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• *GASTRIC CANCER* •

A microarray-based gastric carcinoma prewarning system

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Abstract

AIM: To develop a microarray-based prewarning system consisting of gastric cancer chip, prewarning data and analysis software for early detection of gastric cancer and pre-cancerous lesions.

METHODS: Two high-density chips with 8 464 human cDNA sites were used to primarily identify potential genes specific for normal gastric mucosa, pre-cancerous lesion and gastric cancer. The low-density chips, composed of selected genes associated with normal gastric mucosa, precancerous lesion and gastric cancer, were fabricated and used to screen 150 specimens including 60 specimens of gastric cancer, 60 of pre-cancerous tissues and 30 of normal gastric mucosa. CAD software was used to screen out the relevant genes and their critical threshold values of expression levels distinguishing normal mucosa from pre-cancerous lesion and cancer. All data were stored in a computer database to establish a prewarning data library for gastric cancer. Two potential markers *brcaa1* and *ndr1* were identified by Western blot and immunohistochemistry.

RESULTS: A total of 412 genes associated with three stages of gastric cancer development were identified. There were 216 genes displaying higher expression in gastric cancer, 85 genes displaying higher expression in pre-cancerous lesion and 88 genes displaying higher expression in normal gastric mucosa. Also 15 genes associated with metastasis of gastric cancer and 8 genes associated with risk factors were screened out for target genes of diagnosis chip of early gastric cancer. The threshold values of 412 selected genes to distinguish gastric cancer, pre-cancerous lesion from normal gastric mucosa were defined as 6.01±2.40, 4.86±1.94 and 5.42±2.17, respectively. These selected 412 genes and critical threshold values were compiled into an analysis software, which can automatically provide reports by analyzing the results of 412 genes obtained by examining gastric tissues. All data were compiled into a prewarning database for gastric cancer by CGO software. Northern blot and immunohistochemistry analysis confirmed that gene and protein of *brcaa1* displayed lower expression in normal gastric mucosa and higher expression in gastric cancer tissues, conversely, *ndr1* displayed lower expression in gastric cancer and higher expression in normal gastric mucosa.

CONCLUSION: The microarray-based prewarning system for gastric cancer was developed. This system consisted of gastric cancer-associated gene chip, prewarning data and analysis software, which has a high potential for applications in the early detection of gastric cancer. The two potential markers *brcaa1* and *ndr1* identified may be used to distinguish cancer status fand non-cancer status.

Key words: Microarray; Prewarning; Gastric cancer

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INTRODUCTION

Gastric cancer has high incidence in China and in the whole world. Understanding the biological processes of cancer initiation at the gene expression level is very important for early cancer detection. Study of gene expression levels at different stages of growth, disease, cell cycle, and response to stimulation may help to answer why different stages of cancerous development occur[1]. We have been trying to establish a prewarning system of gastric cancer as a part of a larger effort to develop effective and economical diagnostic tools capable of distinguishing different stages of cancer

development. This system consists of three important parts: a gastric cancer microarray, a prewarning data library and a data analysis software.

Screening characteristic differentially expressed genes associated with different stages of cancer development is of central significance to this study. In our previous studies[2,3], some differentially expressed genes between gastric cancer tissues and precancerous lesions have been obtained. Genes that have been shown to correlate with gastric cancer were used as a part of the target genes in the microarray. Commercially available microarrays with 8 464 human cDNA sites have also been used for identifying specific genes associated with normal mucosa, precancerous lesions and gastric cancer.

The gene microarray technique has the advantage of simultaneously monitoring the expression of thousands of genes in one hybridization experiment. This technique has greatly facilitated the detection of differentially expressed genes and the construction of gene expression profiles. Since 1995, the DNA microarray technique has been widely employed to investigate the functions of genes, especially those genes involved in tumor generation and growth[4]. This technique has a great potential as a practical clinical tool for medical diagnosis[5]. Although many genes are known to be related to the pathological process of gastric carcinoma, so far very few prognostic biomarkers of gastric cancer have actually been used in clinical medicine. In our present study, we tried to identify specific genes involved in gastric carcinogenesis, with the objective of establishing a prewarning system for early diagnosis, therapy and prevention of gastric cancer.

MATERIALS AND METHODS

Resource of tissue specimens

Specimens used in this study were classified into three different categories: those of gastric cancer (including all types of pathologic gastric cancers such as diffuse type and intestinal type), those of paracancerous lesions (according to international classified standard including atrophic gastritis, intestinal gland metaplasia, atypical hyperplasia) and those of normal gastric mucosa (including slight superficial gastritis). A total of 150 specimens including 60 gastric cancers, 60 pre-cancerous lesions and 30 normal gastric mucosa in liquid nitrogen with clear pathological results, were provided by the department of Gastroenterology of the Xi'an Central Hospital. Human cDNA microarrays with 8 464 were purchased from BioDao Company in Shanghai.

Reagents

Total RNA was extracted by using total RNA extract kit from Promega Inc. Reverse transcriptionof mRNA was performed by using Smart PCR cDNA synthesis kit (Clontech). Reaction products were purified with Wizard plus minipreps DNA purification system. Cy3-dUTP, Cy5-dUTP and CSS-25 silylated slides (aldehyde) were purchased from Pharmacia Inc. and Gene Limited Inc. Spot report™ oligo™array validation system (Cat # 252170-7) was purchased from Stratagene® Company. Other reagents were purchased from Sigma Inc.

Fabrication of microarrays

Microarrays consisting of 2 435 fragment sites including 412 genes were fabricated. These synthesized oligonucleotide DNAs were first dissolved in 3× SSC solution. Spot report oligo array validation system (Cat # 252170-7) was used as quality control. Spots with pure 3× SSC solution were selected as background control. The target genes were spotted on silylated slides by MicroGridII spotting robotics (BioRotics Inc.). After spotting, the slides were hydrated (2 h), dried (0.5 h, RT), UV crosslinked (65 mJ/cm), and then treated with 2 g/L SDS (10 min), H_2O (10 min), and 2 g/L $NaBH_4$ (10 min). The slides were dried before being made ready for usage.

Extraction of total RNAs and probe preparation

Total RNA extraction was performed by using total RNA extract kit from Promega Inc. Final total RNA templates were dissolved with non-RNase and non-DNase Milli-Q H_2O. Fluorescent cRNA probes were prepared through reverse transcription and then purified, referring to the protocol of Schena[DNA microarrays, a practical approach. Oxford University Press, 1999:110-126]. The probes from gastric cancer tissues and pre-cancerous tissues were labeled with Cy5-dUTP, those from normal gastric mucosa tissues with Cy3-dUTP. The labeled probes were mixed, fragmented and precipitated by ethanol and dissolved in 20 μL hybridization solution (5×SSC+2 g/L SDS).

Hybridization and washing

After denatured at 95 ℃ for 5 min, the probes were added onto slides, covered with a cover glass and incubated at 42 ℃ for 17 h. The slides were subsequently washed in solutions of 2× SSC+2 g/L SDS, 0.1× SSC+2 g/L SDS and 0.1× SSC, 10 min each time, and then dried at room temperature.

