SPANDIDOS PUBLICATIONS

Identification of differential gene expression between intestinal and diffuse gastric cancer using cDNA microarray

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Abstract. To compare the gene expression profiling between intestinal-type gastric cancer (IGC) and diffuse-type gastric cancer (DGC), cDNA microarray containing 7334 gene elements was performed on 12 paired IGC specimens/its' normal epithelial tissue and 11 paired DGC specimens/its' normal epithelial tissue. Twenty-seven genes were co-over-expressed in IGC and DGC. These overexpressed genes were related to transcription and translation, DNA replications and mitosis, calcium binding, apoptosis and mitochondria protein. Twelve genes were co-underexpressed in IGC and DGC. These underexpressed genes were associated with cell adhesion and migration, organelle movement and intracellular transport, and matrix metalloproteinase. A clustering dendrogram of IGC and DGC with 27 genes significantly differed between IGC and DGC. Nineteen genes were more overexpressed in DGC than in IGC, including annexin A1 (*ANXA1*), chemokine ligand 7 (*CCL7*), and chemokine ligand 8 (*CCL8*). Eight genes were more overexpressed in IGC than in DGC, including claudin 4 (*CLDN4*). The results of quantitative real-time PCR and immunohistochemical staining confirmed the microarray finding. The gene expression profiling between IGC and DGC suggested that they might have unique genetic pathways which share some of the same and some different genetic alterations.

Introduction

Gastric cancer is the second most frequent cause of cancer-related death in the world after lung cancer (1). According to Lauren's classification, gastric cancer can be divided into two histologically distinct types, each of which accounts for half of the cases: IGC and DGC (2). IGC, the predominant type of tumor in high-risk areas, has a glandular pattern and is usually accompanied by papillary formation or solid components (2). DGC, in contrast, consists of poorly cohesive cells diffusely infiltrating the gastric wall with little or no gland formation (2). A special subgroup of this type is the so-called signet ring cell carcinoma, in which the cell nucleus is pushed against the cell membrane creating a classical signet appearance due to an expanded, globoid, optically clear cytoplasm. IGC and DGC may result from the transformation of different epithelial cells or distinct molecular changes in a common cell type (3).

Over the past decade, many studies have clearly demonstrated that the combination of molecular changes differs between IGC and DGC, suggesting that they have unique genetic alterations (4-6). Alterations in specific genes that play important roles in diverse cellular functions, such as cell adhesion, signal transduction, differentiation, development or DNA repair, have been identified (7,8). With regard to genetic alterations in so-called tumor suppressor genes or oncogenes, gastric cancer is no exception. Inactivation due to loss of heterozygosity (LOH) and or mutation of *p53*, *APC* and *DCC* have been reported in gastric cancer. Mutation of *p53* was detected in about 30% of gastric cancers independent of the histological subtype (9,10). Up to 60% of the IGC but 30% of DGC have mutation/LOH of the *APC* gene (11). *DCC* LOH have been detected in 50% of IGC whereas, in DGC, this LOH is absent (12). The *met* proto-oncogene codes for the hepatocyte growth factor receptor which is preferentially amplified and overexpressed in DGC (13). Other growth factor and receptor signal systems that may be altered include EGFR, TGF-α and K-sam (14). Adhesion molecules, such as ß-catenin, have been detected in 30% mutation of IGC but are absent in DGC (15). E-cadherin is the binding partner of ß-catenin and plays a crucial role in establishing the structural integrity of epithelial tissue. *E-cadherin* mutations have been detected in 50% of DGC but are absent in IGC (16).

Because the development and progression of gastric cancer is a very complicated process, we hypothesized that many other genes are as yet undiscovered. However, identifying these genes by conventional methods, such as Northern blots, and serial analysis of gene expression has been either labor intensive or non-systematic. High throughput methods, such

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as cDNA and oligonucleotide microarrays, are increasingly being used to systematically compare molecular features of individual cancers to key clinical parameters (17-20). Previous expression profiling studies of gastric cancer highlighted differences in gene expression between tumors and adjacent mucosa but failed to identify gene expression patterns discriminating histological subtypes (21). Our study uses 12 pairs of IGC and 11 pairs of DGC specimens to view whether the genetic alterations are different between the two types of gastric cancer.

