Expression Profiling and Subtype-Specific Expression of Stomach Cancer

Byungsik Kim, Seunghyun Bang, Seungkoo Lee, Soonok Kim, Yusun Jung, Changhee Lee, Kyungho Choi, Seong-Gene Lee, Kiwhan Lee, Yongsung Lee, Sang-Soo Kim, Yeong-Il Yeom, Yong-Sung Kim, Hyang-Sook Yoo, Kyu-Yong Song, and Inchul Lee

Departments of 1 General Surgery, 2 Biochemistry, 3 Pathology, and 4 Asan Institute for Life Science, University of Ulsan College of Medicine, Seoul; 5 Department of Biochemistry, Hanyang University, Seoul; and 6 Genome Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

ABSTRACT

The expression profiling and molecular grouping of stomach cancers has been a challenging task because of their complexity and variation. We have analyzed gene expression profiles of 22 gastric cancer/normal mucosa couples using 14K cDNA microarray chips designed for gastric cancer analysis. Upon pairwise analysis of the individual couples at the false significance rate 0.91%, 79 and 398 genes were reported to be up-regulated and down-regulated in tumors, respectively. Tumors were clustered into two groups having high and low inflammatory infiltration, respectively. The latter consisted of three subgroups, including diffuse type carcinomas and intestinal types with distinct pathological characteristics of aggressive behavior. When the pooled tumor was hybridized against the pooled normal mucosa samples, more genes were detected to express differentially than those detected by the pairwise analysis at the same threshold level. However, they did not render satisfactory clustering of individual tumors. Our data showed that stomach cancers could be clustered effectively using stomach-specific microarrays and pairwise analysis of tumor/normal mucosa couples. It is suggested that the application of specific goal-oriented experimental design would be advantageous for efficient analysis of expression profiles of such a complex disease as gastric cancer.

INTRODUCTION

Gastric cancers are one of the most frequent fatal malignancies in the world (1) and have a wide pathological and biological variety (2). Helicobacter pylori gastritis has been associated with gastric cancer (3). However, precise molecular pathways of gastric carcinogenesis and clinical progression are yet to be elucidated. To understand the pathways, a comprehensive gene expression profiling of gastric cancers is necessary. It may provide diagnostic molecular markers for gastric cancer as well as clues for developing therapeutic targets on individual basis.

The gene expression profiling of gastric cancer has been a challenging target for DNA microarray analysis (4–6). Gastric cancers usually contain numerous inflammatory and stromal cells, which would interfere the profiling of cancer cells. To eliminate such interference, gastric cancer cell lines have been studied, and certain genes were suggested to be associated with particular behavioral changes (7–10). However, cultured cells may not necessarily reproduce the biological behaviors of tumor cells in the complex microenvironment in vivo.

Gastric cancers have a variety of pathological patterns and biological behaviors. In 1965, Laurén (11) proposed to classify gastric cancers into two groups, i.e., intestinal and diffuse, according to the histopathological pattern of growth. The classification is still valid in predicting the biological behaviors of stomach cancers; diffuse type cancers, which consist of single infiltrating cells, have worse prognosis than intestinal-type cancers of same stage (12). Recently, Hasegawa et al. (13) have reported the expression profiling of intestinal type cancers using microdissection and amplification. They have demonstrated that microdissection and amplification would be an important approach for the analysis of complex tumors in vivo. The expression profiling of diffuse type cancers, however, still poses a considerable technical difficulty because it would not be easy to recognize and microdissect single-infiltrating cells enough to avoid error-prone multistep amplifications. Furthermore, gastric cancers may not always be classified easily based on such a dichotomy; there may be functionally distinct subgroups that would correlate with particular pathological patterns and/or biological behaviors. Molecular markers of those groups would lead to the treatment-oriented classifications and the development of more effective therapeutic agents.

In the expression profiling experiments of complex tumors, several major questions remain to be answered before DNA microarray analysis may be applied to clinical research and/or medical practice. Can the global gene expression profiling detect subgroups of tumors with distinct pathological patterns and/or biological behaviors? What are appropriate experimental designs for efficient clustering and exploring relevant molecular markers? Also, do the experimental designs affect the content of information obtainable using the same samples and microarrays? How much reproducible are the results of expression profiling studies by other experiments using different tissue samples, DNA microarrays, and experimental designs?