Detection and analysis

Microarrays were scanned by using Affymetrix® 428™ array scanner. ImageGene 3.0 software (BioDiscovery Inc.) was used to quantify, correct for background noise and normalize the signals from post-hybridization chip.

Construction of prewarning data library

The data files were incorporated into a computer database by CGO software, including patient disease history and all screened results, such as, name, file number, sex, age, address, telephone, e-mail address, marital status, blood type, body mass, disease history, imaging examination, pathological examination, serum examination, blood examination, cytogenetic report, and gene array report.

Threshold values of expression profiles

Expression gene profiles were established according to the acquired data. CAD software was used in the selection of discriminating candidate genes by their correlation with three kinds of gastric tissues, determination of the optimal set of reporter genes by using a leave-one-out validation procedure, determination of the threshold values of selected gene expression levels to distinguish normal gastric mucosa from pre-cancerous lesions and gastric cancer, and metastatic cancer and no-metastatic cancer.

Analysis software for gastric cancer prewarning data

A total of 412 genes and critical threshold values to distinguish normal gastric mucosa from pre-cancerous lesion and gastric cancer were compiled into an analysis software, which could provide analysis reports by analyzing the microarray test results.

Northern blot analysis

Five micrograms of mRNA was resolved by denaturing formaldehyde agarose gel and transferred onto hybrid membranes (Amersham). The membranes were hybridized with ^{32}p-labeled fragments of cDNA overnight, washed twice in 1 g/L standard saline citrate and 1 g/L SDS for 20 min and then exposed to Kodak BioMax film at -80 ℃ with an intensifying screen for 24 h.

Immunohistochemistry analysis

Standard avidin-biotin complex (ABC) technique was used for immunohistochemical staining of formalin-fixed, paraffin-embedded gastric cancer tissues. Specific antibody (10 mg/L) and PBS were added onto tissue slides previously blocked with rabbit serum and incubated overnight. After washing with PBS, the slides were incubated with a rabbit anti-human IgG conjugated to biotin at room temperature for 1 h, alkaline phosphatase substrate was then added for color development. The slides were counterstained with hematoxylin-eosin.

Statistical analysis

A two-way clustering analysis was performed by using Cluster software and Tree view software from http://www.microarray.org[PNAS 1998; 95:14863]. Statistical analysis was performed by using the *t* test. All *P* values were based on two-sided testing, and a significant difference was defined as *P* less than 0.05.

RESULTS

Screened genes associated with normal gastric mucous, pre-cancerous lesion and gastric cancer

Two high-density chips were used to primarily screen differential genes associated with normal gastric mucosa, pre-cancerous lesion and gastric cancer. According to the obtained partial biochip hybridization results, 393 genes closely associated with three stages of gastric cancer development were primarily screened out (Figure 1). Fifteen genes associated with gastric cancer metastasis and 8 genes associated with risk factor genes of gastric cancer, such as cagA, vacA, Ure, EB, were selected according to the literature[6]. These genes were used as main target genes on the prewarning chip. The oligonucleotides associated with 412 genes were designed, synthesized and fabricated into low-density chip.

One hundred and fifty specimens screened by low-density chip

All the 150 specimens with clear pathological results were screened with the fabricated low-density microarrays. Among these, 60 were known to be cancerous, 60 precancerous and 30 normal (Figure 2). In the 60 cancer specimens, 216 genes were found to exhibit higher expression levels than those in normal gastric mucosa. Among the 216 genes, 156 also exhibited higher expression levels than those in the

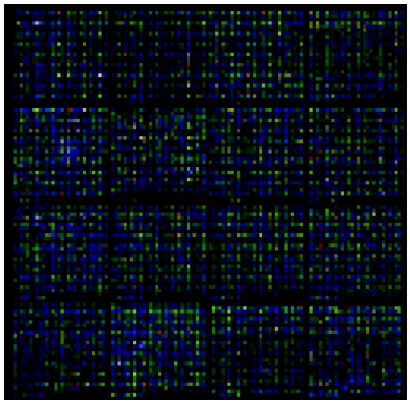

Figure 1 Results of high-density chip hybridization with gastric tissues. Red and yellow: higher gene expression levels. Green and blue: lower gene expression levels.

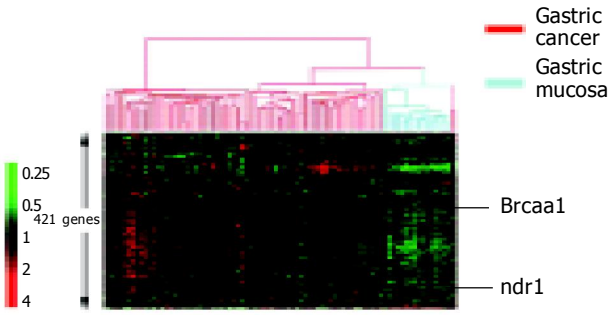

Figure 2 Cluster analysis of low-density-chip-examination results of 150 specimens.

precancerous lesions (Table 1). In the 60 specimens of Pre-cancerous lesions, 126 genes exhibited higher expression levels than those in the normal tissues. Among those, 85 genes also showed higher expression levels than those in the gastric cancer tissues (Table 1). Contrary to our initial expectations, selected risk factor genes such as cagA, vacA, Ure, EB did not show overexpression levels in gastric cancer tissues in comparison with the normal tissues and precancerous lesions. In fact, these genes showed lower expression levels in gastric cancer tissues than in normal tissues and precancerous lesions. This result demonstrated that the risk factor genes due to *H pylori* infection might be more closely associated with the progression of precancerous lesion. Eighty-eight genes in normal tissues exhibited higher expression levels than those found in gastric cancer tissues and pre-cancerous tissues (Table 1). These genes are helpful for distinguishing normal gastric mucosa from precancerous lesions. This is very important in diagnosing the precancerous lesion among common gastric diseases, such as superficial gastritis, because the treatment of precancerous lesion requires special focused methods. If left untreated, precancerous lesion might result in gastric cancer in a limited time.

Construction of prewarning database library of gastric cancer

The gene expression profiles of each specimen obtained by biochip were stored together with patient clinical data

including follow-up treatments until death. The data files were incorporated into a computer database by CGO software, including patients' disease history and all screened results such as name, file number, sex, age, address, telephone, e-mail address, marital status, blood type, body mass, disease history, imaging examination, pathological examination, serum examination, blood examination, cytogenetic report, gene array report. The prewarning data were added with new content. These data would be available on *Gastric Cancer Information Web* presided by Dr. Cui at http://www.37c.com.cn.

Critical threshold values to distinguish normal gastric mucosa from pre-cancerous lesion and gastric cancer

A total of 412 genes were selected as the main diagnostic genes, including 216 genes that displayed higher expression levels in cancer tissues than in non-cancer tissues, 85 genes with higher expression levels in precancerous lesions than in cancer tissues and 88 genes that exhibited higher expression levels in normal tissues than in gastric cancer tissues and pre-cancerous tissues. We selected 15 genes associated with metastasis of gastric cancer as metastasis biomarkers, 8 risk factor genes as reference biomarkers to predict the development of pre-cancerous lesions (Table 1). The critical threshold values to distinguish normal gastric mucosa from pre-cancerous lesion and gastric cancer were decided and were summarized in Table 2.

