Gene expression profiling of gastric cancer by microarray combined with laser capture microdissection

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Abstract

AIM: To examine the gene expression profile of gastric cancer (GC) by combination of laser capture microdissection (LCM) and microarray and to correlate the profiling with histological subtypes.

METHODS: Using LCM, pure cancer cells were procured from 45 cancerous tissues. After procurement of about 5,000 cells, total RNA was extracted and the quality of RNA was determined before further amplification and hybridization. One microgram of amplified RNA was converted to cDNA and hybridized to cDNA microarray.

RESULTS: Among 45 cases, only 21 were qualified for their RNAs. A total of 62 arrays were performed. These included 42 arrays for cancer (21 cases with dye-swap duplication) and 20 arrays for non-tumorous cells (10 cases with dye-swap duplication) with universal reference. Analyzed data showed 504 genes were differentially expressed and could distinguish cancerous and non-cancerous groups with more than 99% accuracy. Of the 504 genes, trefoil factors 1, 2, and 3 were in the list and their expression patterns were consistent with previous reports. Immunohistochemical staining of trefoil factor 1 was also consistent with the array data. Analyses of the tumor group with these 504 genes showed that there were 3 subgroups of GC that did not correspond to any current classification system, including Lauren’s classification.

CONCLUSION: By using LCM, linear amplification of RNA, and cDNA microarray, we have identified a panel of genes that have the power to discriminate between GC and non-cancer groups. The new molecular classification and the identified novel genes in gastric carcinogenesis deserve further investigations to elucidate their clinicopathological significance.

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Key words: Gastric cancer; Microarray; Laser capture microdissection

INTRODUCTION

Gastric cancer (GC) is the second most common cause of cancer-related deaths in the world[1]. In Taiwan, GC ranks the fourth cancer-related death and caused more than 2,000 deaths annually[2]. The prognosis of GC depends mainly on early detection and adequate surgical resection. Although endoscopy with biopsy has been effectively used since 1980s for early diagnosis, the proportion of early to advanced GC being found through this method has not appreciably increased in recent years[3]. In spite of the current surgical techniques and chemotherapy that have made significant improvements, the cure rate for advanced GC remains low and the morbidity remains high[4]. Thus, to improve its detection and therapy, understanding of the pathogenesis and biologic features of GC is crucial.

Gastric carcinogenesis is a multi-factorial and multi-step process accompanied by accumulation of alterations of critical growth regulatory genes[5]. Delineating these genes involved may lead to important new insights into carcinogenesis[6]. Despite the fact that some interesting and promising genetic alterations have been elucidated[7], previous studies of gastric carcinogenesis were incomprehensive and inconclusive. The overall information of the genetic alterations is scanty due to technical problems of analyses. Firstly, the value of even the most sophisticated genetic testing methods will be limited if the inputs of genetic materials are not derived from pure populations or are contaminated by the wrong cells. In solid neoplasm such as GC, stromal and inflammatory cells usually intermingled with cancer cells. Therefore, special procedures to isolate cancer cells from heterogeneous tissues are mandatory. Secondly,
most of previous studies on genetic alterations of GC have focused on selected genes or chromosomal regions known in other cancers. These obstacles may be overcome using the recently developed techniques of laser capture microdissection (LCM) and microarray. LCM allows for the rapid, reliable, and accurate procurement of cells from specific microscopic regions of tissue sections under direct visualization \[8\]. It affords the opportunity to perform molecular genetic analysis of pure populations of malignant cells in their native tissue environment. On the other hand, the advent of high-density cDNA microarray technology with its capacity for simultaneous monitoring of thousands of genes, provides a unique opportunity for high-throughput genetic analysis of cancer\[9\]. For GC, several investigators have demonstrated the use of DNA microarray is beneficial for elucidation of gastric carcinogenesis\[10-24\]. However, to our knowledge, combined analyses of LCM and microarray in GC remain scanty\[25\]. Therefore, we aimed to examine gene expression profiles of GC by these two techniques.

