Gene Expression Analysis Identifies Over-expression of *CXCL1*, *SPARC*, *SPP1*, and *SULF1* in Gastric Cancer

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To elucidate gene expression signatures associated with gastric carcinogenesis, we performed a genome-wide expression analysis of 46 Finnish and 20 Japanese gastric tissues. Comparative analysis between Finnish and Japanese datasets identified 58 common genes that were differentially expressed between cancerous and non-neoplastic gastric tissues. Twenty-six of these genes were up-regulated in cancer and 32 down-regulated. Of these genes, 64% were also differentially expressed in another unrelated publicly available dataset. The expression levels of four of the up-regulated genes, *CXCL1*, *SPARC*, *SPP1* and *SULF*, were further analyzed in 82 gastric tissues using quantitative real-time RT-PCR. This analysis validated the results from the microarray analysis as the expression of these four genes was significantly higher in the cancerous tissue compared with the normal tissue (fold change 3.4–8.9). Over-expression of *CXCL1* also positively correlated with improved survival. To conclude, irrespective of the microarray platform or patient population, a common gastric cancer gene expression signature of 58 genes, including *CXCL1*, *SPARC*, *SPP1*, and *SULF*, was identified. These genes represent potential biomarkers for gastric cancer.

INTRODUCTION

Gastric cancer is the fourth most frequent cancer worldwide and the second leading cause of cancer-related death (Parkin et al., 2005). There is great geographical variation in the incidence of gastric cancer, and the high-risk areas include Japan, China, Korea, Eastern Europe, Portugal, and parts of South and Central America (Ahn et al., 1991; Terry et al., 2002; Parkin et al., 2005; Bertuccio et al., 2009). The highest age-standardized incidence is observed in Japan (Parkin et al., 2005), where gastric cancer causes almost one fifth of all cancer related deaths (Tominaga et al., 1992; Parkin et al., 2005; Bertuccio et al., 2009). Because of the high incidence and mortality, mass screening has been practiced in Japan since 1960s, which has led to earlier diagnosis and therefore better prognosis of this cancer (Tsubono and Hisamichi, 2000). The five-year survival rate in Japan is close to 60% whereas the worldwide rate barely reaches 20% (Tsubono and Hisamichi, 2000; Parkin et al., 2005; Roukos and Kappas, 2005). Despite the improvement in the survival rates, gastric cancer mortality in Japan remains among the highest in the world (Parkin et al., 2005). In Finland, the incidence rate of gastric cancer is about one fifteenth of that in Japan (Parkin et al., 2005).

Understanding of the molecular alterations behind the initiation and progression of gastric carcinogenesis is crucial in finding novel therapeutic and clinical targets for gastric cancer. Specific gene expression profiles which correlate with histology (Hippo et al., 2002; Boussioutas et al., 2003; Chen et al., 2003; Kim et al., 2003; Jinawath et al., 2004), invasiveness (Hasegawa et al., 2002; Hippo et al., 2002) and survival (Chen et al., 2003; Tay et al., 2003; Vecchi et al., 2007) have been identified in gastric cancer including a number of genes with a potential role in gastric carcinogenesis. The number of common genes

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reported in these studies is rather low, which is most likely due to the different patient populations, processing of the samples, microarray platforms, and analysis principles. However, the genes identified as differentially expressed in different patient populations and with different microarray platforms could prove to be the most robust and the most promising clinical biomarker candidates for gastric cancer.

Our aim was to identify a common set of differentially expressed genes to discover potential clinical biomarkers for gastric cancer. We carried out a gene expression study of Finnish and Japanese gastric adenocarcinomas and non-neoplastic gastric tissues using gene expression oligoarrays and real-time quantitative RT-PCR. Furthermore, the results from our microarray analyses were compared with a third unrelated dataset (Tsukamoto et al., 2008) to determine whether the same genes would be identified as differentially expressed irrespective of the used microarray format, the laboratory performing the array hybridizations or the origin of the samples. A subset of genes (*CXCL1*, *SPARC*, *SPP1*, and *SULF*) differentially expressed in all three datasets was validated using real-time qRT-PCR and a set of 82 gastric tissues.

MATERIALS AND METHODS

Patients and Samples

Tissue samples were prospectively collected from patients who underwent gastric surgery or gastroscopy at the Helsinki Universal Central Hospital or Sapporo University Hospital between the years 1999 and 2007. This research project has been reviewed and approved by the Ethical Committee of the Department of Medical Genetics and Surgery and authorized by the Clinical Review Board of Helsinki University Central Hospital. The collection of Japanese samples was approved by local authorities at Sapporo Hospital. Informed consent was obtained from each participating patient. Altogether, the Japanese and Finnish sample sets included 59 cancerous and 37 non-malignant fresh frozen primary gastric tissues (Table 1). Of these, 66 samples were analyzed with gene expression microarrays and 82 with real-time RT-PCR assays. Fresh frozen gastric tissue samples were stored at −80°C. Samples were embedded in Tissue-Tek OCT Compound (Sakura Finetek, Torrance, California) and 5-μm frozen ice-sections were prepared and stained using Trypan Blue before histological evaluation by an experienced pathologist. Ice-sections were used to ensure sufficient tumor content of the sample (>60%). Tissue-Tek was removed from the tissues prior to RNA extraction and RNA was extracted from bulk tissue neighboring the 5-μm ice-sections.

RNA Extraction

Total RNA from the Japanese samples was extracted using TRIzol reagent (Invitrogen, Carlsbad, California) followed by purification of the RNA using Qiagen's RNeasy mini kit columns (Valencia, California) according to the manufacturer's instructions. RNA quality was evaluated using Agilent's 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Only RNAs showing distinct 18S/28S ribosomal peaks in the Bioanalyzer analysis and 260/280 ratios above 2.0 were accepted for further analysis. The RNA extraction and microarray hybridizations of the Finnish samples were performed as described previously (Myllykangas et al., 2008).

Gene Expression Microarray Experiments

Twenty Japanese samples including 12 cancerous and 8 non-malignant gastric tissues were analyzed using the Affymetrix HG-U133-Plus 2.0 arrays. Labeling, hybridization and staining was performed according to the manufacturer's protocol using 2 μg of total RNA as starting material. The arrays were scanned with GeneArray Scanner (Affymetrix) and the data were pre-processed using the GeneChip Operating Software (Affymetrix) according to the manufacturer's default parameters. Scaling was performed using all probe sets with a target intensity of 100. All Affymetrix gene expression data are available at www.cangem.org (Scheinin et al., 2007). Labeling and hybridization of the Finnish samples has been described previously (Myllykangas et al., 2008) and all microarray data are available at www.cangem.org. Briefly, 38 cancerous and eight nonmalignant gastric tissues were analyzed using Agilent Whole Human Genome oligoarrays. 20 μg of total RNA was labeled according to the manufacturer's protocol and hybridized onto the arrays. Arrays were scanned using the DNA Microarray Scanner (Agilent Technologies). Two different types of microarrays were used to be able to determine whether the same genes would be recognized as differentially expressed irrespective of the

TABLE 1. Clinical Parameters of the Gastric Tissues Analyzed with Gene Expression Arrays and/or TaqMan Assay