Table 1 Differentially expressed genes in prewarning microarray of gastric cancer

	GenBank Number	Description of gene
Highly expressed genes in gastric cancer		
1	NM_001962	Homo sapiens ephrin-A5 (EFNA5)
2	XM_017384	Homo sapiens matrix metalloproteinase 7 (MMP7)
3	NM_008610	Mus musculus matrix metalloproteinase 2 (Mmp2)
4	NM_004995	Homo sapiens matrix metalloproteinase 14 (MMP14)
5	AF093573	Bos taurus angiopoietin-1 (ang-1)
6	AF004327	Homo sapiens angiopoietin-2
7	M11730	Human tyrosine kinase-type receptor (HER2)
8	U13948	Human zinc finger/leucine zipper protein (AF10)
9	XM_049646	Homo sapiens similar to octamer-binding transcription factor 3B (OCT-3B)
10	XM_055784	Homo sapiens fibroblast growth factor 2 (basic) (FGF2)
11	XM_056035	Homo sapiens proliferating cell nuclear antigen (PCNA)
12	L24203	Homo sapiens ataxia-telangiectasia group D-associated protein
13	XM_087201	Homo sapiens similar to RED protein, IK cytokine
14	X00663	Human mRNA fragment for epidermal growth factor (EGF) receptor
15	NM_002607	Homo sapiens platelet-derived growth factor alpha polypeptide (PDGFA)
16	XM_165656	Homo sapiens matrix metalloproteinase 2 (MMP2)
17	NM_005918	Homo sapiens malate dehydrogenase 2, NAD (mitochondrial) (MDH2)
18	AF503165	Homo sapiens HUS1 checkpoint homolog (HUS1) gene
19	XM_045667	Homo sapiens antigen identified by monoclonal antibody Ki-67 (MKI67)
20	XM_050913	Homo sapiens frequently rearranged in advanced T-cell lymphomas (FRAT1)
21	XM_032866	Homo sapiens signal transducer and activator of transcription 5A (STAT5A)
22	NM_004103	Homo sapiens protein tyrosine kinase 2 beta (PTK2B)
23	XM_008355	Homo sapiens membrane protein, palmitoylated 2 (MPP2)
24	L18920	Human MAGE-2 gene exon 2, 3, 4
25	M12174	Human ras-related rho
26	NM_012333	Homo sapiens c-myc binding protein (MYCBP)
27	BC016514	Homo sapiens, similar to translocated promoter region (to activated MET oncogene)
28	NM_004324	Homo sapiens BCL-2 associated X protein (BAX)
29	Z26580	cyclin A
30	D45906	LIMK-2
31	D21255	OB-cadherin-2
32	X54925	Type I interstitial collagenase
33	X05232	Stromelysin, matrix metalloproteinase 3
34	M22612	Human pancreatic trypsin 1 (TRY1)
35	XM_055254	Homo sapiens fibronectin 1 (FN1)
36	AF081127	Danio rerio fibronectin (fn2)
37	M15796	Human cyclin protein gene
38	HSFIBEDA	Human fibronectin gene ED-A region
39	HSU66406	Human putative EPH-related PTK receptor ligand LERK-8 (Eplg8)
40	AF068846	Homo sapiens scaffold attachment factor A (SAF-A)
41	HSBTRCP	Homo sapiens mRNA for beta-transducin repeat containing protein
42	AF110763	Homo sapiens skeletal muscle LIM-protein 1 (FHL1) gene
43	HUMHO2SOS1	Human mRNA for heme oxygenase-2
44	HSHMSH16	Human mutator hMSH2 gene
45	HSEHK1	Homo sapiens mRNA for EHK-1 receptor tyrosine kinase
46	HSKLON30	Homo sapiens mRNA for unknown antigen
47	AB005047	Homo sapiens mRNA for SH3 binding protein
48	AF070561	Homo sapiens clone 24703 beta-tubulin
49	HUMCAM1V	Human vascular cell adhesion molecule 1
50	HSRNASMG	Homo sapiens mRNA for Sm protein G
51	X83228	Homo sapiens mRNA for LI-cadherin
52	AF125100	Homo sapiens HSPC039 protein
53	HSU97018	Homo sapiens echinoderm microtubule-associated protein homolog HuEMAP
54	HSU43188	Human Ets transcription factor (NERF-2)
55	HSY17392	Homo sapiens mRNA for prefoldin subunit 1
56	HSU08316	Human insulin-stimulated protein kinase 1 (ISPK-1)
57	HZNF232G2	Homo sapiens zinc finger protein ZNF232, exons 2 and 3
58	HUMP53T	Human p53 cellular tumor antigen
59	J03040	HumanSPARC/osteonectin
60	XM_053809	Homo sapiens similar to chondroitin sulfate proteoglycan 2 (versican)
61	L40379	Homo sapiens thyroid receptor interactor (TRIP10)
62	HSU72069	Human karyopherin beta2
63	HUMPGK2	Human phosphoglycerate kinase (pgk) mRNA, exons 2 to last
64	HSU07139	Human voltage-gated calcium channel beta subunit
65	XM_001472	Homo sapiens v-jun sarcoma virus 17 oncogene homolog (avian) (JUN)
66	AU100088	Human phosphogluconate dehydrogenase (hPGDH) gene
67	HUMKRUPZN	Human Kruppel related zinc finger protein (HTF10)
68	AF077050	Homo sapiens neuroendocrine-specific protein C homolog
69	HUMSC35A	Human splicing factor SC35
70	HUMPTPB	Homo sapiens protein tyrosine phosphatase (CIP2)
71	AF049608	Homo sapiens monocarboxylate transporter 2 (MCT2)
72	HUMHEK	Human receptor tyrosine kinase (HEK)
73	J03210	Human collagenase type IV
74	HSRAB9P40	Homo sapiens mRNA for Rab9 effector p40
75	AF184924	Homo sapiens zinc finger transcription factor BTEB2 gene