The importance of appropriate experimental design of expression profiling has been reviewed recently (14, 15). The pairwise analysis of tumor and normal mucosa couples has been favored to reduce the factors of individual variation and/or pathological changes in the normal mucosa (14). The mucosa of gastrectomy specimen frequently has various pathological changes associated with chronic H. pylori gastritis. The pathological steps of gastric carcinogenesis have not been characterized well thus far (16, 17); mucosal epithelial cells in the chronic gastritis may have considerable changes in their genome or the expression profiles. For instance, the epithelial cells in H. pylori gastritis frequently show malig n cell change, which reflects extensive genomic damage and repair (18).

In an effort to answer aforementioned questions, we have done the expression profiling of 22 gastric cancer/normal mucosa couples by pairwise analysis using 14K cDNA microarrays specifically designed for gastric cancer. Tumors were clustered based on the global expression profiles. For comparison, pooled samples of tumor and normal mucosa were similarly analyzed.

MATERIALS AND METHODS

Patients and Tissue Samples. Twenty-two pairs of gastric cancer and normal mucosa samples were obtained with informed consent from patients who underwent gastrectomy at Asan Medical Center, Seoul, Korea. This study has been approved by the Clinical Research Review Board of Asan Medical Center, Seoul, Korea.

Immediately after the pathological examination, representative tumor samples and adjacent normal mucosa samples were snap-frozen in liquid nitro-
gen. Tumor samples of 1 Cm\(^2\) were taken from the center avoiding necrosis. In case of early gastric cancers, serial sections were taken alternatively for sampling or histopathological examination. Nontumor mucosa samples, 2 \(\times\) 2 Cm, were taken from the same topographical area as the tumors located 3 Cm apart from the margin. Sections were taken for histopathological examination from both tumor and mucosa samples. The tumor samples did not contain normal mucosal tissue, except for occasional entrapped gastric glands. The mucosa samples contained mucosa and a part of adherent submucosa: neither tumor nor dysplasia was included. They showed minimal to mild degree of chronic inflammation and/or atrophy according to the Updated Sydney system (19). Most mucosa samples displayed minimal degree of the malgun cell change, which was characterized by the clear, enlarged nuclei and cyttoplasm (18).

The clinicopathological data of patients are summarized in Table 1. Twenty advanced and 2 early cancers were included. Topographically, 11, 10, and 1 tumors were in the body, antrum, and cardia, respectively. In Korea, most tumors develop in the body and antrum, whereas cardiac tumors are relatively rare. Fifteen patients had regional lymph node metastasis, whereas 7 did not. Tumor-node-metastasis staging was according to the International Union Against Cancer system (20). Tumors were classified according to the Lauren’s classification (11). Some intestinal type tumors were additionally subclassified as papillary or solid type according to the predominant pathological components. The degree of lymphatic permeation, stromal and muscular infiltration, as well as glandular stroma reaction were graded from 0 to 2. The acute and chronic inflammatory infiltration in the tumor was graded from 0 to 3 adopting the Updated Sydney system (19). Some intestinal type tumors were additionally subclassified as papillary or solid type according to the predominant pathological components. The degree of lymphatic permeation, stromal and muscular infiltration, as well as glandular stroma reaction were graded from 0 to 2. The acute and chronic inflammatory infiltration in the tumor was graded from 0 to 3 adopting the Updated Sydney system (19).

cDNA Microarray Analysis. Total RNA was extracted from the frozen samples using Trizol (Invitrogen Co., Carlsbad, CA) according to the manufacturer’s guide. 14K cDNA microarray chips containing 13,376 genes and 704 controls have been fabricated using Korean UniGene Information cDNA clones originated from 14 libraries of gastric cancer cell lines, 1 of gastric carcinoma, and 5 of nontumor mucosa. The libraries were prepared using the oligo-capping and capping methods (21, 22). The information and list of Korean UniGene Information clones may be found at the homepage of 21st Korean UniGene Information clones may be found at the homepage of 21st century Functional Genomics Research Center, Daeduk, Korea. The PCR products were spotted on type-7 glass slides using an Array Spotter Generation II (Amersham Pharmacia, Piscataway, NJ).

For direct labeling of probes with oligodeoxythymidylic acid primer, the protocol of the Brown Lab at Stanford was adopted. For the hybridization, 50 \(\mu\)g of pooled RNA from the tumor and nontumor mucosa was hybridized directly in quadruplication. Hybridized slides were scanned using a GenePix 4000B Scanner (Axon Instruments, Foster City, CA).