Sample	Origin	Tissue type	Histology	T	N	M	Age	Sex	Expression array	TaqMan
126A	Finnish	Normal	Normal				56	f	no	yes
128A	Finnish	Normal	Normal				58	m	yes	yes
134A	Finnish	Normal	Normal				65	f	yes	yes
142A	Finnish	Normal	Normal				50	m	no	yes
146B	Finnish	Normal	Normal				50	f	no	yes
153A	Finnish	Normal	Normal				75	m	no	yes
160B	Finnish	Normal	Normal				67	m	no	yes
163C	Finnish	Normal	Normal				63	m	no	yes
23B	Finnish	Normal	Normal				67	f	yes	yes
T105A	Finnish	Normal	Normal				65	f	yes	yes
T10B	Finnish	Normal	Normal				83	m	no	yes
T114A	Finnish	Normal	Normal				67	m	no	yes
T125A	Finnish	Normal	Normal				66	m	no	yes
T128A	Finnish	Normal	Normal				72	f	no	yes
T131A	Finnish	Normal	Normal				75	f	no	yes
T140B	Finnish	Normal	Normal				64	m	no	yes
T144A	Finnish	Normal	Normal				83	f	no	yes
T171A	Finnish	Normal	Normal				81	f	yes	no
T172A	Finnish	Normal	Normal				68	m	no	yes
T20A	Finnish	Normal	Normal				58	m	yes	yes
T25A	Finnish	Normal	Normal				70	m	no	yes
T27A	Finnish	Normal	Normal				58	m	no	yes
T40A	Finnish	Normal	Normal				53	m	yes	yes
T44A	Finnish	Normal	Normal				75	m	yes	yes
T54A	Finnish	Normal	Normal				63	f	no	yes
T80A	Finnish	Normal	Normal				33	m	no	yes
T88A	Finnish	Normal	Normal				81	m	no	yes
T92A	Finnish	Normal	Normal				72	m	no	yes
T93B	Finnish	Normal	Normal				77	m	no	yes
125A	Finnish	Cancer	Intestinal	3	1	1	80	m	yes	yes
135A	Finnish	Cancer	Intestinal	2	0	0	65	f	yes	yes
141A	Finnish	Cancer	Intestinal	3	3	0	72	m	no	yes
144B	Finnish	Cancer	Intestinal	n/a	n/a	n/a	79	f	yes	yes
154A	Finnish	Cancer	Intestinal	1	0	0	75	m	yes	yes
162A	Finnish	Cancer	Intestinal	3	0	0	67	m	no	yes
164A	Finnish	Cancer	Intestinal	3	2	0	63	m	yes	yes
200A	Finnish	Cancer	Intestinal	2	1	0	57	f	no	yes
3TC	Finnish	Cancer	Intestinal	2	1	0	57	f	no	yes
4T/N	Finnish	Cancer	Intestinal	2	1	0	72	m	no	yes
54B	Finnish	Cancer	Intestinal	3	2	1	72	f	yes	yes
90A	Finnish	Cancer	Intestinal	3	1	0	76	f	yes	yes
96A	Finnish	Cancer	Intestinal	2	1	0	64	m	yes	yes
T102A	Finnish	Cancer	Intestinal	2	1	0	82	m	no	yes
T119B	Finnish	Cancer	Intestinal	3	0	0	76	m	yes	yes
T121A	Finnish	Cancer	Intestinal	n/a	1	0	71	m	no	yes
T12B	Finnish	Cancer	Intestinal	3	1	0	87	f	yes	yes
T142A	Finnish	Cancer	Intestinal	2	1	0	64	m	yes	yes
T143A	Finnish	Cancer	Intestinal	3	2	1	82	f	yes	no
T148A	Finnish	Cancer	Intestinal	2	1	0	83	f	yes	yes
T161A	Finnish	Cancer	Intestinal	2	1	0	91	f	yes	yes
T177A	Finnish	Cancer	Intestinal	4	2	0	75	m	yes	yes
T181A	Finnish	Cancer	Intestinal	2	2	0	61	m	no	yes
T23A	Finnish	Cancer	Intestinal	3	2	0	70	m	yes	yes
T2A	Finnish	Cancer	Intestinal	4	1	0	61	m	yes	no
T34C	Finnish	Cancer	Intestinal	2	0	0	75	m	yes	yes
T47B	Finnish	Cancer	Intestinal	3	2	1	65	m	yes	no
T51B	Finnish	Cancer	Intestinal	4	1	0	69	m	yes	yes
T5A	Finnish	Cancer	Intestinal	3	1	0	99	m	yes	no
T87A	Finnish	Cancer	Intestinal	3	1	0	81	f	yes	yes
T8A	Finnish	Cancer	Intestinal	3	2	0	60	m	yes	yes
T91B	Finnish	Cancer	Intestinal	3	1	1	72	m	yes	yes

(Continued)

TABLE 1. Clinical Parameters of the Gastric Tissues Analyzed with Gene Expression Arrays and/or TaqMan Assay (Continued)

Sample	Origin	Tissue type	Histology	T	N	M	Age	Sex	Expression array	TaqMan
T94A	Finnish	Cancer	Intestinal	3	2	0	77	m	yes	yes
139A	Finnish	Cancer	Diffuse	3	1	1	74	m	yes	yes
148A	Finnish	Cancer	Diffuse	3	3	1	68	f	yes	no
151B	Finnish	Cancer	Diffuse	n/a	n/a	n/a	50	f	yes	yes
T104A	Finnish	Cancer	Diffuse	3	2	0	62	f	yes	no
T112A	Finnish	Cancer	Diffuse	2	0	0	78	f	yes	no
T139A	Finnish	Cancer	Diffuse	3	1	0	64	m	yes	yes
T14B	Finnish	Cancer	Diffuse	3	2	1	80	f	yes	yes
T156A	Finnish	Cancer	Diffuse	3	2	0	81	f	yes	no
T50A	Finnish	Cancer	Diffuse	3	2	1	42	f	yes	no
T53A	Finnish	Cancer	Diffuse	3	2	0	63	f	yes	yes
T78A	Finnish	Cancer	Diffuse	3	1	0	84	f	yes	no
T84A	Finnish	Cancer	Diffuse	3	1	1	53	f	yes	no
T95A	Finnish	Cancer	Diffuse	3	3	1	38	f	no	yes
T97A	Finnish	Cancer	Diffuse	4	2	1	41	m	yes	yes
G22N	Japanese	Normal	Normal				56	f	yes	yes
G23N	Japanese	Normal	Normal				41	f	yes	yes
G24N	Japanese	Normal	Normal				76	m	yes	yes
G28N	Japanese	Normal	Normal				57	f	yes	yes
G30N	Japanese	Normal	Normal				55	m	yes	yes
G32N	Japanese	Normal	Normal				68	m	yes	yes
G33N	Japanese	Normal	Normal				59	m	yes	yes
G37N	Japanese	Normal	Normal				58	m	yes	yes
G38T	Japanese	Cancer	n/a	3	1	0	64	m	yes	yes
G32T	Japanese	Cancer	Intestinal	3	1	0	68	m	yes	yes
G37T	Japanese	Cancer	Intestinal	3	3	1	58	m	yes	no
G22T	Japanese	Cancer	Diffuse	2	2	0	56	f	yes	yes
G24T	Japanese	Cancer	Diffuse	2	1	0	76	m	yes	yes
G28T	Japanese	Cancer	Diffuse	2	1	0	57	f	yes	yes
G31T	Japanese	Cancer	Diffuse	2	0	0	61	f	yes	yes
G33T	Japanese	Cancer	Diffuse	2	2	0	59	m	yes	yes
G39T	Japanese	Cancer	Diffuse	3	1	1	66	m	yes	no
G51T	Japanese	Cancer	Diffuse	2	0	0	79	f	yes	yes
G54T	Japanese	Cancer	Diffuse	2	2	0	81	m	yes	yes
G55T	Japanese	Cancer	Diffuse	3	2	0	56	f	yes	yes

applied microarray format (Agilent versus Affymetrix).