76	HUMC5A2A	Human fibrillar collagen (proa2 (V)) gene
77	HUMGAPA	Human GTPase-activating protein ras p21 (RASA)
78	HUMGLURS	Human glutamate receptor subunit (GluH1)
79	AF047715	Homo sapiens A-kinase anchoring protein (AKAP18)
80	HSU40282	Homo sapiens integrin-linked kinase (ILK)
81	HSATPF1M	Human mRNA for mitochondrial ATP synthase (F1-ATPase) alpha subunit
82	AF152485	Homo sapiens protocadherin alpha 7 short form protein (PCDH-alpha7)
83	HSRP19	Human mRNA for 19 ku protein of signal recognition particle (SRP)
84	U17195	Homo sapiens A-kinase anchor protein (AKAP100)
85	HSU79299	Human neuronal olfactomedin-related ER localized protein
86	XM_037859	Human focal adhesion kinase (FAK)
87	HSU04209	Human-associated microfibrillar protein
88	D82878	Hemicentrotus pulcherrimus mRNA for p34cdc2
89	AF060515	Homo sapiens cyclin K (CPR4)
90	D21262	Human mRNA for KIAA0035 gene
91	NM_005641	Homo sapiens TATA box binding protein-associated factor, RNA polymerase II, 85 ku
92	HSU07550	Human chaperonin 10
93	X82153	Homo sapiens mRNA for cathepsin 0
94	HSU41766	Human metalloprotease/disintegrin/cysteine-rich protein precursor (MDC9)
95	AB017019	Homo sapiens mRNA for JKTBP2
96	HUMFNC	Human cellular fibronectin
97	U93033	Homo sapiens thyroglobulin (TG)
98	AF0304354	Homo sapiens proteoglycan 3 (PRG3) gene
99	HUMCOL3IX	Homo sapiens collagen alpha 3 type IX (COL9A3)
100	NM_002427	Homo sapiens matrix metalloproteinase 13 (MMP13)
101	AF039747	Homo sapiens cadherin-10 (CDH10)
102	AF072242	Homo sapiens methyl-CpG binding protein MBD2 (MBD2)
103	HSMYCC	Human c-myc oncogene
104	HSTSPM	Homo sapiens tissue specific mRNA
105	HSU64317	Human Crk-associated substrate related protein Cas-L
106	HSVACM1	Homo sapiens mRNA for vasopressin activated calcium mobilizing receptor-like protein
107	HUMPA1V	Human pro-alpha-1 (V) collagen
108	AF059611	Homo sapiens nuclear matrix protein NRP/B (NRPB)
109	HSU004845	Human a6 (I V) collagen (COL4A6)
110	M87860	Human S-lac lectin L-14-II (LGALS2) gene
111	AF492837	Human mRNA for osteopontin
112	HSCOX7BM	Homo sapiens coxVIIb mRNA for cytochrome *c* oxidase subunit VIIb
113	U01244	Human fibulin-1D
114	U52153	Human inwardly rectifying potassium channel Kir3.2
115	S66427	RBP1=retinoblastoma binding protein 1 [human, Nalm-6 pre-B cell leukemia, mRNA, 4834 nt]
116	AF117108	Homo sapiens IGF-II mRNA-binding protein 3 (IMP-3)
117	HSU49083	Human cell surface heparin binding protein HIP
118	HSU59289	Human H-cadherin
119	HSU95032	Human growth-arrest-specific protein 2
120	HSU18018	Human E1A enhancer binding protein (E1A-F)
121	HUMCGRPB	Homo sapiens (clone HSNME29) CGRP type 1 receptor
122	X59543	Human mRNA for M1 subunit of ribonucleotide reductase
123	AF072810	Homo sapiens transcription factor WSTF
124	AF005068	Homo sapiens breast and ovarian cancer susceptibility protein splice variant (BRCA1)
125	HSU66197	Human fibroblast growth factor homologous factor 1 (FHF-1)
126	HUMVTNR	Human cell adhesion protein (vitronectin) receptor alpha subunit
127	HSA6417	Homo sapiens mRNA for beta-tubulin folding cofactor D
128	AF109126	Homo sapiens stromal cell-derived receptor-1 beta
129	AB030078	Homo sapiens mRNA for K-sam-II03
130	HUMMFAP	Homo sapiens extracellular matrix protein (MFAP3) gene
131	HUMCOLVA	Human alpha-2 type V collagen gene
132	HUMAAMP1X	Homo sapiens angio-associated migratory cell protein (AAMP)
133	Y08319	Homo sapiens mRNA for kinesin-2
134	HSVWFR1	Human mRNA for pre-pro-von Willebrand factor
135	S60085S2	ADMLX=putative adhesion molecule [human, mRNA, 4121 nt, segment 2 of 2]
136	HSU51334	Homo sapiens signal transducing adaptor molecule 2A (STAM2)
137	AF435957	Homo sapiens Ly-6 antigen/uPA receptor-like domain-containing protein
138	NM_000245	Homo sapiens met proto-oncogene (hepatocyte growth factor receptor)
139	XM_044659	Homo sapiens c-src tyrosine kinase (CSK)
140	AF061573	Homo sapiens protocadherin (PCDH8)
141	HUMMFAP	Homo sapiens extracellular matrix protein (MFAP3) gene
142	AF081535	Homo sapiens CDC45L (CDC45L)
143	HUMCA1XIA	Human alpha-1 type XI collagen (COL11A1)
144	AB016625	Homo sapiens OCTN2 gene
145	AF151899	Homo sapiens CGI-141 protein
146	HSU12535	Human epidermal growth factor receptor kinase substrate (Eps8)
147	HSFCRIB	Human mRNA for high affinity Fc receptor (FcRI) 'b form'
148	HSU74628	Homo sapiens cell division control related protein (hCDCrel-1)
149	AF039564	Homo sapiens retinoblastoma binding protein (RBBP9)
150	HUMGAPA	Human GTPase-activating protein ras p21 (RASA)
151	HUMSTK2A	Human protein serine/threonine kinase stk2
152	AF144700	Homo sapiens small zinc finger-like protein (TIM13)
153	HUMTUBAK	human alpha-tubulin
154	HUMADCY	Homo sapiens adenyl cyclase-associated protein (CAP)
155	HSU89329	Human alternatively spliced microtubule-associated protein 2C (MAP2)
156	BC00051	Homo sapiens, Insulin-like growth factor 2
157	HSU89329	Human alternatively spliced microtubule-associated protein 2C (MAP2)
158	BC00051	Homo sapiens, insulin-like growth factor 2
159	AB000529	Homo sapiens, prostate differentiation factor
160	HSMAP01	Human microtuble-associated protein-2 (MAP-2) gene, exon 1
161	X67951	Human mRNA for proliferation-associated gene (pag)
162	M94250	Human retinoic acid inducible factor (MK) gene
163	XM_046278	Homo sapiens core promoter element binding protein (COPEB)
164	HSBM40	Human mRNA for extracellular matrix protein BM-40
165	HSU76381	Homo sapiens fibroblast growth factor (FGF-12b)
166	HSCALM2S04	Homo sapiens calmodulin (CALM2) gene, exons 3-6
167	HUMID2X	Human helix-loop-helix protein(ID-2)
168	U20758	Human osteopontin gene
169	AF152307	Homo sapiens protocadherin alpha 11 (PCDH-alpha11)
170	HUMAAMP1X	Homo sapiens angio-associated migratory cell protein (AAMP)
171	HUMMXI1A	Human MXI1
172	AF143536	Homo sapiens colon cancer-associated protein Mic1 (MIC1)