**MATERIALS AND METHODS**

**Tissues samples for LCM and RNA isolation**

A total of 45 cancerous tissues and their respective non-cancerous tissues obtained at operation from patients with GC were collected and immediately frozen in liquid nitrogen. Gastric cancer and normal cells were stained by Histogene LCM Frozen Section Staining Kit and laser capture microdissected by using a Pix Cell II LCM system (Arturus, USA). Malignant and normal cells were captured in a number of about 5 000 cells and their total RNAs were isolated by using PicoPure RNA extraction kit (Arturus, USA). The quality of RNA was determined by Bioprocessor before further amplification and hybridization.

**RNA amplification, probe labeling, and hybridization**

Linear RNA amplification was performed by using the RiboAmp kit (Arturus, USA). Two rounds of RNA amplification were performed to obtain enough amplified RNA (aRNA) for a microarray experiment. To serve as reference in cDNA microarray comparison, a human reference RNA pooled from 9 cell lines (Stratagene, USA) was amplified identically. cDNA was transcribed from aRNA at a quantity of 1.5 μg per channel in the presence of Cy3- or Cy5-dUTP by using Cyscribe First-Strand cDNA Labelling kit (Amersham Biosciences, USA). Free conjugated dUTP was removed by Millipore Microcon YM-30 column. Cy5-labeled cDNA was pooled with Cy5-labeled reference probe in 30 μL of hybridization solution and hybridized to an Agilent human 1 cDNA microarray (Agilent Technology, USA). Hybridization was carried out at 65 °C for 17 h in a humidified dark chamber (Genetix, UK). After hybridization, slides were washed at the following condition: 2 X SSC/0.1 g/L SDS at 60 °C for 10 min, 2X SSC at room temperature for 10 min, and 0.2X SSC at room temperature for 10 min.

**Scanning, image analysis, and data processing**

Washed microarrays were scanned with a Virtek fluorescence reader (Virtek, CA, USA) at 535 nm for Cy3 and 625 nm for Cy5. Scanned images were analyzed by using Array-Pro image acquisition software (Media Cybernetics, USA), an image analysis algorithm was used to quantify the signal and background intensity for each target element. Data normalization was performed by lowess method using R package (written by Terry Speeds Microarray Data Analysis Group, University of Berkeley, USA). Measurements from dye-swap replicates were average after normalization. A software developed by researchers in Stanford University-Prediction Analysis of Microarray (PAM) and Spotfire software was utilized to analyze the data.

**Immunohistochemistry**

Immunostaining for trefoil factor 3 protein was performed by using a standard avidin-biotin-peroxidase complex detection system\[25\]. The monoclonal antibody used for this study was purchased from Signet Laboratory (Bedham, MA, USA). In brief, 5-μm sections were dewaxed, microwaved, and rehydrated. Endogenous peroxidase activity and non-specific bindings were blocked by incubation with 30 mL/L hydrogen peroxide (H2O2) and non-immune serum, respectively. The slides were then incubated sequentially at 4 °C with the primary mouse monoclonal antibody overnight, a biotinylated goat anti-mouse secondary antibody for 30 min, peroxidase-conjugated streptavidin for 10 min and finally diaminobenzidine tetrahydrochloride/H2O2 chromogen substrate for 10 min. Slides were then counterstained with Mayer's hematoxylin. Negative control sections were prepared by substituting the primary antibody with buffered saline. The percentage of positively stained cells was determined for each tumor section as well as its adjacent intestinal metaplasia and non-metaplastic epithelium. The immunostaining for trefoil factor 3 was registered as negative only if less than 5% of the cells showed a positive staining.