Statistical Analysis of the Microarray Data

The gene expression changes in the Finnish and Japanese sample sets were analyzed independently using the Chipster software (http://chipster.csc.fi/). Background correction for the Agilent data were performed using the normexp method (Ritchie et al., 2007) with a background offset of 50 and the data were normalized according to the loess method. Control probes were removed from the analysis prior to normalization. Affymetrix data were normalized using the GCRMA method. The probes were reannotated using alternative CDF environments (hs133phsentrezg (hgu133plus2)). During the reannotation process, ambiguous probes that map to more than one position in the genome were discarded from further analysis, and after the reannotation, 17,589 probesets remained for further analysis. Microarray quality was evaluated using the default quality control metrics for Agilent 2-color and Affymetrix arrays included in the Chipster software package. The quality of the arrays was acceptable according to the manufacturers' quality parameters (number of outliers, signal to noise ratio, 3′ to 5′ signal ratios for the house-keeping genes *GAPDH* and *ACTB*). After normalization, genes were filtered according to their standard deviation from the gene mean using 1SD (67% of probes showing the least variation in the whole data set were filtered out). Differential gene expression between the cancerous and non-malignant gastric tissues was determined using the Emperical Bayes as the statistical testing method, Benjamini-Hochberg

for multiple testing correction and *P* value threshold of 0.05 (Smyth, 2004). Gene expression fold changes were calculated by dividing the mean expression of the cancerous samples by the mean expression of the nonmalignant samples. The differentially expressed genes identified with the statistical testing were then subjected to hypergeometric test for gene ontology (GO) to find over-represented GO categories. Finally, the results from the Finnish and Japanese datasets were compared to find common alterations.

To delineate differential gene expression for gastric tumors of different invasive properties (T1-2 versus T3-4 tumors) and different histological background (intestinal versus diffuse), the Finnish gastric cancers ($n = 38$) were also analyzed as a separate data set. Background correction, normalization, removal of control probes, and filtering was performed as described above. Differential gene expression was determined using the Emperical Bayes as the statistical testing method, Benjamini-Hochberg for multiple testing correction and *P* value threshold of 0.05. In the Japanese sample set the number of samples in each subgroup was too low for a similar comparison analysis.

To study whether the same genes would be identified in a completely independent and unrelated dataset, we compared our results with a previously published gene expression data by Tsukamoto et al., (2008). Here, we re-analyzed the Tsukamoto data set (24 gastric cancer and five normal gastric tissue samples analyzed on Agilent 1-color arrays) using the Chipster software. Data were quality controlled using the default quality control metrics for Agilent 1-color arrays included in the Chipster software package and normalized using the quantile normalization. Control probes were removed from further analysis. Differential gene expression between the cancerous and nonmalignant gastric tissues was determined using the Emperical Bayes as the statistical testing method, Benjamini-Hochberg for multiple testing correction and *P* value threshold of 0.05.

Real-Time RT-PCR Analysis

Real-time RT-PCR was performed for four genes *CXCL1* (chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)), *SULF1* (sulfatase 1), *SPP1* (secreted phosphoprotein 1 (osteopontin, bone sialoprotein, early T-lymphocyte activation 1)) and *SPARC* (secreted protein, acidic, cysteine-rich (osteonectin)). The expression levels were measured in 82 gastric tissues including 46 cancerous and 36 nonmalignant tissues (Table 1). 1 μg of total RNA was converted to cDNA using Moloney-murine leukemia virus reverse transcriptase (Promega, Madison, Wisconsin) and random primers (Invitrogen) in a volume of 50 μl for 1 hr at 37°C. The reaction was heat-inactivated (95°C, 3 min) and filled to a final volume of 200 μl with molecular grade water. The transcripts were quantitated using the Assays-on-DemandTM gene expression products (Hs00236937_M1 for *CXCL1*, Hs00234160_M1 for *SPARC*, Hs00960942_M1 for *SPP1*, Hs00290918_M1 for *SULF1*, and 4319413E for S18 rRNA) according to the manufacturer's protocol (Applied Biosystems, Foster City, California). All primers were located on exon-exon boundaries. Briefly, 2 μl of cDNA template was mixed with 1.25 μl of specific primers and probes labeled with FAM-reporter dye. 12.5 μl of TaqMan® Universal PCR Mastermix and RNase-free water were added to a total volume of 25 μl. Human 18S rRNA served as an endogenous control to normalize the expression levels in the subsequent quantitative analysis. The 18S probe was labeled with VIC-reporter dye to allow multiplex PCR with the target genes. The PCR conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Each sample was measured in triplicate and the data were analyzed by the delta-delta method for comparing relative expression results ($2^{-[\Delta Ct\ \text{sample}-\Delta Ct\ \text{control}]}$). Gene expression fold changes were calculated by dividing the mean expression of the cancerous samples by the mean expression of the nonmalignant samples.

Statistical Analysis of the qRT-PCR Data

A nonparametric Mann-Whitney test for two independent samples was applied to determine the statistical significance of differences in the relative mRNA expression levels of *CXCL1*, *SPARC*, *SPP1*, and *SULF1* in nonmalignant and cancerous gastric samples as well as in gastric cancer samples of different histological subtypes or TNM-stages. A *P* value below 0.05 was considered statistically significant (SPSS 17.0). Kaplan Meier method and log rank test were used to calculate the cumulative survival in patients showing ≥5-fold over-expression of a specific gene compared with patients with <5-fold over-

expression. A P value below 0.05 was considered statistically significant (SPSS 17.0).

RESULTS

Gastric Cancer-Related Gene Expression Changes

In the Japanese data set, 149 genes were differentially expressed between the cancerous and normal tissues ($P < 0.05$) (Supporting Information 1). Of these, 77 were over-expressed and 72 under-expressed genes. Among the 10 most highly expressed genes were *THSB2*, *SPP1*, *SFRP2*, *CTHRC1*, *SULF1*, *CXCL1*, *CXCL9*, *NID2*, *COL6A3*, and *THBS4* (fold change (FC) 7.6–19.1), whereas the lowest expression was detected for *ATP4A*, *SST*, *DPCR1*, *AKR1C2*, *VSIG2*, *MUC5AC*, *CAPN9*, *CYP2C18*, *GSTA1*, and *VSIG1* genes (FC 9.9–33.8).

In the Finnish data set, 2,948 genes were differentially expressed between the cancerous and normal tissues ($P < 0.05$) (Supporting Information 2). Of these, 747 were over-expressed and 2201 under-expressed genes. Among the 10 most highly expressed genes were *OLFM4*, *CEACAM6*, *COL1A2*, *TFF3*, *S100A10*, *COLA1*, *RGS1*, *LCN2*, *DMBT1*, and *KCNE3* (FC 4.1–15.4) whereas *LIPF*, *PGA5*, *ATP4A*, *PGC*, *GKN1*, *ATP4B*, *GIF*, *GKN2*, *PSCA*, and *KCNE2* showed the lowest expression (FC 15.1–458.0). Eighty-six genes were expressed differentially in the T1-2 versus T3-4 tumors (Supporting Information 3). Of these, seven genes were over-expressed and 79 under-expressed in the T3-T4 tumors compared to the T1-T2 tumors ($P < 0.05$). There were no statistically significant differences in the gene expression between the two histological subtypes. The large difference between the number of differentially expressed genes in the Finnish and Japanese sample sets is probably partly due to the removal of the probesets that map to more than one location in the Affymetrix data (about two thirds of the probesets) and partly due to the lower number of samples in the Japanese data set.