Data Analysis. The log ratio values from all hybridizations were merged into a tabular file. For the unsupervised clustering analyses, the pairwise ratio of gene expression was calculated for every tumor/nontumor mucosa couple by subtracting the log ratio value of a nontumor mucosa from that of the matching tumor sample. Array elements on <80% of samples were excluded. Data of remaining 6075 genes were centered by subtracting the median log values. Hierarchical clustering was applied to both genes and samples using web-available software Cluster and TreeView written by Michael B. Eisen. The pairwise log ratios were analyzed using the SAM method, which provides the serial lists of both up-regulated and down-regulated genes at a given false significance rate of choice (23). The pooled tumor/nontumor samples were also analyzed using SAM at the similar threshold level as the pairwise analysis to compare the efficiency of detection of differentially expressed genes. The hierarchical clustering was repeated using the genes selected by the individual pairwise analysis and pooled sample study, respectively.

In Situ Hybridization. The gene expression was verified using in situ hybridization and/or immunohistochemistry. For In situ hybridization, 5-µm thick frozen sections were prepared using the same tissues analyzed for cDNA microarray. Frozen sections were fixed in 4% paraformaldehyde to make appropriate probes. cDNAs were subcloned into pBluescript II SK (Stratagene, La Jolla, CA) vector to make linear DNA. Probes were made using T7 RNA polymerase and \(^35\)S-UTP and purified using Quick Spin column (Roche Molecular Biochemicals, Mannheim, Germany). Using the probes, the sections were hybridized at 58°C for 16 h. After washing, slides were coated with Kodak liquid emulsion NTB2 and exposed in dark for 1–2 weeks. Slides were developed in Kodak Developer D-19 and Fixer. Then, H&E staining was done.

Semiquantitative RT-PCR. Single-stranded cDNA was synthesized with oligodeoxynucleotid acid primer from 3 \(\mu\)g of total RNA using Superscript II reverse transcriptase (Invitrogen Co.). Each single-stranded cDNA was diluted for subsequent PCR amplification by monitoring \(\beta\)-actin as a quantitative control. Each PCR was carried out in a 20-µl volume of 1× PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl\(_2\), 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.2 mM each of the appropriate primers, and 5 U of Taq DNA polymerase. The amplification was performed in a DNA thermal cycler for 30 cycles, each consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min.
for 10 min at 94°C for initial denaturing, followed by 25 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, in the GeneAmp PCR system 9700 (Perkin-Elmer Applied Biosystems, Foster City, CA). The primer sequences are summarized in Table 2.

**RESULTS**

Hierarchical Clustering of Gastric Cancer. Upon two-way hierarchical clustering of prefiltered genes, gastric tumors appeared to be clustered into two groups. It was shown clearly based on hierarchical clustering of prefiltered genes, gastric tumors appeared to be clustered into two groups. It was shown clearly based on 538 differentially expressed genes selected by SAM at the false significance rate 0.09% and \( \delta = 1.614 \) (Fig. 1). The age, differentiation, location, and lymph node metastasis did not correlate with the tumor clustering. The group A consisted of intestinal types, except for a case having mixed intestinal and diffuse type (Table 1). Compared with the other group, they tended to have intense chronic and/or acute inflammatory infiltration (Fig. 2A). Two early gastric cancers and 3 papillary carcinomas belonged to this group.

The group B tumors did not have significant inflammatory infiltration. They consisted of three subgroups with distinct pathological features: B1, intestinal type with predominant lymphatic permeation (Fig. 2B); B2, diffuse type (Fig. 2C); and B3, intestinal type tumors with extensive infiltration (Fig. 2D). In the subgroup B1, >30% of the tumor volume was in the dilated lymphatic space. In the subgroup B3, tumor cell aggregates infiltrated extensively into the lamina propria, muscularis and perineural space without significant stromal desmoplastic reaction. Neither B1 nor B3 pattern was present in group A tumors. In the diffuse type subgroup, B2, three tumors consisted of single-infiltrating cells entirely, whereas two cases had intestinal components as well. There was also a case of mixed type tumor in the group A; it had more inflammatory infiltration than the group B counterparts.