173	HSU70322	Human transportin (TRN)
174	HUMMNMP	Human major nuclear matrix protein
175	AF071057	mRNA differentially expressed in GC7901 and GES-1
176	AF219140	Homo sapiens gastric cancer-related protein GCYS-20
177	HSU40282	Homo sapiens integrin-linked kinase (ILK)
178	HSCA2VR	Human mRNA for pro-alpha 2 (V) collagen chain
179	HUMPECAM27	Homo sapiens platelet/endothelial cell adhesion molecule-1 (PECAM-1) gene
180	XM_165823	Homo sapiens tumor necrosis factor (TNF superfamily, member 2) (TNF)
181	D21063	Homo sapiens MCM2 minichromosome maintenance deficient 2, mitotin
182	XM_168045	Homo sapiens CD24 antigen (small cell lung carcinoma cluster 4 antigen) (CD24)
183	XM_030326	Homo sapiens CD44 antigen (CD44)
184	XM_034862	Homo sapiens interferon regulatory factor 1 (IRF1)
185	AB025106	Homo sapiens mRNA for E-cadherin
186	U73704	Homo sapiens 48 ku FKBP-associated protein FAP48
187	AF380298	Oncorhynchus mykiss interferon regulatory factor 1 gene, promoter region and partial sequence
188	L24203	Homo sapiens ataxia-telangiectasia group D-associated protein
189	D45906	Homo sapiens mRNA for LIMK-2
190	D21255	Human mRNA for OB-cadherin-2
191	X54925	Homo sapiens mRNA for type I interstitial collagenase
192	X05232	Human mRNA for stromelysin, matrix metalloproteinase 3
193	M22612	Human pancreatic trypsin 1 (TRY1)
194	HUMGOS8PPC	Human helix-loop-helix basic phosphoprotein (GOS8)
195	HUMTHBS3	Homo sapiens thrombospondin 3 (THBS3) gene
196	HSVECAD	Homo sapiens VE-cadherin
197	HSBTRCP	Homo sapiens mRNA for beta-transducin repeat containing protein
198	HUMPROFII	Human profilin II
199	HSCALT	Homo sapiens mRNA for caltractin
200	AF091214	Homo sapiens WRN (WRN)
201	AF070561	Homo sapiens clone 24703 beta-tubulin
202	HUMCD14MCA	Human monocyte antigen CD14 (CD14)
203	HUMCAM1V	Human vascular cell adhesion molecule 1
204	AF032900	Homo sapiens timing protein CLK-1
205	AF070561	Homo sapiens clone 24703 beta-tubulin
206	HSUPUE	Homo sapiens mRNA for unknown protein of uterine endometrium
207	HUMIL8RB	Homo sapiens interleukin 8 receptor beta (IL8RB)
208	HSERK3	Homo sapiens ERK3
209	AF208045	Homo sapiens breast cancer-associated antigen BRCAA1 (BRCAA1)
210	AF081259	Homo sapiens testis-specific chromodomain Y-like protein (CDYL)
211	AB022918	Homo sapiens mRNA for alpha2,3-sialyltransferase ST3Gal VI
212	AF057036	Homo sapiens acetylcholinesterase collagen-like tail subunit (COLQ)
213	AF152497	Homo sapiens protocadherin beta 4 (PCDH-beta4)
214	M86752	Stress-induced phosphoprotein 1
215	L04270	TNF C receptor
216	AF009674	Axin 1

Highly expressed genes in precancerous lesions

1	HSU72621	Human LOT1
2	HUMNMOR	Human NAD(P)H: menadione oxidoreductase
3	AF009227	Homo sapiens gamma-heregulin
4	HSU44839	Human putative ubiquitin C-terminal hydrolase (UHX1)
5	HUMAAE	Homo sapiens dbpB-like protein
6	HSU08316	Human insulin-stimulated protein kinase 1 (ISPK-1)
7	HUMCD3621	Human antigen CD36 (clone 21)
8	Z11899	Homo sapiens OTF3 mRNA for encoding octamer binding protein 3B
9	XM_003226	Homo sapiens vasoactive intestinal peptide receptor 1 (VIPR1)
10	HUMPAI2B	Human plasminogen activator inhibitor 2 (PAI-2)
11	HUMACTIIA	Human activin type II receptor
12	M93718	Human nitric oxide synthase
13	HSU46837	Human RNA polymerase II holoenzyme component SRB7 (SRB7)
14	HUMPCNA	Human proliferating cell nuclear antigen (PCNA) gene
15	HSPCAR	Human mRNA for calcium dependent protease (small subunit)
16	HSU12140	Human tyrosine kinase receptor p145TRK-B (TRK-B)
17	HSTOP2A10	Homo sapiens topoisomerase II alpha (TOP2A) gene, exons 34 and 35
18	HUMYWXD703	Homo sapiens ADP/ATP carrier protein (ANT-2) gene
19	HUMKGF	Human keratinocyte growth factor
20	HS40KDAP	Homo sapiens 40 ku protein kinase related to rat ERK2
21	HUMPAFAA	Human mRNA for platelet activating factor acetylhydrolase IB gamma-subunit
22	HUMLPL	Human lipoprotein lipase
23	HUMMYLCC	Human smooth muscle myosin alkali light chain (MLC 1sm)
24	HSU10564	Human CDK tyrosine 15-kinase WEE1Hu (Wee1Hu)
25	AF022655	Homo sapiens cep250 centrosome associated protein
26	D49737	Homo sapiens mRNA for cytochrome b large subunit of complex II
27	HUMCD53GLY	Human CD53 glycoprotein
28	L02867	Homo sapiens 62 ku paraneoplastic antigen
29	HUMCALBETB	Human voltage-dependent calcium channel beta-1 subunit
30	HUMEPSURAN	Human surface antigen
31	AB020647	Homo sapiens mRNA for KIAA0840 protein
32	HSU88966	Human protein rapamycin associated protein (FRAP2) gene
33	HUMHGLUT1	Human mRNA for glutamate transporter
34	U70663	Human zinc finger transcription factor hEZF(EZF)
35	HSPTS1R	Homo sapiens mRNA for peroxisomal targeting signal 1 (SKL type) receptor
36	HSU61276	Human transmembrane protein Jagged 1 (HJ1)
37	HUMMYONM	Human nonmuscle myosin heavy chain (NMHC)
38	AF016270	Homo sapiens thyroid hormone receptor coactivating protein
39	HSU66243	Human p38 gamma MAP Kinase
40	HSU41766	Human metalloprotease/disintegrin/cysteine-rich protein precursor (MDC9)
41	HUMELF2	Human translational initiation factor 2 beta subunit (eIF-2-beta)
42	HUMCYCAA	Human somatic cytochrome *c* (HCS) gene
43	NM_013217	Homo sapiens gene for AF-6
44	AB017642	Homo sapiens mRNA for oxidative-stress responsive 1
45	AF110956	Homo sapiens SUMO-1 activating enzyme subunit 1 (SAE1)
46	HUMALR	Human aldehyde reductase
47	HUMATPSAS	Human gene for ATP synthase alpha subunit (exon 1 to 12)
48	AF052497	Homo sapiens clone B18
49	AB000889	Homo sapiens mRNA for phosphatidic acid phosphatase 2b
50	HUMTPARN	Homo sapiens mRNA for tissue plasminogen activator.
51	AF006082	Homo sapiens actin-related protein Arp2 (ARP2)
52	HSU21090	Human DNA polymerase delta small subunit
53	HUMVENHK1	Human voltage-gated potassium channel (HK1)

