779; actin binding; experimental evidence
207909-x-at	-5.2	-1.2	Deleted in azoospermia	GO:3676; rrm; nucleic acid binding activity; 4.3e-10; extended:inferred from electronic annotation GO:3723; RNA binding; predicted/computed
213071-at	-5.3	-1.3	Dermatopontin	GO:5515; protein binding; traceable author statement GO:5194; cell adhesion molecule activity; inferred from electronic annotation
201497-x-at	-5.4	-1	Myosin, heavy polypeptide 11, smooth muscle	GO:3779; actin binding; inferred from electronic annotation GO:5516; calmodulin binding; inferred from electronic annotatio GO:5524; ATP binding; inferred from electronic annotation GO:3774; motor activity; inferred from electronic annotation
207912-s-at	-5.9	-1.3	Deleted in azoospermia	GO:3676; rrm; nucleic acid binding activity; 4.3e-10;extended:inferred from electronic annotation GO:3723; RNA binding;predicted/computed
220630-s-at	-6.3	-1.8	Eosinophil chemotactic cytokine	GO:16787; Glyco_hydro_18; hydrolase activity; 2.7e-81; extended:Unknown GO:16798; hydrolase activity, acting on glycosyl bonds;inferred from electronic annotation GO:8061; chitin binding;inferred from electronic annotation GO:8843; endochitinase activity; inferred from electronic annotation
209612-s-at	-6.9	-1.4	Alcohol dehydrogenase IB (class I), beta polypeptide	GO:4024; alcohol dehydrogenase activity, zinc-dependent;traceable author statement GO:4327; 1.2.1.1; formaldehyde dehydrogenase (glutathione) activity; 3.69e-120; extended: inferred from electronic annotation GO:4023; alcohol dehydrogenase activity, metal ion-independent; inferred from electronic annotation GO:4025; alcohol dehydrogenase activity, iron-dependent;inferred from electronic annotation GO:8270; zinc ion binding; traceable author statement GO:4552; 1.1.1.73; octanol dehydrogenase activity; 4.76e-120; extended: non-traceable author statement GO:16491; oxidoreductase activity; inferred from electronic annotation GO: 5489; electron transporter activity; 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[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

drugs. Varis *et al*[30], reported that Topo II expression level was obviously up-regulated in human gastric cancer tissues. Skotheim[31] reported that Topo II expression level was obviously up-regulated in malignant peripheral nerve sheath tumors. In our study the Topo II expression was also up-regulated in gastric cancer and pericancerous epithelium (SLR cancer was 2.3, pericancerous was 1.4). It might be related to a degree of malignancy and resistance. In the present study, we found that the cell cycle protein M4 (SLR cancer was 4.5, pericancerous was 3.7) was highly expressed. It might indicate that tumor cells had a high proliferation ratio.

In our study, the expression of E-cadherin (SLR cancer was 2.3, pericancerous was 1.8) and integrin β 4 (SLR cancer was 2.7, pericancerous was 2.0) was significantly increased in gastric cancer as reported by Mori *et al*[16], Hippo *et al*[25] and Oue *et al*[34].

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*[32] reported that cell skeleton protein was up-regulated in gastric cancer. Inhibitor 3 of proteins belongs to external matrix of cells (SLR cancer was 2.3, pericancerous was 1.7). Wang *et al*[33] reported that external matrix of gastric cancer cells was up-regulated. We found that 4/5 patients had a lymph-node metastasis, and related metastatic genes had a higher expression, further suggesting that the overexpression of cell skeleton tissues and external matrix is associated with tumor invasion and metastasis.

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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