Tumor-Specific Gene Expression. Upon the pairwise analysis of the 22 couples, 477 genes were detected to express differentially using SAM at the false significance rate 0.91% and \( \delta = 1.106 \) (Fig. 3): the up-regulated and down-regulated genes were 79 and 398, respectively (Table 3). Up-regulated genes included mitochondrial isocitrate dehydrogenase 2, IGFBP7, acid cluster protein 33, cathepsin B, midline, tissue inhibitor of metalloproteinase 1, BAP1, NME1, ANXA2, protein phosphatase 4, TGFBI, and other genes and expressed sequence tags.

Down-regulated genes are summarized in Table 4: they included LIPA, LIPF, APOB, lactotransferrin, PGC, SNC73, PSCA, translin-associated factor X, cathepsin E, TFF1, TFF2, mesoderm-specific transcript homologue, SEPP1, KLK4, LTBP4, calpain 2, basic leucine-zipper protein BZAP45, IFN-\( \alpha \)-inducible protein 27, immunoglobulin genes, and other genes and expressed sequence tags.

We then analyzed tumor type-specific expression in the groups A, B, B1, B2, and B3 in comparison with others. For instance, 137 up-regulated and 145 down-regulated genes were detected in subtype B2, diffuse-type cancers, upon SAM analysis at the false significance rate 0.60% and \( \delta = 1.194 \), respectively. The overlapping genes with those of all tumor pairwise analysis are summarized in the right columns of Tables 3 and 4; the numbers represent the serial ranking of differential expression in the given subtypes. More down-regulated genes than up-regulated genes overlapped among various subtypes and all tumor pairwise analysis. For instance, APOB was down-regulated in the group A tumors predominantly. CA11 was down-regulated in the group B tumors, especially in the subtype B2, diffuse-type cancers.

**Pooled Sample Analysis.** To compare the efficiency of selecting differentially expressed genes in tumors, pooled samples of tumors and nontumor mucosa were hybridized directly using four chips. Upon the SAM analysis at the false significance rate 0.93%, which was comparable with that of the pairwise analysis above, 1389 genes were reported to express differentially in comparison with 477 genes detected by the pairwise analysis (Fig. 4). Alternatively, when the thresholds were set to detect comparable number of genes as that of the pairwise analysis, the false significance rate was 0.36%, which was much lower than that of the pairwise analysis (Table 5). Our data suggested that the pooling analysis might be more efficient for the detection of differential expression common to gastric carcinomas than the pairwise analysis.

The concordance rate between the gene populations detected by the pairwise and pooling analyses was calculated at different thresholds (Table 5). Among 477 genes reported by the former, 28.7 and 45.7% were included in the list of the latter at the false significance rate 0.36 and 0.93% \( (\delta = 1.064 \text{ and } 0.750) \), respectively. The overlapping genes are summarized in the right columns of Tables 3 and 4. No clustering was achieved upon the hierarchical clustering of 22 tumors based on...
the genes detected by the pooling analysis (data not shown). The low concordance rate and failure of clustering suggested that the gene populations of the analyses did not necessarily concur.

**Verification of Selected Genes.** The expression of candidate genes was verified by *in situ* hybridization and/or semiquantitative RT-PCR analysis. By *in situ* hybridization, ANXA2 was strongly up-regulated in tumor (Fig. 5A), whereas nontumor mucosa was largely negative (Fig. 5B). In contrast, TFF2 was expressed in normal glands (Fig. 5C), whereas the tumor was negative (Fig. 5D). No convincing expression of PSCA, KLF4, IFNα-inducible protein 27, and SEPP1 was detected in tumors, either. In addition, we have studied the expression of 2 genes, which were detected by the pooling analysis but not by the pairwise analysis; suppressor of Ty (Saccharomyces cerevisiae) 6 homologue and FXYD domain-containing ion transport regulator 3 were also down-regulated in tumors in comparison with nontumor mucosa (data not shown).

The expression of selected genes was also analyzed using semi-quantitative RT-PCR analysis (Fig. 6). Compared with the β-actin control, the expression of ANXA2, IGFBP7, BAP1, and galectin 4 was verified to be up-regulated, whereas LIPF, TFF2, NAD(P)H dehydrogenase, and quinone 1 were down-regulated. We have also studied 2 genes that were detected to be differentially expressed by the pooling analysis but not pairwise analysis; CALU and paraspeckle protein 1 were confirmed to be up-regulated in tumors (Fig. 6). Taking the data of *in situ* hybridization and RT-PCR analysis together, it was concluded that both pairwise and pooling analyses yielded verifiable results.