Common Gene Expression Alterations

Comparative analysis of the Finnish and Japanese samples showed that altogether 58 genes were differentially expressed in cancerous versus nonmalignant gastric tissues in both data sets (Table 2). Twenty-six of these genes were over-expressed in cancer (FC 1.4-19.1) and 22 under-expressed (FC 1.4-184.9). The comparison of our results with the Tsukamoto et al., data set revealed that 37 of the 58 genes (64%) that were differentially expressed in our two datasets were also up- or down-regulated in this third unrelated data set (Table 2). Of these, 37 common genes 15 were over-expressed in all three datasets and 22 genes were under-expressed. We calculated a median gene expression fold change for these 37 genes in the three microarray datasets. The most highly expressed (FC >3.0) genes were *CXCL1* (FC 9.4), *COL1A2* (FC 5.6), *SULF1* (FC 5.5), *COL1A1* (FC 4.8), *SPP1* (FC 4.3), *COL3A1* (FC 4.0), *SPARC* (FC 3.6), and *THBS2* (FC 3.3). Four of these up-regulated genes, including *CXCL1*, *SULF1*, *SPP1*, and *SPARC*, were chosen for real-time RT-PCR analysis to validate the microarray results.

Pathway Analysis

The gene ontology analysis of the differentially expressed genes in each of the two data sets showed that 182 and 73 BP (biological process) GO terms were over-represented ($P < 0.05$) among the differentially expressed genes in Finnish and Japanese sample sets, respectively, whereas the number of over-represented MF (molecular function) terms was 91 and 28 ($P < 0.05$) (Supplement 4). Among the over-represented GO terms for BP with the highest statistical significance ($P < 0.001$) were processes such as angiogenesis, apoptosis, cell adhesion and immune response. Among the over-represented GO terms for MF with the highest statistical significance ($P < 0.001$) were growth factor binding and functions related to extracellular matrix structure. Twenty-one of the over-represented gene ontology terms (9 BP and 12 MF) were shared between the Finnish and Japanese gastric cancers (Table 3).

Expression of *CXCL1*, *SPARC*, *SPP1*, and *SULF1* in Gastric Cancer

The qRT-PCR analysis of *CXCL1*, *SPARC*, *SPP1*, and *SULF1* mRNA expression levels in 82 gastric tissues showed over-expression ($P < 0.001$, fold change 8.6–45.4) of these genes in cancerous gastric tissue compared with the non-malignant gastric tissue (Table 4). In addition, *SPP1* was over-expressed in metastasized tumors ($P < 0.05$, fold change 1.4). Tumor (T) or node (N) staging did not have a statistically significant effect on gene expression of these four genes nor

TABLE 2. 58 Common Differentially Expressed Genes in Finnish and Japanese Primary Gastric Cancers

Symbol	Location	Fold change in Japanese cases	Fold change in Finnish cases	*P*-value in Japanese cases	*P*-value in Finnish cases
ABCA1	9q31.1	3.1	2.2	0.0463	0.0023
ABCC5	3q27	−3.1	−3.1	0.0484	0
AGPAT9	4q21.23	−3.2	−1.6	0.0484	0.008
ATP4A	19q13.1	−33.7	−184.9	0.0425	0
CAPN13	2p22-p21	−4.1	−1.7	0.0229	0.0007
CAPN9	1q42.11-q42.3	−11.1	−1.6	0.0299	0.0053
CDH11	16q22.1	5.8	2.3	0.0164	0.0102
CMTM4	16q22.1	−2.3	−2.0	0.0484	0
COL1A1	17q21.33	4.8	4.9	0.0199	0
COL1A2	7q22.1	6.4	5.6	0.0091	0
COL3A1	2q31	4.6	4.0	0.0164	0.0004
COL4A2	13q34	3.6	2.4	0.0252	0.0001
COL5A1	9q34.2-q34.3	4.3	1.4	0.0217	0.0131
COL5A2	2q14-q32	4.5	2.3	0.0282	0.0012
CTSK	1q21	5.3	1.7	0.0255	0.0233
CXCL1	4q21	9.4	3.2	0.0199	0.0023
CYP2C18	10q24	−10.5	−1.7	0.0199	0.0003
DHCR24	1p33-p31.1	−3.8	−2.0	0.0429	0.0007
DPCR1	6p21.33	−15.3	−3.7	0.0255	0.0032
ELOVL6	4q25	−4.2	−1.8	0.0231	0.0003
EPN3	17q21.33	−3.3	−2.4	0.0229	0
FA2H	16q23	−2.0	−2.0	0.0484	0.0328
FMO5	1q21.1	−3.3	−1.6	0.0425	0.0004
GEM	8q13-q21	7.0	1.8	0.0164	0.005
IFITM1	11p15.5	2.6	2.7	0.0282	0.001
IGFBP7	4q12	3.8	2.4	0.0173	0.0204
IL1R2	2q12-q22	−6.1	−1.5	0.0425	0.0366
KIAA1949	15q24	4.3	1.8	0.046	0.0101
LOC400451	15q26.1	−2.3	−1.9	0.0425	0.0002
MAL	2cen-q13	−7.4	−3.8	0.046	0
MUC5AC	11p15.5	−11.2	−3.4	0.0255	0.0045
MXRA5	Xp22.33	2.7	2.0	0.0249	0.01
MYOC	1q23-q24	−2.6	−1.8	0.0463	0.0071
NEDD4L	18q21	−2.7	−2.3	0.0396	0
OLFML2B	1q23.3	4.9	1.6	0.0376	0.0003
PDGFRB	5q31-q32	2.8	2.9	0.0232	0.0001
PDIA2	16p13.3	−7.4	−2.1	0.0252	0
PKIB	6q22.31	−2.8	−1.9	0.0461	0.0004
PLXDC1	Xp22.33	2.6	2.0	0.0282	0.0114
PMEPA1	20q13.31-q13.33	3.1	2.3	0.0385	0.016
RAB31	18p11.3	3.0	2.2	0.0484	0.001
RASEF	9q21.32	−5.2	−3.7	0.0425	0
RASSF6	4q13.3	−5.1	−1.4	0.0199	0.0141
SERINC2	1p35.1	−2.5	−1.4	0.0484	0.0192
SFRP4	7p14.1	5.9	4.0	0.0444	0.0032
SLC44A2	19p13.1	−2.4	−1.4	0.0219	0.0362
SLC7A8	14q11.2	−2.6	−2.5	0.0219	0
SPARC	5q31.3-q32	4.1	3.6	0.0196	0.0002
SPP1	4q21-q25	14.2	2.5	0.0232	0.0243
SST	3q28	−15.6	−1.9	0.0458	0.0115
SULF1	8q13.2-q13.3	9.6	3.9	0.0229	0.0001
SYTL2	11q14	−2.7	−1.9	0.0484	0.0221
THBS2	6q27	19.1	1.8	0.0091	0.0276
THY1	11q22.3-q23	5.9	2.0	0.0226	0.0003
TMEM171	5q13.2	−3.3	−1.7	0.0476	0.0001
VSIG1	Xq22.3	−9.9	−2.9	0.0229	0
VSIG2	11q24	−12.3	−2.8	0.0173	0
XK	Xp21.1	−5.3	−1.6	0.0252	0.0268

The 37 differentially expressed genes in all three microarray datasets are underlined. Fold change defines gene expression fold change between the cancerous and nonmalignant samples. (−) under-expression, (+) over-expression.