Materials and methods

Patients and specimens. Tumor and normal specimens were collected from patients with GC requiring subtotal or total gastroectomy resection in Chang Gung Memorial Hospital (CGMH) in Taiwan. Written informed consent was obtained before collection and this study was approved by the IRB. Table I summarizes the characteristics of patient samples. Each pair of normal samples were isolated. Tumors were resected and divided (fresh frozen in -80˚C freezer and formalin-fixed) within 15 min *ex vivo*. Formalin-fixed tissue sections were stained with H&E and classified by a pathologist. These results were compared with the pathology records from CGMH. The final pathology was determined by consensus and review if necessary. Each specimen was attributed a diagnosis and scored for Lauren's classification. Pathological AJCC stages were obtained from clinical records with patient permission. *H. pylori* serological testing was performed using PANBIO *H. pylori* IgG ELISA on preoperative serum collected on patients.

Table I. Clinical and pathological parameters of patient samples included for microarray analysis

	IGC	DGC
Total	12	11
Gender		
Male	8	4
Female	4	7
Age	63.1 (37-87)	57.2 (37-73)
AJCC stage		
IA	1	2
IB	1	0
II	4	1
IIIA	1	1
IIIB	2	4
V	3	3
Lymph node status		
N0	5	3
N1 (1-6)	2	1
N2 (7-15)	2	6
N3 (>15)	3	1
H. pylori infection		
-	8	9
+	4	2

Microarray analysis. Total RNA was isolated by phenol-chloroform extraction (TRIzol; Invitrogen) and column chromatography (RNeasy; Qiagen). The total RNA was then qualified and quantified by RNA LabChip on Bioanalyzer 2100B (Agilent). Total RNA (10 μg) was labeled using reverse transcription with Superscript II (Invitrogen) and Cy5-dCTP (Amersham) for tumor specimens and with Cy3-dCTP (Amersham) for normal specimens. The procedure was similar to previous methods (22). Labeled cDNA was cohybridized in 1X hybridization buffer (Amersham) and 50% formamide for 14-16 h at 42˚C to spotted cDNA arrays printed at the CGMH Microarray Core Facility. These comprised 7344 elements representing 6394 unique cDNAs (Unigene Cluster) and were printed onto an aminosilane-coated slide (ArrayIt) using a robotic arrayer (Genetix). Slides were washed in 1X SSC with 0.2% SDS, then 0.1X SSC, and finally 0.1X SSC at room temperature and scanned using a confocal scanner ChipReader (Virtek). Data were managed using MATLAB 6.0 software (The MathWorks).

Quantitative PCR. Four representative genes (*claudin 4*, *annexin A1*, *CCL7* and *CCL8*) were selected that were differentially expressed, based on hierarchical clustering of gene expression signatures. Reverse transcription was performed on RNA samples using random hexamers (Gibco BRL) as primers and superscript reverse transcriptase (Gibco BRL), as described in the instruction manual provided by the manufacturer. The cDNA amplification was performed using an ABI PRISM 7700 sequence detection system, using the 5' nuclease method (TaqMan) with a two-step PCR protocol (95˚C for 10 min followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min). All primers and probes were purchased from ABI. The lot numbers of claudin 4, annexin A1, CCL7, and CCL8 were Hs00533616-S1, Hs00167549-m1, Hs00171147-m1 and Hs00428422-q1, respectively. The number of PCR cycles required for the detection of each transcript was defined (cycle threshold: Cτ). Serial dilutions of a standard cDNA were included to ensure linearity and reproducibility of the assay. The quantitative value of each sample was calculated as the ratio between the amount of the test gene and the endogenous control of the house-keeping gene, GAPDH.

Immunohistochemistry. The tissue block were constructed according to the method of Schraml *et al* (23) and the best representative morphological areas of tumors were used in this study. The specimen sections were deparaffinized, then blocked endogenous peroxidase with 3% hydrogen peroxide and microwaved after pretreatment in citric acid to retrieve antigenicity. The sections were incubated with blocking solution containing PBS and 1% bovine serum albumin for 20 min at room temperature, and then incubated with primary antibody (goat anti-human polyclonal antibody from Santa Cruz Biotechnology) overnight at 4˚C. After washing 4 times with TBS, the sections were incubated with secondary antibody (rabbit anti-goat IgG, Santa Cruz Biotechnology). The immuno-complex was visualized by the immunoglobulin enzyme bridge technique using the Dako LSAB 2 System, HRP kit (Dako Corp. Carpinteria, CA) with 3,3' diamino-

Genes significantly co-overexpressed in IGC and DGC.