**DISCUSSION**

Using stomach-specific cDNA microarrays, we could cluster gastric cancers into two main groups and subgroups that correlated with distinct pathological patterns. As far as we are aware of, this is the first study of effective clustering of gastric cancers based on the gene expression profiling. Although more samples should be analyzed for comprehensive clustering of diverse gastric cancers, our data suggested the feasibility of clustering of gastric cancers and the possibility of finding biologically relevant subgroups and molecular markers based on the global gene expression profiling.

Tumors in groups A and B had high and low levels of inflammatory infiltration, respectively, suggesting that the expression of inflammatory cells contributed to the profiling and clustering. However, the clustering of group B as three pathologically distinct subgroups of

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Fig. 2. Pathologic features of tumor subgroups: A, group A, intestinal type carcinoma, moderately differentiated. Dense inflammatory infiltration is a common feature among group A tumors (H&E, ×200); B, subgroup B1, intestinal type, moderately differentiated. Note extensive lymphatic permeation that exceeds 30% of the tumor by volume (H&E, ×200); C, subgroup B2, diffuse type (H&E, ×400); D, subgroup B3, intestinal type, poorly differentiated. Note extensive perineural infiltration. The subgroup B3 is characterized by extensive infiltration without significant stromal reaction (H&E, ×100).

Fig. 3. Pairwise analysis of 22 tumor/nontumor couples. Upon SAM, 477 genes were detected to express differentially at the false significance rate 0.91% and $81.106$. 

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high-grade carcinomas suggested that it was largely based on the expression profiles of tumor cells. The fact that all early and papillary type cancers were clustered in the group A supported the notion. Also, all diffuse type carcinomas, except for one, were clustered in the group B. In our lists of differentially expressed genes did not correlate well with those of previous studies (4–6, 13). Among the up-regulated genes, midkine, SIAH binding protein 1, and NME1 were in the previous studies as well; they have been implicated in the carcinogenesis or metastasis (24–26). Among the down-regulated genes, CA11, TFF1, TFF2, APOB, and PGC were included in the previous studies (13). The down-regulation of CA11 (27) and TFFs 1 and 2 (28, 29) have been reported in gastric cancers. In our data, CA11 was down-regulated predominantly in the group B, particularly the diffuse type tumors, suggesting that the down-regulation of CA11 might be related to high-grade tumors. The down-regulation of APOB, PGC, LIPF, LIPA, and lactotransferrin appeared to reflect the loss of normal mucosal function in cancer: the predominant down-regulation in diffuse type tumors, suggesting that the down-regulation of CA11 might be related to high-grade tumors. The down-regulation of APOB, PGC, LIPF, LIPA, and lactotransferrin appeared to reflect the loss of normal mucosal function in cancer: the predominant down-regulation in diffuse type tumors, suggesting that the down-regulation of CA11 might be related to high-grade tumors.
global gene expression profiling? There is no consensus on how to select differentially expressed genes for further analysis. Technical errors and individual variation can lead to apparently discrepant ranking of differentially expressed genes. Obviously, it would be advantageous to use organ- or disease-specific datasets. However, this may also be related to the discrepancy of results. Regardless of the general quality of microarrays, different coverage of genes would result in apparently discrepant ranking of differentially expressed genes. Obviously, it would be advantageous to use organ- or disease-specific microarrays that would cover most related genes expressed in the tissue and/or disease of interest.

Second, the selected gene lists may vary according to the thresholds applied in the prefiltering and subsequent analysis. Particularly, genes with low expression levels have posed a problem in DNA microarray analysis. They tend to be excluded from the analysis to reduce the unknown effects of individual variation. The considerable influence of experimental false significance rate would permit and to validate the data independently.

Third, the difference of DNA microarrays used in various studies may also be related to the discrepancy of results. Regardless of the general quality of microarrays, different coverage of genes would result in apparently discrepant ranking of differentially expressed genes. Obviously, it would be advantageous to use organ- or disease-specific microarrays that would cover most related genes expressed in the tissue and/or disease of interest.