TABLE 3. Over-represented Biological Process (BP) and Molecular Function (MF) Gene Ontology (GO) Terms in Finnish and Japanese Gastric Cancers

GO term	GO Description	*P*-value in Japanese cases	*P*-value in Finnish cases	No. of genes in Japanese cases	No. of genes in Finnish cases
GO:0008154 (BP)	Actin polymerization and/or depolymerization	0.012	0.017	3	15
GO:0022610 (BP)	Biological adhesion	0.000	0.009	19	129
GO:0007155 (BP)	Cell adhesion	0.000	0.009	11	124
GO:0008283 (BP)	Cell proliferation	0.037	0.027	3	28
GO:0007586 (BP)	Digestion	0.040	0.000	3	28
GO:0030198 (BP)	Extracellular matrix organization and biogenesis	0.007	0.047	3	12
GO:0002376 (BP)	Immune system process	0.031	0.000	12	172
GO:0006817 (BP)	Phosphate transport	0.000	0.029	11	21
GO:0051338 (BP)	Regulation of transferase activity	0.035	0.018	5	44
GO:0003823 (MF)	Antigen binding	0.005	0.031	3	11
GO:0004197 (MF)	Cysteine-type endopeptidase activity	0.003	0.004	4	20
GO:0008234 (MF)	Cysteine-type peptidase activity	0.027	0.034	4	30
GO:0004175 (MF)	Endopeptidase activity	0.036	0.000	7	95
GO:0005201 (MF)	Extracellular matrix structural constituent	0.000	0.000	10	30
GO:0005539 (MF)	Glycosaminoglycan binding	0.000	0.016	7	24
GO:0019838 (MF)	Growth factor binding	0.000	0.036	5	17
GO:0008201 (MF)	Heparin binding	0.000	0.002	7	22
GO:0005506 (MF)	Iron ion binding	0.022	0.031	6	51
GO:0016491 (MF)	Oxidoreductase activity	0.032	0.001	11	135
GO:0001871 (MF)	Pattern binding	0.000	0.028	3	24
GO:0030247 (MF)	Polysaccharide binding	0.000	0.013	7	25

TABLE 4. Statistical Significance of *CXCL1*, *SPARC*, *SPP1*, and *SULF1* Expression in Gastric Cancer Tissues

Gene	Cancer vs. normal	M1 vs. M0	T3-4 vs. T1-2	N1-3 vs. N0	Intestinal vs. Diffuse
SPARC	$P < 0.001$ (8.6-fold over-expression)	$P = 0.835$	$P = 0.261$	$P = 0.553$	$P = 0.258$
SULF1	$P < 0.001$ (11.6-fold over-expression)	$P = 0.282$	$P = 0.751$	$P = 0.344$	$P = 0.336$
SPP1	$P < 0.001$ (45.4-fold over-expression)	$P < 0.05$ (1.4-fold over-expression)	$P = 0.156$	$P = 0.327$	$P = 0.485$
CXCL1	$P < 0.001$ (12.3-fold over-expression)	$P = 0.153$	$P = 0.340$	$P = 0.229$	$P = 0.500$

P-values from the nonparametric Mann-Whitney test are shown for each comparison.

did the histology of the tumor tissue (intestinal versus diffuse). The survival rates were better for patients showing ≥5-fold over-expression of *CXCL1* ($P < 0.05$) (Fig. 1). No association between the over-expression (≥5-fold versus <5-fold) of the other genes and cumulative survival was found (data not shown).

DISCUSSION

The gene expression microarray analysis of Finnish and Japanese gastric cancers revealed gastric cancer specific gene expression signatures that clearly discriminated gastric cancers from normal tissues. The differentially expressed genes identified in our study showed a good correlation with a previously published gene expression microarray data set (Tsukamoto et al., 2008) since the majority (64%, $n = 37$) of the genes identified in our study was also differentially expressed in this unrelated data set. These 37 commonly up- and down-regulated genes include 17 genes previously reported as differentially expressed in gastric cancer compared to normal gastric tissues (*CAPN9*, *COL1A1*, *COL1A2*, *COL3A1*, *CTSK*, *IFITM1*, *IGFBP7*, *MAL*,

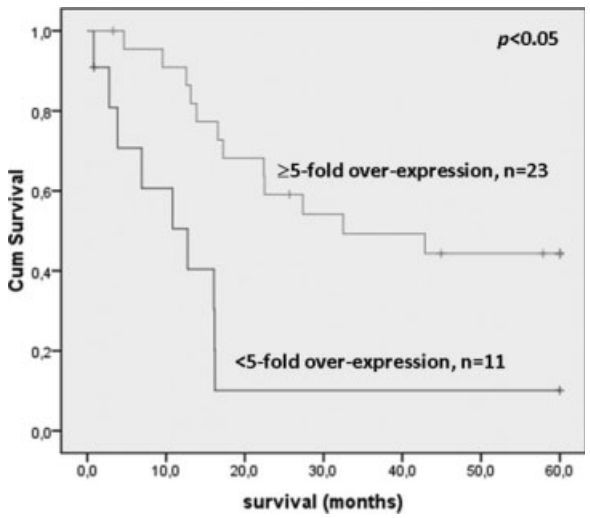

Figure 1. Cumulative 5-year survival of gastric cancer patients according to the *CXCL1* gene expression.

MUC5AC, *PDGFRB*, *RAB31*, *SLC7A8*, *SPARC*, *SPP1*, *SST*, *SULF1*, and *THBS2*) (Boussioutas et al., 2003; Kim et al., 2003; Tay et al., 2003; Jinawath et al., 2004; Krueger et al., 2005; Yang et al., 2005; Higashiyama et al., 2007; Yang et al., 2007b; Chen et al., 2009). Moreover, 20 novel gastric cancer-related genes were identified (Table 2).

The previous gene expression microarray studies of gastric cancer have reported several genes that show deregulated gene expression in gastric cancer (Hasegawa et al., 2002; Hippo et al., 2002; Boussioutas et al., 2003; Chen et al., 2003; Tay et al., 2003; Jinawath et al., 2004; Kim et al., 2007; Vecchi et al., 2007; Yang et al., 2007b; Myllykangas et al., 2008; Takeno et al., 2008; Tsukamoto et al., 2008). Our study correlated well with these previous studies as 17 of the 37 genes (46%) identified in our study were deregulated also in at least one of the previous studies. However, none of the previous studies has combined data from different gene expression microarray formats or datasets, whereas in our study, patients from two different populations (Finland and Japan) and data from three different microarray formats (Agilent 1- and 2-color arrays and Affymetrix arrays) were combined to identify common gastric cancer gene expression signatures.