54	HUMVTNR	Human cell adhesion protein (vitronectin) receptor alpha subunit
55	AF091242	Homo sapiens ATP sulfurylase/APS kinase 2
56	HUMIGFBP1	Human insulin-like growth factor binding protein-1 (IGFBP1) gene
57	AF047439	Homo sapiens unknown
58	AF117386	Homo sapiens ubiquitin-specific protease (UBP)
59	AF092129	Homo sapiens guanine nucleotide binding protein gamma-3 subunit
60	HUMCOXIV	Human cytochrome *c* oxidase COX subunit IV (COX IV)
61	J05412	Human regenerating protein (reg) gene
62	AF054162	Gccys-1, mRNA differentially expressed between GC7901 and GES-1
63	AF054163	Gccys-2, mRNA differentially expressed between GC7901 and GES-1
64	AF054164	Gccys-3,mRNA differentially expressed between GC7901 and GES-1
65	AF054165	Gccys-4, mRNA differentially expressed between GC7901 and GES-1
66	AF054166	Gccys-5, mRNA differentially expressed between GC7901 and GES-1
67	AF054167	Gccys-6, mRNA differentially expressed between GC7901 and GES-1
68	NM_003542	Homo sapiens H4 histone family, member G(H4FG)
69	XM_032781	Homo sapiens tubulin, gamma 1 (TUBG1)
70	XM_083852	Homo sapiens ribonucleotide reductase M1 polypeptide(RRM1)
71	HSU51586	Human siah binding protein 1 (siahBP1)
72	X55181	Human ETS2 gene
73	NM_004526	Homo sapiens MCM2 minichromosome maintenance deficient 2, mitotin (MCM2)
74	XM_040900	Homo sapiens MAP/microtubule affinity-regulating kinase 3 (MARK3)
75	XM_083852	Homo sapiens ribonucleotide reductase M1 polypeptide(RRM1)
76	NM_012145	Homo sapiens deoxythymidylate kinase (thymidylate kinase) (DTYMK)
77	X59543	Ribonucleotide reductase M1 polypeptide
78	M74542	Human aldehyde dehydrogenase type III (ALDHIII)
79	M61855	Human cytochrome P4502C9 (CYP2C9)
80	S37730	Homo sapiens insulin-like growth factor binding protein-2
81	AB015982	Homo sapiens EPK2 mRNA for serine/threonine kinase
82	X67951	Human mRNA for proliferation-associated gene (pag)
83	AF127506	Homo sapiens adenomatosis polyposis coli tumor suppressor (APC) gene
84	HT880	Human Gastric mucin 6
85	M63154	Gastric intrinsic factor

Highly expressed genes in normal gastric mucous

1	X05997	Human mRNA for gastric Lipase
2	U75272	Human gastricsin
3	M63154	Human intrinsic factor
4	AF043909	Homo sapiens gastric mucin (MUC5AC)
5	L07518	Homo sapiens mucin
6	M61853	Human cytochrome p4502C18 (CYP2C18)
7	M10942	Human metallothionein-Ie gene (hMT-Ie)
8	L15533	Homo sapiens pancreatitis-associated protein (PAP) gene
9	Z49107	Homo sapiens galectin
10	U52191	HumanSMCY (H-Y)
11	NM_005522	Homo sapiens homeo box A1 (HOXA1)
12	M57732	Human hepatic nuclear factor 1 (TCF1)
13	X59770	Homo sapiens IL-1R2 mRNA for type II interleukin-1 receptor
14	X76223	Homo sapiens MAL gene exon 4
15	U05259	Human MB-1 gene
16	XM_052013	Homo sapiens polymeric immunoglobulin receptor (PIGR)
17	U90065	Human potassium channel KCNO1
18	M55422	Human Krueppel-related zinc finger protein (H-plk)
19	S78825	Id1, transcription regulator helix-loop-helix protein
20	U19948	Human protein disulfide isomerase (PDIp)
21	U43522	Human cell adhesion kinase beta (CAKbeta)
22	U12139	Human alphal (XI) collagen (COL11A1) gene, 5′ region and exon 1
23	M14539	Human factor XIII subunit
24	X65614	Homo sapiens mRNA for calcium-binding protein S100P
25	AF000560	Homo sapiens TTF-I interacting peptide 20
26	AF002224	Homo sapiens Angelman Syndrome Gene, E6-AP ubiquitin protein ligase 3A
27	U57096	Human janus kinase 3 (Jak3)
28	U42600	Human calcium-activated potassium channel beta subunit
29	NM_017406	cAMP responsive element binding protein-like 1
30	U04806	Human FLT3/FLK2 ligand
31	D84361	Human p52 and p64 isoforms of N-Shc
32	Z30425	Homo sapiens orphan nuclear hormone receptor
33	M16364	Human creatine kinase-B
34	X96924	Homo sapiens encoding mitochondrial citrate transport protein
35	HSNM23H1	Homo sapiens nm23H1 gene
36	NM_014792	Homo sapiens KIAA0125 gene product (KIAA0125)
37	M34041	Human alpha-2-adrenergic receptor (aipha-2 c2) gene
38	XM_002444	Homo sapiens serine threonine kinase 39 (Stk39)
39	NM_001690	Homo sapiens ATPase, H+ transporting, lysosomal 70 ku, V1 subunitA
40	L12398	Human sapiens dopamine receptor D4 (DRD4)
41	L76465	Homo sapiens NAD+ dependent15 hydroxyprostaglandin dehydrogenase (PGDH)
42	U57094	Human small GTP-binding protein
43	Z14978	Homo sapiens mRNA for actin-related protein
44	X53961	Human lactotransferrin
45	M62628	Human alpha-1 Ig germline C-region membrane-coding region
46	M84526	Human adipsin/complement factor D
47	X04391	Human lymphocyte glycoprotein T1/Leu-1
48	X044533	Homo sapiens sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphoring) 4B (SEMA4B)
49	AF071054	Gcys-11, mRNA differentially expressed in cell lines GC7901 and GES-1
50	AF063015	Homo sapiens cell division protein
51	AF071056	Gcys-17, mRNA differentially expressed in cell lines GC7901 and GES-1
52	AF071058	Gcys-15, mRNA differentially expressed in cell lines GC7901 and GES-1
53	NM_001730	Homo sapiens Kruppel-like factor 5 (intestinal) (KLF5), mRNA
54	AB047278	Arabidopsis thaliana AtNdr 1 mRNA for Ndr kinase
55	XM_061005	Homo sapiens similar to Mucin 2 precursor (Intestinal mucin2)
56	HUM20D9	Human gene for 2-oxoglutarate dehydrogenase
57	HSCDC2	Human CDC2 gene involved in cell cycle control
58	AF202051	Homo sapiens NM23-H8 (NME8)
59	NM_005423	Homo sapiens trefoil factor 2 (spasmolytic protein 1) (TFF2)
60	D50419	Homo sapiens OTK18
61	HSU88870	Human cell division control-related protein 2b (hcdcrel2b)
62	NM_031942	Homo sapiens cell division cycle associated 7 (CDCA7)
63	HSU09716	Human mannose-specific lectin (MR60)
64	HSU14394	Human tissue inhibitor of metalloproteinases-3