Function and gene ID number	Gene name (gene symbol)	Protein function (refs.)
Transcription and translation		
04918	X-box binding protein (*XBP*)	A transcription factor by recognizing the X2 promoter element (25)
00927	S-phase kinase-associated protein 1A (*SKP1A*)	Involved in transcription regulation for the development or maintenance of specialized functions of the inner ear (26)
01671	Nuclear receptor coactivator 1 (*NCOA1*)	Steroid receptor coactivator-1, a coactivator that is required for full transcriptional activity of the steroid receptor superfamily (27)
07137	Activating transcription factor 4 (*ATF-4*)	cAMP-dependent transcription factor-4 (28,29)
02397	Zinc finger protein 36 (*ZFP36*)	An unusual zinc finger structure (30)
06945	POU domain, class 2, transcriptional factor 2, (*POU2F2*)	An octamer-binding transcription factor (31)
03812	Tyrosyl-tRNA synthetase (*YARS*)	Catalyzes the aminoacylation of tRNA by their cognate amino acid (32)
DNA replications and mitosis		
02023	PCTAIRE protein kinase 1 (*PCTK1*)	Serine/threonine-specific protein kinase regulating the initiation and passage through mitosi (33)
Calcium binding		
04992	S100 calcium binding protein (*S100*)	Regulates sarcoplasmic reticulum calcium ion handling and myofibrillar calcium ion responsiveness (34)
Apoptosis		
03664	Testis enhanced gene transcript (*TEGT*)	A BAX inhibitor (35)
Mitochondria protein		
06341	Cytochrome C oxidase subunit IV (*COX4*)	Reduction of oxygen to water is accompanied by the extrusion of four protons from the intramitochondrial compartment involved in respiratory chain
06879	ATP synthase, H^+ transporting, mitochondrial F1 complex	Catalyzes ATP synthesis
05278	Creatine kinase (*CKMT2*)	Catalyzes the reversible transfer of a phosphoryl group from creatine phosphate to ADP to form ATP
Enzyme		
2389	Progastricsin (pepsinogen C), inactive precursor of protein	The zymogen, cleaves itself in an acidic environment to form active pepsin
5155	Lipase A, lysosomal acid, cholesterol esterase	Involved in intracellular degradation of cholesteryl esters
03906	Cathepsin D, lysosomal aspartyl protease	Proteinase, a member of the peptidase C1 family, has a specificity similar to but narrower than that of pepsin A
03916	Phosphate cytidylyltransferase 1, choline, α isoform	Catalyzes rate-limiting synthesis of phosphotidyl-choline; plays a key role in cell membrane synthesis and in supply of lipid second messenger
06006	Aldehyde dehydrogenase	Ubiquitous enzyme located in virtually all mammalian tissues; catalyzes oxidation of aldehyde substrates to carboxylic acids
00171	Malate dehydrogenase	Inhibits endocytosis of lactate dehydrogenase M4 by liver macrophages
02209	Protein phosphotase 2, regulatory subunit B (B56), γ	A heterotrimeric serine/threonine phosphatase

Table II. Continued.

Function and gene ID number	Gene name (gene symbol)	Protein function (refs.)
06144	NADH dehydrogenase (ubiquinone) 1 α subcomplex	Transfers electrons from NADH to ubiquinone
04287	Dicarbonyl/L-xylulose reductase α	An enzyme that has both diacetyl reductase and L-xylulose reductase activities
02483	Cytochrome b-5 (*CYB5*)	Reduces the heme iron atom to the ferrous form
Others		
05559	ATPase, H^+/K^+ exchanging, ß polypeptide (*ATP4B*)	In gastric parietal cells, H(+),K(+)-ATPase plays an essential role in the formation of hydrochloric acid
00371	Metallothionein 1F (*MT1F*)	A family of low molecular weight, heavy metal-binding proteins characterized by a high cysteine content and lack of aromatic amino acids
04473	H^+ transporting ATPase	Translocates protons into intracellular organelles or across the plasma membrane
05759	Lactotransferrin (*LTF*)	A family of iron-binding proteins that modulate iron metabolism, hemopoiesis and immunological reactions

benzidine tetrachloride as the substrate. The sections were lightly counterstained with hematoxylin, dehydrated with graded alcohols, cleared with xylene and mounted with a coverslip (24).