The gene ontology analysis showed that the differentially expressed genes in our two datasets represent many biological processes and molecular functions relevant to carcinogenesis. Comparison of the enriched GO terms in Finnish and Japanese data sets showed 21 common GO terms that represented processes central to carcinogenesis such as cell adhesion, cell proliferation, extracellular matrix organization, and growth factor binding.

The real-time qRT-PCR analysis confirmed the results from the gene expression microarray analysis as all of the four validated genes showed a clear over-expression of these genes in gastric cancer tissues. As expected, the over-expression of these genes was higher according to the qRT-PCR analysis (fold change 8.6–45.4) compared to the microarray analysis (fold change 2.5–14.2). The highest over-expression was detected for *SPP1* (average fold change 45.4), whereas the over-expression of the other three genes (*CXCL1*, *SPARC*, and *SULF1*) was approximately 10-fold compared with the normal tissues.

SPP1 (also known as osteopontin) and *SPARC* (also known as osteonectin) both belong to the group of bone matrix-associated factors (Denhardt et al., 1993; Lane et al., 1994). They are expressed primarily during tissue reneval and tissue remodeling (Bornstein et al., 1995) and are able to bind type I collagens such as COL1A1 and COL1A2 (Termine et al., 1981; Chen et al., 1992). COL1A1 and COL1A2 were also among the most highly over-expressed genes identified in this study and thus the interactions between these four genes might be important for the interaction between tumor cells and the surrounding tissue matrix.

SPP1 is involved in stress response, cell adhesion, inflammation, wound healing, chemotaxis, prevention of apoptosis and immune response (El-Tanani, 2008; Johnston et al., 2008; Wang and Denhardt, 2008). In addition to the over-expression in gastric cancer samples in our study, *SPP1* expression was also higher in the metastasized tumors compared to the nonmetastasized tumors. This is in line with the previous reports of *SPP1*'s role as a promoter of angiogenesis and metastasis in gastric cancer (Higashiyama et al., 2007; Tang et al., 2007; Tang et al., 2008). Furthermore, the over-expression of *SPP1* in gastric cancer has been reported in previous studies (Higashiyama et al., 2007; Tang et al., 2008). High *SPP1* expression has also been associated with lung, prostate, breast, pancreatic and ovarian cancers (Kim et al., 2002; Carlinfante et al., 2003; Donati et al., 2005; Hu et al., 2005; Kolb et al., 2005).

SPARC interacts with a number of ECM components such as thrombospondin I and

vitronectin and thereby influences ECM synthesis and tissue repair (Bradshaw and Sage, 2001). SPARC may have a role in the communication of cancer cells with their surrounding stroma which enables their proliferation and invasion beyond the original growth site. SPARC is known to bind to growth factors such as PDGF and VEGF and control also the activity of other growth factors such as bFGF and TGF-β (Bradshaw and Sage, 2001). *SPARC* over-expression in gastric cancers has previously been reported by Wang et al. (2004) who detected a 4.27-fold over-expression of this gene in tumor tissue compared to non-malignant tissue. The expression of *SPARC* was higher in T1 tumors compared with the T2-4 tumors, and over-expression of *SPARC* was associated with lymph node metastasis, lymph node invasion and perineural invasion. In our data, *SPARC* showed an 8.6-fold over-expression in tumor tissue compared to the non-malignant tissue, but the over-expression did not correlate with T, N, or M staging. *SPARC* has also been reported to be over-expressed in breast cancers (Watkins et al., 2005) as well as gliomas and melanomas (Ikuta et al., 2005; Shi et al., 2007; Smit et al., 2007), which indicates its role as an oncogene. However, in other types of tumors *SPARC* seems to function as a tumor suppressor, since hypermethylation and loss of SPARC gene expression has been detected in lung cancers, ovarian, and colorectal cancers. Increased *SPARC* expression is associated with a better prognosis of these tumors (Suzuki et al., 2005; Yang et al., 2007a; Tai and Tang, 2008; Socha et al., 2009).

CXCL1 is a chemokine that has roles in the development, homeostasis, and the immune system. It also contributes to many protumorigenic processes such as inflammation and angiogenesis (Eck et al., 2003; Acosta et al., 2009). In gliomas, the up-regulation of *CXCL1* has been shown to increase tumorigenicity through its ability to increase the *SPARC* expression, although the exact mechanism for this interaction is not known (Zhou et al., 2005). This gene has also been reported to be over-expressed in bladder and ovarian carcinomas (Kawanishi et al., 2008; Yang et al., 2006) but over-expression of *CXCL1* in gastric cancers compared with the nonmalignant tissue has not been previously reported. However, Eck et al., (2003) reported that the protein level expression of this gene was significantly higher in diffuse subtype compared to the intestinal subtype of gastric cancer, but no normal samples were included in their study. Interestingly, the survival analysis showed that survival was better if patients were showing at least a 5-fold over-expression of *CXCL1* compared with patients with less than 5-fold over-expression. Thus, even although gastric cancers in general show a 12.3-fold over-expression of this gene, it seems that among the gastric cancer patients better survival is associated with patients showing the higher (>5-fold) over-expression. Acosta et al. (2008) reported that signaling by *CXCL1* (also known as GROα) reinforces senescence early in tumorigenesis and might thereby inhibit tumor growth. This could explain the dual role of *CXCL1* in carcinogenesis.

SULF1 is a heparin sulfate 6-0 endosulphatase that removes 6-O-sulfate groups from heparin sulfate. Heparan sulfate proteoglycans (HSPGs) on the other hand, are central to the cell signaling in the extracellular matrix. Hence, since SULF1 modifies HSPG, and this leads to changes in HSPG-related signal transductions, deregulation of SULF1 may have significant impact on cell growth and carcinogenesis (Dai et al., 2005; Chen et al., 2009). Hypermethylation and consequential down-regulation of *SULF1* in gastric and breast cancers has been reported (Narita et al., 2007; Chen et al., 2009). However, in pancreatic cancers (Li et al., 2005) this gene was shown to be significantly over-expressed (22.5-fold) in cancerous tissue compared with normal controls. In our study, *SULF1* expression was 11.6-fold higher in gastric cancer tissue compared with the nonmalignant gastric tissue. This is the first study to report gastric cancer-associated over-expression of this gene. It has been suggested that the up-regulated *SULF1* expression is related to *MYC* oncogene amplification in hepatocellular cancers (Lai et al., 2004). These two genes are both located at the 8q chromosomal region which is frequently amplified in gastric cancers (Weiss et al., 2004; Myllykangas et al., 2008; Tsukamoto et al., 2008.) *MYC* has been reported to be amplified in 26–30% of gastric tumors (Koo et al., 2000; Kozma et al., 2001) and the coamplification of *MYC* and *SULF1* might therefore explain the over-expression of *SULF1* also in gastric cancers.

In conclusion, the integrated gene expression microarray analysis of the three independent datasets pointed out several interesting genes that show deregulated gene expression in cancerous gastric tissue. Real-time qRT-PCR validated the results obtained from the microarray analysis well as all of the tested genes showed significant over-expression in gastric cancers. These and

other genes identified as up- and down-regulated in gastric cancer may prove to be potential biomarkers for gastric cancer although further studies of these genes needs to be performed.

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REFERENCES

Acosta JC, O'Loghlen A, Banito A, Guijarro MV, Augert A, Raguz S, Fumagalli M, Da Costa M, Brown C, Popov N, Takatsu Y, Melamed J, d'Adda di Fagagna F, Bernard D, Hernando E, Gil J. 2008. Chemokine signaling via the CXCR2 receptor reinforces senescence. Cell 133:1006–1018.