65	Z48314	Apomucin
66	M63154	Gastric intrinsic factor
67	J05412	Regenerating protein
68	M57732	Hepatic nuclear factor 1
69	U70663	Kruppel-like factor 4
70	AB002559	Syntaxin binding protein 2
71	U80226	GABA transaminase
72	U05259	CD79A
73	X04391	CD5
74	U60800	CD100
75	M74542	Aldehyde dehydrogenase 3
76	X66839	Carbonic anhydrase IX
77	L00972	Cystathionine-beta-synthase
78	L41688	UDP-galactose-4 epimerase
79	J03915	Chromogranin A
80	S76942	Dopamine receptor D4
81	D14695	Herp
82	D50915	KIAA0125
83	D86961	HMGIC fusion partner-like 2
84	X96924	Mitochondrial citrate transporter
85	M16364	Creatine kinase, brain
86	M14539	Factor XIII precursor
87	U19948	Protein disulfide isomerase
88	X65614	S100 calcium binding protein P
Highly expressed genes associated with metastasis		
1	NM_004994	Homo sapiens matrix metalloproteinase 9 (gelatinase B, 92 ku type IV collagenase) (MMP9)
2	XM_053256	Homo sapiens mucin 1, transmembrane (MUC1)
3	XM_010702	Homo sapiens cathepsin K (pycnodysostosis) (CTSK)
4	NM_002628	Homo sapiens profiling 2 (PFN2), transcript variant 2
5	NM_002128	Homo sapiens high-mobility group protein 1 (HMG1)
6	M28130	Human interleukin 8 (IL8) gene
7	S3488	Metastasis-associated gene (human, highly metastatic lung cell subline Anip)
8	NM_005231	Homo sapiens ems1 sequence, transcript variant 1
9	XM_059020	Homo sapiens similar to GPI-anchored metastasis-associated protein homolog
10	NP_571483	Vascular endothelial growth factor (VEGF)
11	I56986	OPN-a-human (fragment)
12	AAG31602	CD44 isoform v3-v6
13	AF018733	92 ku type IV collagenase precursor (matrix metalloproteinase-9) (MMP-9)
14	AF00196	Octamer-binding transcription factor 2 (OTF-2)
15	XM_055254	Homo sapiens fibronectin 1 (FN1)
Risk factor genes		
1	V01555	Epstein-Barr virus (EBV) genome, strain B95-8
2	AF275307	*H pylori* plasmid pHPM8 (cagA)
3	AF275307	*H pylori* plasmid vacA
4	AF275307	*H pylori* plasmid Urase
5	AF431736	Human herpesvirus 1 strain KOS ICPO gene
6	Z86099	Herpes simplex virus type 2 (strain HG52)
7	AF477385	Human papillomavirus type 16 E7 gene
8	AX742207	Human hepatitis virus 11 type

Analysis software for prewarning data of gastric cancer

All 412 genes and critical threshold values to distinguish normal gastric mucosa from precancerous lesion and gastric cancer were compiled into an analysis software, which can automatically provide analysis reports by analyzing the provided microarray test results. The analysis software for examination results of prewarning system of gastric cancer locates on the website http://shasta.mpi-stuttgart.mpg.de/array/form.html. The software cannot be downloaded until it is confirmed to be very effective and complete.

Northern blot of brcaa1 and ndr1

Two new biomarkers *brcaa1* and *ndr1* (NM_007271) were identified. *Brcaa1* (AF208045) showed no or low-expression levels in normal gastric mucosa and high-expression level in gastric cancer There was a statistically significant difference in expression levels between normal gastric mucous tissues and gastric cancer tissues ($P<0.01$, Figure 3), indicating that higher expression of *brcaa1* was closely associated with gastric cancer stage. Further analysis indicated that higher expression of *brcaa1* appeared to have no correlation with pathological types of gastric cancer ($P>0.05$, data not shown). Conversely, *ndr1* (NM_007271) displayed higher expression levels in normal gastric tissues and no or lower expression in gastric cancer, and there was a statistically significant difference in expression levels between normal gastric mucous tissues and gastric cancer tissues ($P<0.01$), indicating that higher expression level of *ndr1* was closely associated with normal stage of gastric mucosa tissues.

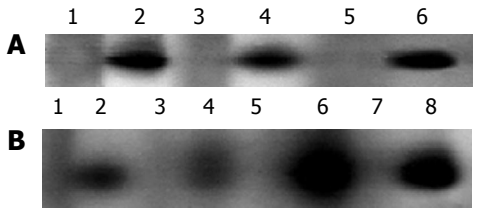

Figure 3 Northern blot analysis of *brcaa1* and *ndr1*. A: *brcaa1*: lanes 1, 3, 5: Normal gastric tissues; lanes 2, 4, 6: Gastric cancer tissues; B: *ndr1*: lanes 1, 3, 5, 7: Gastric cancer tissues; lanes 2, 4, 6: Normal gastric tissues.

Immunohistochemistry analysis of brcaa1 and ndr1

Brcaa1 protein exhibited higher expression in 60 gastric cancer tissues, lower or no expression in 30 normal gastric

Table 2 Gene expression threshold for distinguishing three kinds of gastric mucosa

Gene classification	Gastric cancer tissue (GC/N)	Precancerous lesion (PC/N)	Normal gastric mucosa (N*/GC or N*/PC)
216 genes associated with gastric cancer	6.01±2.40	1.18 ±0.47	< 0.75
85 genes associated with precancerous lesions	1.32±0.53	4.86±1.94	2.54±0.41
88 genes associated with normal mucosa	1.31±0.54	2.50±0.75	5.42±2.17
15 genes associated with metastasis of gastric cancer	5.81±2.32 (M) 2.32±1.19 (N^1)	1.13±0.58	0.65±0.35
8 genes associated with risk factors		>2.0	

Specification: The above data indicate the relative expression levels between GC/N, PC/N, N/PC and N/GC mean ratio and minimum values. M: Metastasis; N^1: No metastasis. GC: Gastric cancer; PC: Precancerous lesion; N: Normal mucosa. N*: Selected gene expression levels in normal gastric mucosa.