Data analysis. Data were imported into GeneSpring (Silicon Genetics) and intensity-dependent normalization carried out using LOWESS. A group of 7344 elements satisfied filtering criteria based on the presence of a significant signal in ≥80% of hybridizations. Hierarchical clustering was performed using Pearson correlation as a measure of similarity, after average linkage and median centering of values using the program, Cluster, and viewed with Treeview. Discriminant genes and differences between the two groups were analyzed using two-tailed ANOVA with Benjamini Hochberg multiple testing correction factor at $P \leq 0.05$.

Results

Co-overexpressed and co-underexpressed gene expression in IGC and DGC. We compared the gene expression of IGC and DGC with that of normal stomach tissue using a cDNA microarray containing 7344 gene elements. To identify genes that exhibited the most significant and consistent expression changes in IGC and DGC compared with normal stomach tissue, we analyzed the gene expression ratios as described in Materials and methods. We first selected outlying genes that have an average expression ratio >2.0 SD from the mean. This 2.0 SD cut-off represents a 95% confidence interval. There were a total of 39 genes that met these analysis criteria for co-overexpression and co-underexpression (27 for over-expressed and 12 for underexpressed). They accounted for 0.53% of the 7344 unique cDNA clones included in the microarray.

The co-overexpressed genes are listed in Table II and co-underexpressed genes are listed in Table III. The 27 genes whose expressions were co-overexpressed in IGC and DGC represented a variety of functional groups. There are seven genes involved in transcription and translation; *XBP* (a transcription factor recognizing both the X2 promoter element of both the human DR-α and DP-ß) (25), *SKP1A* (involved in transcription regulation for the development or maintenance of specialized functions of the inner ear) (26), *NCOA1* (steroid receptor coactivator-1, a coactivator that is required for full transcriptional activity of the steroid receptor superfamily) (27), *ATF-4* (cAMP-dependent transcription factor-4) (28,29), *ZFP36* (an unusual zinc finger structure) (30), *POU2F2* (an octamer-binding transcription factor) (31) and *YARS* (catalyze the aminoacylation of tRNA by their cognate amino acid) (32). *PCTK1* (serine/threonine-specific protein kinase regulating the initiation and passage through mitosis) (33) is involved in DNA replications and mitosis and *S100* (regulating sarcoplasmic reticulum calcium ion handling and myofibrillar calcium ion responsiveness) (34) is involved in calcium binding. *TEGT* (a BAX inhibitor) (35) is associated with apoptosis and three genes are mitochondria protein: MTCO4, ATP synthase and CKMT2. There are ten genes which function as enzymes and four genes listed under 'others' (Table II). The 12 genes who were co-underexpressed in IGC and DGC represented a variety of functional groups (Table III). *ITGB1* (36) and *fibronectin* (37) are involved in cell adhesion and migration. The functions of *KIF2C* (38) and *CFL1* (39) are associated with organelle movement and intracellular transport. Two genes, *TUBA6* and *TUBB5*, are involved in the cytoskeleton.

Differential gene expression profiling between IGC and DGC. A clustering dendrogram of IGC and DGC with 27 genes significantly differs between IGC and DGC. The upper 19 genes were more overexpressed in DGC than in IGC. These genes included *ANXA1*, *CCL7* and *CCL8*. The bottom 8 genes were more overexpressed in IGC than in DGC. These genes included *CLDN4* and *CCT3* (Fig. 1).

Genes significantly co-underexpressed in the IGC and DGC.