**RESULTS**

Among the 45 subjects with GC, only 21 (12 intestinal and 9 diffuse subtypes by Lauren classification; 19 advanced and 2 early gastric cancer by depth of invasion) of their respective RNAs were qualified for further analyses after capture of malignant cells by LCM. Figure 1 shows a representative example of LCM. A total of 62 arrays were then performed, including 42 arrays for cancer (21 cases with dye-swap duplication) and 20 arrays for non-tumorous cells (10 cases with day-swap duplication) with universal reference. PAM analyses showed differential expression of 504 genes (Tables 1 and 2) could distinguish the cancerous and non-cancerous groups with more than 99% accuracy. Of the 504 genes, trefoil factors 1, 2, and 3 were in the list and their expression patterns (down-regulation of trefoil factors 1 and 2, and up-regulation of trefoil factor 3 in cancer cases) were consistent with
Table 1 Up-regulated genes noted in gastric cancer

| Biosynthesis | Argininosuccinate synthetase [BC009243], hydroxymethylbilane synthase [BC005020], hypothetical protein CL640 [BC008804], methyleneetrahydrofolate dehydrogenase (NADP+ dependent), methylenetetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase [BC001014] |
| Cell adhesion | Collagen, type XVIII, alpha 1 [AF019088], mesothelin [U40434], CD9 antigen (p24) [X60111], collagen, type VI, alpha 3 [J52023], thrombospondin 2 [L12250], immunoglobulin superfamily containing leucine-rich repeat [AB003184] |
| Cell death | Nerve growth factor receptor (TNFR superfamily, member 16) [M1764], phosphophoryn enriched in astrocytes 15 [AF153274], programmed cell death 5 [AF19495] |
| Cell growth and/or maintenance | Cell division cycle 25B [S7878], cyclin D2 [D13639], hippocalin [BC001777], anillin, actin binding protein (scrap homolog, Drosophila) [AF273437], afamin [L23240], chromosome 14 open reading frame 58 [AK000378], Cig and tumor necrosis factor related protein 1 [AF032884], low density lipoprotein receptor-related protein 8, apolipoprotein e receptor [D96407], enhancer of rudimentary homolog (Drosophila) [U68671], tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) [D11139], insulin receptor substrate 2 [AF073310], low density lipoprotein receptor-related protein 8, apolipoprotein e receptor [D50678], thyroid hormone receptor inhibitor 10 [AA000441], collagen, type I, alpha 1 [Z74615], ATPase, Na+ / K+ transporting, beta 3 polypeptide [AF005896], T-LAK-cell originated protein kinase [A072249], platelet-derived growth factor receptor, beta polypeptide [E03278], nuclear protein 1 120 kDa [BC001566], keratin [BC001207], oncostatin M receptor [BC001943], tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) [BC008666], insulin-like growth factor 2 (somatomedin B) [S7035], karyopherin (importin) beta 1 [L39951], nuclear protein 2 120kDa [X55540], thymosin beta-10 [S45405] |
| Cell motility | Hyaluronan-mediated motility receptor (RHAMM) [AF032866], sorcin [M32886], troponin T1, skeletal, slow [BC01063] |
| Cell cycle signaling | Ephrin-A2 [J52969], midkine (neurite growth-promoting factor 2) [X55110], prostat glandulin 12 (prostacyclin) receptor (IP) [D52481] |
| Immune response | Carcinogenicmycin antigen-related cell adhesion molecule 1 (biliary glycoprotein) [X14831], Human 1-8D gene from interferon-inducible gene family [X5735], GTP binding protein overexpressed in skeletal muscle [U1059], indoleamine-pyrrole 2,3 dioxygenase [NM64455], interferon induced transmembrane protein 1 [2-97] [J14641] |
| Macromolecule metabolism | Cathespis L2 [AB001928], PAK2 [AF09213], ribosomal protein L2, large P [L17887], hypothetical protein FLJ22761 [AK026414], KIAA0205 [D96690], protosomase (prosome, macropain) subunit, alpha type 1, [D00779], protein tyrosine phosphatase type IV, member 1 [U48596], peptidylpyrolyl isomerase (cyclophilin)-like 1 [BC003048], ribosomal protein L14 [BC009294], pyruvate kinase, muscle [BC007252], protosomase (prosome, macropain) subunit, alpha type 7, [BC008195], nuclease dehydrogenase 3 (NAD+) alpha [U5678], transglutaminase 4 (prostate) [L3190] |
| Organogenesis | Secreted protein, acidic, cysteine-rich (osteomucin) [M25743], caudal type home box transcription factor 2[C2DX2] [J278434], transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha) [M67671] |
| Regulation of metabolism | Homebox b7 [BC153545], B-cell receptor-associated protein 37 [AF162021], Mediterranean fever [AA03147], homebox b7 [M16937], homebox d4 [XJ7360], TFP641 [AB012122], sir-related polypeptide, 18 kDa [U33636] |
| Response to stress | Complement component 2 [X04481], component complement 3 [K02765], inhibitor, beta A (activin, activin AB alpha polypeptide) [X57579], serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antitrypsin, antiprotease), member 1 [K02212], serine (or cysteine) proteinase inhibitor, clade C (heat shock protein 47), member 1, binding protein 1 [D38174], metiotic recombination (S. cerevisiae) 11 homolog A [AF073362], Rec-Q protein 4 [BC013277] |
| Signal transduction | Secreted frizzled-related protein 4 [AF026692], lyphocyte antigen 6 complex, locus E [RIG-E] [Z61879], dimethylarginine dimethylamionohydrolase 2 [BC001435] |
| Unclassified | Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 [BB86471], apolipoprotein C-I [AV70943], argininosuccinate synthetase [BC030520], ATPase, Ca++ transporting, plasma membrane 4 [AB008533], ATPase, Na+/K+ transporting, beta 3 polypeptide [AU151263], B-cell associated protein [BG771222], BTG family, member 2 [BC07501], cadherin 3, type 1, F-cadherin (placental) [NM001795], calcium modulating ligand [AY474591], carbonyl reductase 3 [AA013066], zinc finger protein 267 [AU12843], transKetolase [BI195990], ribosomal protein L35 [BF310946], ribosomal protein L37 [BG032793], ribosomal protein S16 [BG765030], ribosomal protein S3 [AA59872], S100 calcium-binding protein A [BC003048], nucleolar and coiled-body phosphoprotein [BC006473], platelet-derived growth factor receptor, beta polypeptide [E03278], nuclear protein 1 120 kDa [BC001566], keratin [BC001207], oncostatin M receptor [BC001943], tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) [BC008666], insulin-like growth factor 2 (somatomedin B) [S7035], karyopherin (importin) beta 1 [L39951], nuclear protein 2 120kDa [X55540], thymosin beta-10 [S45405] |