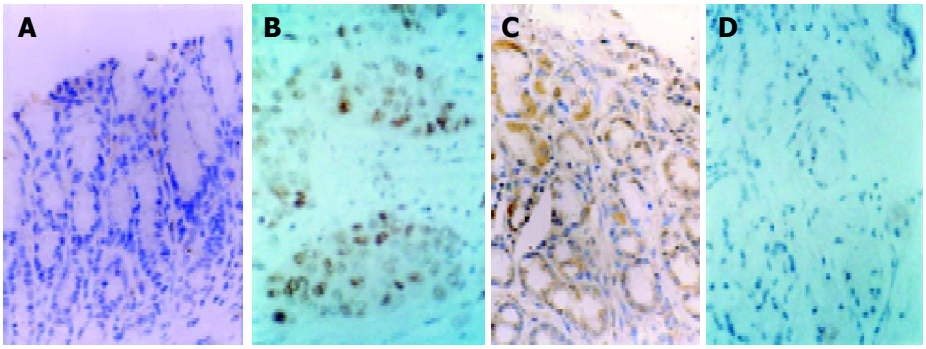

Figure 4 Immunohistochemistry analysis of *brcaa1* and *ndr1*. A: *Brcaa1* expression in normal gastric mucosa tissues (200 size); B: *Brcaa1* expression in gastric cancer tissues (400 size); C: *Ndr1* expression in normal gastric mucous tissues (200 size); D: *Ndr1* expression in gastric cancer tissues (200 size).

mucosa tissues. There was a statistically significant difference in expression levels between gastric cancer tissues and normal gastric mucous tissues ($P<0.01$, Figure 4A). The result indicated that higher expression of *brcaa1* was associated with gastric cancer stage. *Ndr1* protein exhibited higher expression in 30 normal gastric mucosa tissues, lower or no expression in 60 gastric cancer tissues. There was a statistically significant difference in expression levels between normal gastric mucous tissues and gastric cancer tissues ($P<0.01$, Figure 4B). The result indicated that higher expression of *ndr1* was closely associated with normal stage of gastric mucous tissues.

DISCUSSION

The development of normal gastric mucosa into gastric cancer is a complex process. Previous research in the pathology of gastric cancer demonstrated that normal gastric mucosa could gradually develop into pre-cancerous lesions under special conditions, eventually evolving toward gastric carcinoma. During the periods from normal gastric mucosa to gastric cancer, it has not been shown how many genes are involved at different stages of cancer development. The cDNA microarray technology could provide an efficient tool to address the difficulties in screening and quantifying expression levels of a large number of genes[7-10]. So far there are some reports associated with gene expression profiles of gastric cancer based on biochip[11,12]. However, the problem of early gastric cancer detection is still not solved satisfactorily. In the present study, we tried to establish a prewarning system of gastric cancer based on biochip and CAD technique to solve the problem of early gastric cancer detection.

Firstly, two high-density microarrays with 8 464 human cDNA sites were used to screen two pairs of gastric cancer tissues and 389 genes associated with three stages of gastric cancer development such as normal gastric mucosa, precancerous lesion and gastric cancer were obtained. The selected 389 genes were used as main diagnostic genes on the prewarning chip, 15 genes associated with metastasis of gastric cancer as diagnostic genes of metastasis stages, 8 risk factor genes as reference biomarkers to predict the development of precancerous lesions.

A total of 412 genes were selected to fabricate the low-density chip, which was used to screen 150 clinical specimens. It was found that the gene expression levels in normal, pre-cancerous lesion and cancer tissues were significantly different as expected. CAD software and statistical methods were used to identify key genes and their critical threshold values characterizing different tissue status. Two hundred and sixteen genes displayed higher expression levels in cancer tissues than in non-cancer tissues, 85 genes exhibited higher expression levels in precancerous lesions than in cancer tissues, and 88 genes exhibited higher expression levels in normal tissues than in gastric cancer and precancerous tissues (Table 1). The critical threshold values to distinguish normal gastric mucosa from precancerous lesion and gastric cancer were identified (Table 2). With the above-mentioned standards, the 150 specimens could be clearly grouped according to their tissue status determined in pathology diagnosis. Therefore, we considered that the established standard had a great potential in the detection of early gastric cancer. Based on these selected genes and critical threshold values characterizing three stages of gastric cancer development, an analysis software was developed which could analyze the examination results of 412 genes achieved by biochip and provide automatically an analysis report. The software remained to be optimized. These expression profiles obtained from all these specimens and available clinical data had been compiled into a prewarning data library of gastric cancer by CGO software, and these detailed data would be very useful for the further research and therapy of gastric cancer.

From Table 2, it appeared reasonable to define integrate markers of GC, PC, NU consisting of many genes, instead of individual genes, to distinguish three kinds of gastric tissues status. Once gastric cancer was diagnosed, the expression levels of 15 metastasis genes could be subjected to focal studies to identify whether the cancer metastasized, and to speculate the prognosis of the cancer patients. These results could also be complemented with supporting evidence from patient's disease history, for example, discomfort or pain in the gastric area, body mass loss in a short time, *etc*. If a precancerous lesion was diagnosed, the expression levels of risk factor genes might be analyzed as indicators on how fast such lesion would lead to cancer[13]. One may also establish and search the prewarning database library to compare similar patients to make a best treatment plan. The diagnosis and treatment information associated with

gastric cancer can also be obtained from gastric cancer information web presided over by Dr. Cui http://www.37c.com.cn. The prewarning database of gastric cancer is available on gastric cancer information web. The analysis software of examination results of the prewarning system of gastric cancer locates on the website http://shasta.mpi-stuttgart.mpg.de/array/form.html.

Two new biomarkers have been identified of diagnostic value, *brcaa1* (AF208045)[14] and *ndr1* (NM_007271). *Brcaa1* showed no or low-expression levels in normal gastric mucosa and high-expression level in gastric cancer, and appeared to have no correlation with pathological types of gastric cancer. Conversely, *ndr1* displayed high-expression levels in normal gastric tissues and no or lower expression in gastric cancer. These results were also confirmed by Northern blot and immunohistochemistry analysis. These two biomarkers may be very useful for distinguishing benign from malignant gastric mucosa lesions.

Gastric cancer specimens from different patients were found to display some variability in gene expression profiles. The reasons could be attributed to variations in specimens, lesion types and the number of cells collected. Moreover, variations among individuals may pose a serious challenge to diagnosis accuracy. In cases of doubt, it would be advisable to analyze microarray results together with clinical symptoms of patients and pathological results. It is very difficult to devise gene expression profiles to further classify the specimens consistent with pathology types such as atrophic gastritis, intestinal gland metaplasia, atypical hyperplasia, *etc*. Of course, new methods of disease classification can be defined according to gene expression profiles and DNA levels (mutation, deletion and amplification). Such methods may not be fully consistent with pathology classification, but nevertheless may be appropriate for future clinical applications. In the near future, pathological diagnosis will remain a useful and complementary diagnostic tool.

To test the generality of this standard, we collected randomly some autopsy specimens and screened them with fabricated gastric microarrays. Simultaneously, pathology diagnosis was performed on the same specimens. We found that the results achieved by the microarray were highly identical with traditional pathological results. In another paper, we have reported these results in detail[15,16].

In summary, further studies will lead to a more complete prewarning database library. The prewarning database, together with miniaturized microarray techniques, will be used to further improve the accuracy and reliability of the prewarning system for gastric cancer[16].

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