Acosta JC, Gil J. 2009. A role for CXCR2 in senescence, but what about in cancer? Cancer Res 69:2167–2170.

Ahn YO, Park BJ, Yoo KY, Kim NK, Heo DS, Lee JK, Ahn HS, Kang DH, Kim H, Lee MS, Park TS. 1991. Incidence estimation of stomach cancer among Koreans. J Korean Med Sci 6:7–14.

Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J Jr., Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM. 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503–511.

Bertuccio P, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, Malvezzi M, La Vecchia C. 2009. Recent patterns in gastric cancer: A global overview. Int J Cancer 125:666–673.

Boussioutas A, Li H, Liu J, Waring P, Lade S, Holloway AJ, Taupin D, Gorringe K, Haviv I, Desmond PV, Bowtell DD. 2003. Distinctive patterns of gene expression in premalignant gastric mucosa and gastric cancer. Cancer Res 63:2569–2577.

Bradshaw AD, Sage EH. 2001. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J Clin Invest 107:1049–1054.

Carlinfante G, Vassiliou D, Svensson O, Wendel M, Heinegård D, Andersson G. 2003. Differential expression of osteopontin and bone sialoprotein in bone metastasis of breast and prostate carcinoma. Clin Exp Metastasis 20:437–444.

Chen X, Leung SY, Yuen ST, Chu KM, Ji J, Li R, Chan AS, Law S, Troyanskaya OG, Wong J, So S, Botstein D, Brown PO. 2003. Variation in gene expression patterns in human gastric cancers. Mol Biol Cell 14:3208–3215.

Chen Z, Fan JQ, Li J, Li QS, Yan Z, Jia XK, Liu WD, Wei LJ, Zhang FZ, Gao H, Xu JP, Dong XM, Dai J, Zhou HM. 2009. Promoter hypermethylation correlates with the Hsulf-1 silencing in human breast and gastric cancer. Int J Cancer 124:739–744.

Dai Y, Yang Y, MacLeod V, Yue X, Rapraeger AC, Shriver Z, Venkataraman G, Sasisekharan R, Sanderson RD. 2005. HSulf-1 and HSulf-2 are potent inhibitors of myeloma tumor growth in vivo. J Biol Chem 280:40066–40073.

Eck M, Schmausser B, Scheller K, Brändlein S, Müller-Hermelink HK. 2003. Pleiotropic effects of CXC chemokines in gastric carcinoma: Differences in CXCL8 and CXCL1 expression between diffuse and intestinal types of gastric carcinoma. Clin Exp Immunol 134:508–515.

El-Tanani MK. 2008. Role of osteopontin in cellular signaling and metastatic phenotype. Front Biosci 13:4276–4284.

Hasegawa S, Furukawa Y, Li M, Satoh S, Kato T, Watanabe T, Katagiri T, Tsunoda T, Yamaoka Y, Nakamura Y. 2002. Genome-wide analysis of gene expression in intestinal-type gastric cancers using a complementary DNA microarray representing 23,040 genes. Cancer Res 62:7012–7017.

Higashiyama M, Ito T, Tanaka E, Shimada Y. 2007. Prognostic significance of osteopontin expression in human gastric carcinoma. Ann Surg Oncol 14:3419–3427.

Hippo Y, Taniguchi H, Tsutsumi S, Machida N, Chong JM, Fukayama M, Kodama T, Aburatani H. 2002. Global gene expression analysis of gastric cancer by oligonucleotide microarrays. Cancer Res 62:233–240.

Hu Z, Lin D, Yuan J, Xiao T, Zhang H, Sun W, Han N, Ma Y, Di X, Gao M, Ma J, Zhang J, Cheng S, Gao Y. 2005. Overexpression of osteopontin is associated with more aggressive phenotypes in human non-small cell lung cancer. Clin Cancer Res 11:4646–4652.

Ikuta Y, Nakatsura T, Kageshita T, Fukushima S, Ito S, Wakamatsu K, Baba H, Nishimura Y. 2005. Highly sensitive detection of melanoma at an early stage based on the increased serum secreted protein acidic and rich in cysteine and glypican-3 levels. Clin Cancer Res 11:8079–8088.

Jinawath N, Furukawa Y, Hasegawa S, Li M, Tsunoda T, Satoh S, Yamaguchi T, Imamura H, Inoue M, Shiozaki H, Nakamura Y. 2004. Comparison of gene-expression profiles between diffuse- and intestinal-type gastric cancers using a genome-wide cDNA microarray. Oncogene 23:6830–6844.

Johnston NI, Gunasekharan VK, Ravindranath A, O'Connell C, Johnston PG, El-Tanani MK. 2008. Osteopontin as a target for cancer therapy. Front Biosci 13:4361–4372.

Kawanishi H, Matsui Y, Ito M, Watanabe J, Takahashi T, Nishizawa K, Nishiyama H, Kamoto T, Mikami Y, Tanaka Y, Jung G, Akiyama H, Nobumasa H, Guilford P, Reeve A, Okuno Y, Tsujimoto G, Nakamura E, Ogawa O. 2008. Secreted CXCL1 is a potential mediator and marker of the tumor invasion of bladder cancer. Clin Cancer Res 14:2579–2587.

Kim JH, Skates SJ, Uede T, Wong KK, Schorge JO, Feltmate CM, Berkowitz RS, Cramer DW, Mok SC. 2002. Osteopontin as a potential diagnostic biomarker for ovarian cancer. JAMA 287:1671–1679.

Kim B, Bang S, Lee S, Kim S, Jung Y, Lee C, Choi K, Lee SG, Lee K, Lee Y, Kim SS, Yeom YI, Kim YS, Yoo HS, Song K, Lee I. 2007. Expression profiling and subtype-specific expression of stomach cancer. Cancer Res 63:8248–8255.

Kolb A, Kleeff J, Guweidhi A, Esposito I, Giese NA, Adwan H, Giese T, Büchler MW, Berger MR, Friess H. 2005. Osteopontin influences the invasiveness of pancreatic cancer cells and is increased in neoplastic and inflammatory conditions. Cancer Biol Ther 4:740–746.

Koo SH, Kwon KC, Shin SY, Jeon YM, Park JW, Kim SH, Noh SM. 2000. Genetic alterations of gastric cancer: Comparative genomic hybridization and fluorescence In situ hybridization studies. Cancer Genet Cytogenet 117:97–103.

Kozma L, Kiss I, Hajdú J, Szentkereszty Z, Szakáll S, Ember I. 2001. C-myc amplification and cluster analysis in human gastric carcinoma. Anticancer Res 21:707–710.

Krueger S, Kalinski T, Hundertmark T, Wex T, Küster D, Peitz U, Ebert M, Nägler DK, Kellner U, Malfertheiner P, Naumann M, Röcken C, Roessner A. 2005. Up-regulation of cathepsin X in Helicobacter pylori gastritis and gastric cancer. J Pathol 207:32–42.

Lai JP, Chien JR, Moser DR, Staub JK, Aderca I, Montoya DP, Matthews TA, Nagorney DM, Cunningham JM, Smith DI, Greene EL, Shridhar V, Roberts LR. 2004. hSulf1 Sulfatase promotes apoptosis of hepatocellular cancer cells by decreasing heparin-binding growth factor signaling. Gastroenterology 126:231–248.