Function and gene ID number	Gene name (gene symbol)	Protein function (refs.)
Cell adhension and migration		
06431	Integrin, ß1 (*ITGB1*)	Fibronectin receptors (36)
07001	Fibronectin	Glycoprotein on the surface of cell (37)
Organelle movement and intracellular transport		
00994	Kinesin family member 2C (*KIF2C*)	Moves cargo along microtubules (38)
00540	Cofilin (*CFL1*)	An intracellular actin-modulating protein which involvedin the translocation of actin-cofilin complex from cyto plasm to nucleus (39)
Cytoskeleton		
00081	Tubulin α6 (*TUBA6*)	α subunit of microtubules, are constituent parts of a diverse variety of eukaryotic cell structures, e.g., the mitotic apparatus, cilia, flagella and elements of the cytoskeleton
06394	ß5-tubulin (*TUBB5*)	ß subunit of microtubules, are constituent parts of a diverse variety of eukaryotic cell structures, e.g., the mitotic apparatus, cilia, flagella and elements of the cytoskeleton
MMP		
00835	Matrix metalloproteinase 2 (*MMP-2*)	A member of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes
00618	Matrix metalloproteinase 10 (*MMP-10*)	A member of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes
Others		
04819	Calumenin (*CALU*)	A calcium-binding protein localized in the endoplasmic reticulum and is involved in such ER functions as protein folding and sorting
00539	Stratifin (*SFN*)	Mediates signal transduction by binding to phosphoserine-containing proteins
06315	Phosphoglycerate kinase 1 (*PGK1*)	Major enzyme in glycolysis catalyzes the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate, generating one molecule of ATP
01017	Annexin A2 (*ANXA2*)	A family of Ca(2+)-dependent phospholipid binding proteins which have a molecular weight of approximately 35000-40000 and are preferentially located on the cytosolic face of the plasma membrane

Confirmation of microarray results with quantitative real-time PCR. To validate our RNA expression results, we selected four genes, *CLDN4*, *ANXA1*, *CCL7* and *CCL8*, conducted on 3 IGC and 2 DGC samples to perform quantitative real-time PCR as an assessment of relative RNA abundance. Table IV shows that there was an excellent concordance of the results of quantative real-time PCR analysis and microarray finding.

Immunohistochemical analysis of protein expression. We selected four differentially expressed genes between IGC and DGC to compare their transcript and protein expression. Three of these genes, *ANXA1*, *CCL7* and *CCL8,* were over-expressed in DGC compared with in IGC. *CLDN4* was over-expressed in IGC compared with in DGC. Immunohistochemical staining of IGC and DGC tissue sections with the four antibodies confirmed the pattern of expression seen in our microarray experiments (Fig. 2).

Discussion

In the present study, we used cDNA microarray to explore the variations in gene expression between IGC and DGC.

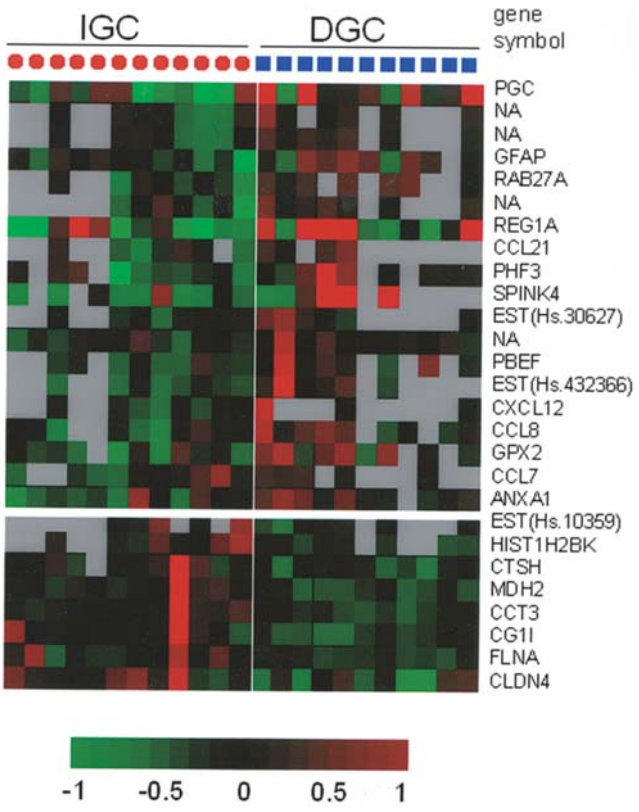

Figure 1. Significantly differential gene expression between 12 IGC and 11 DGC with 27 genes. The standard deviation is >0.4 and the p-vaule is <0.05 using Student's t-test. Red and green indicate gene expression respectively above and below the median (black) for each gene across all cases and gray indicate the missing data.