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| [ J. Gersellbank accession number ] | |
Table 2
Down-regulated genes noted in gastric cancer

Biosynthesis
5-aminomimidazole-4-carboxamide ribonucleotide formyltransferase/IPM cyclodohydrolase [D89796], adenosine monophosphate deaminase 1 (isoform M [M60692], histidine decarboxylase [D16583], Human histidine decarboxylase (HDC) [M60645].

Catabolism
Human bilirubin UDP-glucuronosyltransferase isozyme 1 [M57899], Human placental cDNA coding for 5’ nucleotidase (EC 3.1.3.5) [X55740].

Cell adhesion
Collagen, type IV, alpha 6 [D21337], collagen, type XVII, alpha 1 [J76589], tenascin C (hexabrachion) [X78565], dermatopontin [AL049455], Human pancreatitis associated protein [M84337], sialic acid binding Ig-like lectin 11 [AF337818], Macaque brain cDNA clone:Qf1A-14173, full insert sequence [AB062939].

Cell death
Baculoviral IAP repeat-containing 1 [U80017], CD3E antigen, epsilon polypeptide (T13 complex) [X03884], programmed cell death 4 (neoplastic transformation inhibitor) [U96628].

Cell growth and/or maintenance
Core-binding factor, runt domain, alpha subunit 2; translocated to 1; cyclin D-related [S69002], ATPase, Ca++ transporting, ubiquitous [Z68985]. ATP-binding cassette, sub-family C (FTR/MRP), member 5 [AB080659], B-cell CLL/lymphoma 3 [M1732], CDC7 (cell division cycle 7, S. cerevisiae, homolog)-like 1 [AF015592], gatrinome 1 [AB038866], Human chondroderbin mRNA, complete cds [AF371328], CDC28 protein kinase regulatory subunit 2 [X54942], solute carrier family 39 (zinc transporter), member A2 (BF679509), aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase) [AB032150], aldolase C (6-phosphogluconate dehydrogenase) [U82017], cytochrome b oxidase subunit Vic [BC000187], cytochrome P450, family 4, subfamily F, polypeptide 11 [AF23608], cytochrome P450, family 2, subfamily A, polypeptide 1 [AF21346], cytochrome C oxidase subunit Vb [BC007520].