Fourth, the experimental designs may also affect the data considerably. In our study, we applied pairwise analysis of tumor/nontumor mucosa from the same patients to reduce the unknown effects of individual variation. The considerable influence of experimental de-
signs on the obtainable results was demonstrated by the comparison of pairwise analysis with pooling analysis of same samples. The pooling analysis was more efficient than the pairwise analysis for the detection of genes with differential expression in cancers. Because severe sample variations were to be considered as experimental errors in the statistical analysis, the pooling analysis was expected to yield more sensitive detection of differentially expressed genes than the individual case analysis did. Indeed, the reported genes of the former were more than those of the latter by about three times at the same false significance rate. However, less than half of genes in the selected lists of two experiments coincided, and no satisfactory clustering of tumors was achieved based on genes detected by the pooling analysis. Despite the discrepancy, the expression of genes in both pools was verifiable by in situ hybridization and/or semiquantitative RT-PCR. Taken together, the microarray analysis of tumors may yield variable results and efficiency according to the experimental designs applied. It is suggested that DNA microarray experiments should be designed depending on the purpose of experiment.

In our lists, there were genes implicated in gastric cancer and/or carcinogenesis. Cathepsin B has been reported to correlate with the depth of invasion in early gastric cancers (30). Tissue inhibitor of metalloproteinase 1 has been reported to be particularly up-regulated in schirrhous-type carcinomas (31), and the up-regulation in stomach cancers was reported to correlate with serosal invasion, lymphatic permeation, and poor prognosis (32). ANXA2 is one of a calcium-dependent phospholipid-binding protein family that plays a role in the regulation of cellular growth and in signal transduction pathways (33).

There were genes the down-regulation of which has been implicated in cancer. KLF4 is a zinc finger-containing transcription factor, which arrests the cellular proliferation at the G1-S boundary of the cell cycle (34, 35). Programmed cell death 4 inhibits tumor promoter-induced neoplastic transformation (36). The down-regulation of LTB4 has been implicated in the prostate cancer (37). It is noteworthy that TGF-β1, which has been implicated in carcinogenesis (38), is in our list of up-regulated genes in gastric cancer. The down-regulation of LTB4 along with up-regulation of TGF-β suggests that the TGF-β pathway may be important in the pathobiology of gastric cancer.

### Table 5 Overlapped genes between pairwise and pooling analyses

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<thead>
<tr>
<th>Analysis</th>
<th>Pairwise</th>
<th>Pool Pool</th>
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<tr>
<td>Genes</td>
<td>Significant genes</td>
<td>477</td>
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<td></td>
<td>Overlapped genes</td>
<td>137/477 (28.7%)</td>
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*fs, false significance rate.*

**Fig. 4.** Pooled sample analysis using four DNA chips. Upon SAM at a similar false significance rate as that in Fig. 4 (0.93%, δ 0.75), 1389 genes are reported to express differentially in tumors.

**Fig. 5.** In situ hybridization of selected genes. ANXA2 is positive in tumor (A) but negative in normal mucosa (B), whereas TFF2 is positive in normal glands (C) but negative in tumors (D). (A, B, and D: X200; C, ×300).

**Fig. 6.** Semiquantitative RT-PCR analysis of selected genes. Eight couples of nontumor mucosa and tumor are analyzed using β-actin as a control. Genes in the top bracket are up-regulated, whereas those in the bottom bracket are down-regulated. ANXA2, BAP1, IgfBP7, LIPF, and TFF2 represent the ones detected by both pairwise and pooling analyses, whereas PSP1 (paraspeckle protein 1) and CALU are the ones reported by only the pooling analysis.
SEPP1 has a protective role against peroxynitrite-mediated oxidation and reduces phospholipid hydroperoxide in vitro (39). It has been reported that SEPP1 was down-regulated in prostate cancers and inversely correlated with the progression of prostate cancer (40). Gastric cancers appear to develop in the background of extensive cellular genomic damage (18), which, in turn, develops frequently in the milieu of longstanding oxidative stress by acute foeventis of pylori gastritis (41). The down-regulation of SEPP1 may have gastric epithelial cells to be more vulnerable to the inflammation-related oxidative stress.

Our data suggest that the molecular classification of stomach cancer is feasible despite the considerable complexity and variety. It needs to be confirmed whether the pairwise analysis of tumor/normal tumor couples is indeed advantageous for the clustering. In any case, the fact that different data may be obtained by the experiments using different samples, microarrays, and experimental designs suggest that multiplicated global microarray analyses are required to get a comprehensive global expression profiling and relevant clustering of complex tumors, preferably using standardized protocols that would facilitate the comparison and interpretation of the data among the research groups.

ACKNOWLEDGMENTS

After the submission of this manuscript, S. T. Tay et al. reported that stomach cancers could be subtyped based on the expression profiling (42).

REFERENCES

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