Twenty-seven genes were identified as significantly co-over-expressed in IGC and DGC. These genes can be categorized into several groups on the basis of their known functions (Table II). The first major group of genes was transcription or translated genes and included transcriptional factors such as X-box binding protein (*XBP*); activating transcription factor 4 (*ATF-4*); and POU domain, class 2, transcriptional factor 2 (*POU2F2*); and transcriptional or translational machinery-related genes. The overexpression of these genes was not mentioned in gastric cancer progression although they are involved in the regulation of cell growth and proliferation.

DNA replications and mitosis-related genes were another important group of genes overexpressed in gastric cancer, including *PCTK1*. One calcium binding protein, S100 and one anti-apoptotic protein, TEGT were also highly expressed in gastric cancer. Three mitochondria proteins involved in the respiratory complex and ten enzymes with various catalytic functions were overexpressed in gastric cancer (Table II).

Twelve genes were identified as significantly co-under-expressed in IGC and DGC. These genes can be categorized into several groups on the basis of their known functions (Table III). Cell adhesion and migration-related genes were the major group of genes underexpressed in gastric cancer. This group included integrin, ß1; and fibronectin. Bittner *et al* also showed that the expression of integrin, ß1 was reduced in gene expression profiling of cutaneous malignant melanomas (40). Fibronectin is an extracellular glycoprotein that serves as a ligand for the integrin family of cell adhesion receptors and regulates cytoskeletal organization. Fibronectin expression has been linked with tumorigenesis (41) and metastasis (42), although these studies were only correlative. Peptides that mimic the cell adhesive region of fibronectin were, however, known to inhibit metastasis (43), which may indicate that tumor cells must interact with molecules such as fibronectin to metastasize. Another group consisted of organelle movement and intracellular transport genes, including *KIF2C* and *CFL1*. The reasons for the down-regulation in expression of these two genes involved in gastric cancer progression was unknown. Recent reports showed that *KIF2C* was a microtubule depoly-merase that forms an ATP-hydrolyzing complex at microtubule ends (38) but *CFL1* promoted actin polymerization and defined

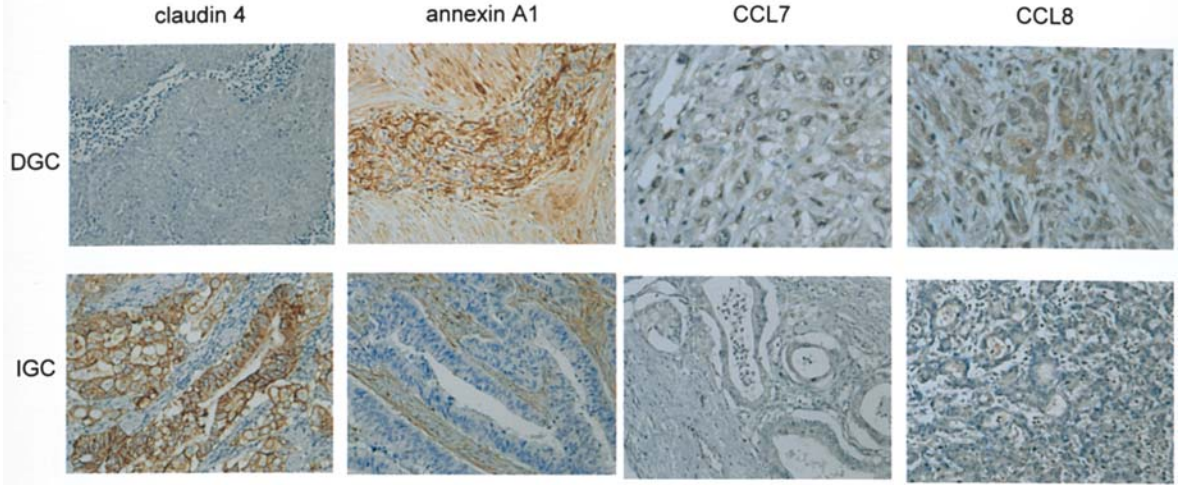

Figure 2. Immunohistochemical staining of claudin 4, annexin A1, CCL7 and CCL8 on representative IGC and DGC tissue sections. Claudin 4, magnified view (x200) of DGC with negative claudin 4 staining and IGC with positive claudin 4 staining. Annexin A1, magnified view (x200) of DGC with positive annexin A1 staining and IGC with negative annexin A1 staining. CCL7, magnified view (x400) of DGC with positive CCL7 staining and magnified view (x200) of IGC with negative CCL7 staining. CCL8, magnified view (x400) of DGC with positive CCL8 staining and magnified view (x200) of IGC with negative CCL8 staining.