Digestion
Japanese Macaque (Macaca fuscata) mRNA for peptidinogen A-2/3. [X59755], Abelson murine leukemia virus (p210) [M68831], ribonuclease, RNase A family, 4 [BC015520], carbonic anhydrase II [J03037], carbonic anhydrase IX [Z54349], transcription termination factor, mitochondrial [Y09565], hydroxyprostaglandin dehydrogenase 15-NAD [J76465], dehydrogenase/reductase (SDR family) member 9 [Y0107349], ubiquitin carboxy-terminal hydrolase L1 [U96826], ubiquitin carboxy-terminal hydrolase L1 [U96826], ubiquitin carboxy-terminal hydrolase L1 [U96826].

Immune response
Dicyclic glycerol kinase, delta 130 ku [D63479], Fr fragment of IgG, high affinity Ia, receptor for (CD64) [X14356], beta-satellite protein, activating enzyme 2 [AL163285], X-box binding protein 1 [L13850], X-box binding protein 1 [L13850].

Macromolecule metabolism
Sphingomyelin phosphodiesterase, acid-like 3A [Y08136], calpain 9 (ncl-4) [AB083463], carboxypeptidase D2 (pancreatic) [BC070092], 2,4-dienoyl CoA reductase 2, peroxisomal [AI006463], protein kinase (AP-3-dependent, catalytic) inhibitor beta [BAA25513], calpain 13 [AK027176], calpain 9 (ncl-4) [AF022799], fructose-1,6-bisphosphatase 1 [L10320], fructose-1,6-bisphosphatase [Y10812], gastric lipase [X05997], heat shock 10 ku protein [A250915], progastricrin (pepsinogen C) [M25077], protein tyrosine phosphatase, receptor type, N polypeptide 2 [U6702], carboxypeptidase D2 (pancreatic) [U19977], prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) [M59979], tyrosine-protein kinase, Fms-like tyrosine kinase 3, dominant negative [BC009949], tyrosine-protein kinase, Fms-like tyrosine kinase 3, dominant negative [BC009949], tyrosine-protein kinase, Fms-like tyrosine kinase 3, dominant negative [BC009949].

Organogenesis
GATA binding protein 4 [L34357], aldehyde dehydrogenase 1, member A2 [U46689], tropomyosin 4 [BC02827].

Regulation of blood pressure
Chromogranin A (parathyroid secretory protein 1) [BC009384].

Regulation of coagulation
Annamycin A10 [AF238799].

Regulation of metabolism
Estrogen-related receptor gamma [AB020639], zinc finger protein 345 [X78933], Kruppel-like factor 2 (lung) [AF134053], Rel-related orphan receptor C [U16997], Rett syndrome-related protein gene 2 [AL145926].

Response to stress
Checkpoint suppressor 1 [BC007506], CHK1 (checkpoint kinase 1) [M69226], histidine decarboxylase homolog [AF105682], for protein disulfide isomerase-related [D49490], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947], glutathione S-transferase A3 [BC573805], glutathione synthetase [AK000947].

Unclassified
Actin binding LIM protein 1 [NM006719], activated leucocyte cell adhesion molecule [AI050952], adrenocortical beta-2, receptor, surface [NM000204], alcohol dehydrogenase 1C (class I), gamma polypeptide [NM006899], aldehyde dehydrogenase 1 family, member A1 [AI064957], aldehyde dehydrogenase 3 family, member A2 [B407909], aldo-keto reductase family 1, member C1 (diiodohydrogen dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase).
Lauren's classification. There were 3 subgroups of GC (Figure 3) that did not non-cancerous group with these 504 genes showed that status of trefoil factor 3 in GC by immunohistochemical previous reports. In addition, we verified expression [   ]: GeneBank accession number