Lane TF, Sage EH. 1994. The biology of SPARC, a protein that modulates cell-matrix interactions. FASEB J. 8:163–173.

Li J, Kleeff J, Abiatari I, Kayed H, Giese NA, Felix K, Giese T, Büchler MW, Friess H. 2005. Enhanced levels of Hsulf-1 interfere with heparin-binding growth factor signaling in pancreatic cancer. Mol Cancer 4:14.

Myllykangas S, Junnila S, Kokkola A, Autio R, Scheinin I, Kiviluoto T, Karjalainen-Lindsberg ML, Hollmén J, Knuutila S, Puolakkainen P, Monni O. 2008. Integrated gene copy number and expression microarray analysis of gastric cancer highlights potential target genes. Int J Cancer 123:817–825.

Narita K, Chien J, Mullany SA, Staub J, Qian X, Lingle WL, Shridhar V. Loss of HSulf-1 expression enhances autocrine signaling mediated by amphiregulin in breast cancer. J Biol Chem 282:14413–14420.

Parkin DM, Bray F, Ferlay J, Pisani P. 2005. Global cancer statistics, 2002. CA Canc J Clin 55:74–108.

Peddanna N, Holt S, Verma RS. 1995. Genetics of gastric cancer. Anticancer Res 15:2055–2064.

Ritchie ME, Silver J, Oshlack A, Silver J, Holmes M, Diyagama D, Holloway A, Smyth GK. 2007. A comparison of background correction methods for two-colour microarrays. Bioinformatics 23:2700–2707.

Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, López-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T, Staudt LM; Lymphoma/Leukemia Molecular Profiling Project. 2002. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346:1937–1947.

Roukos DH, Kappas AM. 2005. Perspectives in the treatment of gastric cancer. Nat Clin Pract Oncol 2:98–107.

Socha MJ, Said N, Dai Y, Kwong J, Ramalingam P, Trieu V, Desai N, Mok SC, Motamed K. 2009. Aberrant promoter methylation of SPARC in ovarian cancer. Neoplasia 11:126–135.

Shi Q, Bao S, Song L, Wu Q, Bigner DD, Hjelmeland AB, Rich JN. 2007. Targeting SPARC expression decreases glioma cellular survival and invasion associated with reduced activities of FAK and ILK kinases. Oncogene 26:4084–4094.

Smit DJ, Gardiner BB, Sturm RA. 2007. Osteonectin downregulates E-cadherin, induces osteopontin and focal adhesion kinase activity stimulating an invasive melanoma phenotype. Int J Cancer 121:2653–2660.

Smyth GK. 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genetics Mol Biol 3:1–25.

Suzuki M, Hao C, Takahashi T, Shigematsu H, Shivapurkar N, Sathyanarayana UG, Iizasa T, Fujisawa T, Hiroshima K, Gazdar AF. 2005. Aberrant methylation of SPARC in human lung cancers. Br J Cancer 92:942–948.

Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lonning P, Borresen-Dale AL. 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98:10869–10874.

Takeno A, Takemasa I, Doki Y, Yamasaki M, Miyata H, Takiguchi S, Fujiwara Y, Matsubara K, Monden M. Integrative approach for differentially overexpressed genes in gastric cancer by combining large-scale gene expression profiling and network analysis. Br J Cancer 99:1307–1315.

Tang H, Wang J, Bai F, Hong L, Liang J, Gao J, Zhai H, Lan M, Zhang F, Wu K, Fan D. 2007. Inhibition of osteopontin would suppress angiogenesis in gastric cancer. Biochem Cell Biol 85:103–110.

Tang H, Wang J, Bai F, Zhai H, Gao J, Hong L, Xie H, Zhang F, Lan M, Yao W, Liu J, Wu K, Fan D. 2008. Positive correlation of osteopontin, cyclooxygenase-2 and vascular endothelial growth factor in gastric cancer. Cancer Invest 26:60–67.

Tay ST, Leong SH, Yu K, Aggarwal A, Tan SY, Lee CH, Wong K, Visvanathan J, Lim D, Wong WK, Soo KC, Kon OL, Tan P. 2003. A combined comparative genomic hybridization and expression microarray analysis of gastric cancer reveals novel molecular subtypes. Cancer Res 63:3309–3316.

Terry MB, Gaudet MM, Gammon MD. 2002. The epidemiology of gastric cancer. Semin Radiat Oncol 12:111–127.

Tsubono Y, Hisamichi S. 2000. Screening for gastric cancer in Japan. Gastric Cancer 3:9–18.

Tsukamoto Y, Uchida T, Karnan S, Noguchi T, Nguyen LT, Tanigawa M, Takeuchi I, Matsuura K, Hijiya N, Nakada C, Kishida T, Kawahara K, Ito H, Murakami K, Fujioka T, Seto M, Moriyama M. 2008. Genome-wide analysis of DNA copy number alterations and gene expression in gastric cancer. J Pathol 216:471–482.

Vecchi M, Nuciforo P, Romagnoli S, Confalonieri S, Pellegrini C, Serio G, Quarto M, Capra M, Roviaro GC, Contessini Avesani E, Corsi C, Coggi G, Di Fiore PP, Bosari S. 2007. Gene expression analysis of early and advanced gastric cancers. Oncogene 26:4284–4294.

Wang KX, Denhardt DT. 2008. Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev 19:333–345.

Wang CS, Lin KH, Chen SL, Chan YF, Hsueh S. 2004. Overexpression of SPARC gene in human gastric carcinoma and its clinic-pathologic significance. Br J Cancer 91:1924–1930.

Watkins G, Douglas-Jones A, Bryce R, Mansel RE, Jiang WG. 2005. Increased levels of SPARC (osteonectin) in human breast cancer tissues and its association with clinical outcomes. Prostaglandins Leukot Essent Fatty Acids 72:267–272.

Weiss MM, Kuipers EJ, Postma C, Snijders AM, Pinkel D, Meuwissen SG, Albertson D, Meijer GA. 2004. Genomic alterations in primary gastric adenocarcinomas correlated with clinicopathological characteristics and survival. Cell Oncol 26:307–317.

Yang Y, Lee JH, Kim KY, Song HK, Kim JK, Yoon SR, Cho D, Song KS, Lee YH, Choi I. 2005. The interferon-inducible 9–27 gene modulates the susceptibility to natural killer cells and the invasiveness of gastric cancer cells. Cancer Lett 221:191–200.

Yang G, Rosen DG, Zhang Z, Bast RC Jr., Mills GB, Colacino JA, Mercado-Uribe I, Liu J. 2006. The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. Proc Natl Acad Sci USA 103:16472–16477.

Yang E, Kang HJ, Koh KH, Rhee H, Kim NK, Kim H. 2007. Frequent inactivation of SPARC by promoter hypermethylation in colon cancers. Int J Cancer 121:567–575.

Yang S, Shin J, Park KH, Jeung HC, Rha SY, Noh SH, Yang WI, Chung HC. 2007. Molecular basis of the differences between normal and tumor tissues of gastric cancer. Biochim Biophys Acta 1772:1033–1040.

Zhou Y, Zhang J, Liu Q, Bell R, Muruve DA, Forsyth P, Arcellana-Panlilio M, Robbins S, Yong VW. 2005. The chemokine GRO-alpha (CXCL1) confers increased tumorigenicity to glioma cells. Carcinogenesis 26:2058–2068.