Quantitative real-time PCR values and cDNA ratios for representative genes.

	Gene ratios							
	Claudin 4		Annexin A1		CCL7		CCL8	
Specimens	RT	Array	RT	Array	RT	Array	RT	Array
GA92[a]19-DGC	16.33	2.38	4.34	1.35	-	1.33	5.97	2.53
GA92[a]24-DGC	13.36	1.67	3.81	2.44	-	1.37	1.91	1.52
GA92[a]52-IGC	5.61	2.82	1.14	1.14	15.34	-	0.42	-
GA92[a]59-IGC	12.81	1.76	1.69	1.28	9.64	-	0.51	-
GA92[a]62-IGC	3.11	4.00	0.11	0.75	-	0.72	0.57	1.04

-, Indicates that data is missing on cDNA array or none of cDNA is available for real-time PCR.

the direction of cell motility (39). The down-regulation in expression of the cytoskeleton-related genes, tubulin α6 and tubulin ß5, was unexpected because tubulin was a chemotherapeutic target. The taxanes, paclitaxel and docetaxel, which act on microtubules to arrest mitosis, were the best antineoplastic drugs against a wide spectrum of cancers, including ovarian cancer, breast cancer, small and non-small cell lung cancer, colon cancer, head and neck cancer, multiple myeloma, melanoma, and Kaposi's sarcoma (44). The identification of down-regulated expression in MMP-2 and MMP-10 was unexpected because one would expect the MMP genes to be overexpressed in tumor cells, especially in metastastic tumor cells (45).

The expression of the adherens junction varies with the disruption of glandular morphology and loss of differentiation. It has been suggested that the loss of tight junction function is related to tumor differentiation and our results showed that claudin 4 was down-regulated in DGC. Lee *et al* also showed that the reduced expression of claudin 4 was more frequent in DGC than in IGC and the reduced expression of claudin 4 correlated with poor differentiation (46).

Our results showed that annexin A1 was down-regulated more frequently in IGC than in DGC. Annexin A1 belongs to a family of Ca(2+)-dependent phospholipid binding proteins which have a molecular weight of approximately 35000-40000 and are preferentially located on the cytosolic face of the plasma membrane. Annexin A1 protein had an apparent relative molecular mass of 40 kDa, with phospholipase A2 inhibitory activity. Hu *et al* showed that allelic loss of annexin A1 occured frequently, whereas somatic mutations were rare, suggesting that annexin A1 was not inactivated in esophageal squamous cell carcinoma via a two-hit mechanism (47). A decrease in annexin A1 protein expression was confirmed, consistent with a quantitative decrease in mRNA expression, and appeared to be related to tumor cell differentiation. Hu *et al* concluded that annexin A1 was not the tumor suppressor gene corresponding to the high levels of loss of heterozygosity observed on chromosome 9 in esophageal squamous cell carcinoma; however, dysregulation of mRNA and protein levels was associated with esophageal squamous cell carcinoma (47).

The CCL7 and CCL8 chemokines subdivided into C-C chemokines having 2 adjacent cysteine residues. C-C chemokines predominantly attract monocytes/macrophages and have been involved in macrophage recruitment during inflammation and cancer invasion. These factors are often produced by invasive cancer cells (48). This is consistent with our results that CCL7 and CCL8 were more overexpressed in DGC than in IGC.

Many studies in the past decade have clearly demonstrated that multiple genetic alterations are responsible for the development and progression of gastric cancer. Alterations in specific genes that play important roles in diverse cellular functions such as cell adhesion, signal transduction, transcription and translation, DNA replication and mitosis, and DNA repair have been identified. With regard to genetic alterations in so-called tumor suppressor genes or oncogenes, gastric cancer is no exception. Such tumor-associated gene alterations have frequently been reported in both histological subtypes of gastric cancer. However, the combination of molecular changes differs between IGC and DGC, suggesting that they may have unique genetic pathways and our microarray results support this hypothesis.

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