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DISCUSSION

The completion of human genome sequencing has facilitated high-throughput quantitative analysis of gene expression alterations. Such transcriptional analyses utilizing DNA microarray offer a new avenue to understand the biological diversity of human cells and
Microarray analysis of malignancies from other organ sites has revealed molecular subtypes of tumors that are histologically indistinguishable and clinically informative [26-29]. To elucidate the molecular portrait of GC, we extracted RNA from microdissected normal gastric epithelium and corresponding tumor cells for examining gene expression profiles in the cells as they exist in vivo. Coupling with common “reference” sample as an internal standard, we found this strategy is a rapid and efficient way to identify differentially expressed genes and can readily distinguish GC into 3 molecular subtypes.

Most previous gene expression studies on GCs have used whole tumor tissues [10-21]. The overall information of genetic alterations with this approach was comprehensive but might be hampered by the fact that stromal and inflammatory cells usually intermingled with cancer cells. Therefore, special procedures to isolate cancer cells from heterogeneous tissues are mandatory. To overcome the obstacles of the contamination of cancer cells with wrong cells, we adopted LCM. LCM allows for the rapid, reliable and accurate procurement of cells from the specific microscopic regions of tissue sections under direct visualization. The proportion of contaminated cells with this method is estimated to the less than 0.3% [30]. It thus affords the opportunity to perform molecular genetic analysis of pure populations of malignant cells in their native tissue environment.

However, the RNAs obtained from procured cancer cells were not sufficient to hybridize with cDNA microarray. The recent advances of amplification techniques have provided the key to resolve this problem [31]. In particular, linear RNA amplification could not only provide sufficient RNAs for further analyses but also preserve the overall genetic information.

By the successfully developed techniques of LCM, linear amplification of RNA and cDNA microarray, we found that 504 genes could distinguish cancerous and non-cancerous groups with more than 99% accuracy. Of the genes discovered, some have known association with gastrointestinal cancers. Among them, trefoil factors 1, 2, and 3 were in the list. Furthermore, the expression patterns of these 3 genes were in agreement with previous reports in GCs [32]. These results confirmed our microarray data indirectly. Moreover, we selected trefoil factor 1 for further verification by immunohistochemical staining and the data were also consistent. The remaining novel genes differentially expressed in cancerous and non-cancerous tissues provided a starting point for identification of new biomarkers in GC and are worthy of future in-depth studies to elucidate their roles in GCs.

By comparing expression patterns of the identified 504 genes in individual tissues, we noticed a phylogenetic tree that not only showed clear segregation between normal and cancerous gastric tissues but also assorted GC into 3 subtypes. Although most previous studies suggested classification of GC based on gene expression largely recapitulate that based on histology [33], we did not find such correlation in our system. This discrepancy may be, in part, due to methodological differences and small case numbers in our study. In addition, one intriguing possibility is that tumors sharing similar genes expression profile arise from a common molecular genetic lesion but present with different histological appearances [16,18].

Enrollment of a larger number of tumor samples with well-documented information is under way to further clarify the clinicopathological significance of this molecular classification.

Another unique feature distinct from previous
Microarray studies of GC is that we hybridized each tumor or normal mRNA against a “universal reference” of mRNA. This concept was advocated by investigators from Stanford University, who prepared cDNA for microarray experiments from a pool of mRNA isolated from 11 different cell lines[29]. Although normal epithelium from a spectrum of histological changes from atrophy to metaplasia could be easily microdissected by LCM, the so-called normal epithelium adjacent to tumor may not be similar to epithelium distant from the tumor at the molecular level. Therefore, the definition of control normal tissue will need to be carefully assessed in future global analysis of gene expression.

In conclusion, combined LCM and cDNA microarray is a rapid and efficient way to identify differentially expressed genes and can readily distinguish GCs by their molecular portraits. Further characterization of the genes identified in this study and prospective translational studies to determine their potential clinical value may lead to a deeper insight into the pathogenesis of GCs and facilitate the development of novel biomarkers for diagnostic and therapeutic applications.

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