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ORIGINAL PAPER

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Gastric and intestinal phenotypic cell marker expressions in gastric differentiated-type carcinomas: association with E-cadherin expression and chromosomal changes

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Abstracts Gastric and intestinal phenotypic cell markers are widely expressed in gastric carcinomas, irrespective of their histological type. In the present study, the relations between the phenotypic marker expression of the tumour, histological findings, expression of cell adhesion molecules, and the chromosomal changes in gastric differentiated-type carcinomas were examined. The phenotypic marker expression of the tumour was determined by the combination of the expression of the human gastric mucin (HGM), MUC6, MUC2 and CD10, and was evaluated in comparison with the expression of cell adhesion molecules, such as E-cadh erin and β-catenin, and chromosomal changes by comparative genomic hybridization (CGH) in 34 gastric differentiated-type carcinomas. Tumours were classified into the gastric- (G-), gastric and intestinal mixed- (GI-), intestinal- (I-), or unclassified- (UC-) phenotype according to the immunopositivity of staining for HGM, MUC6, MUC2, and CD10. G-phenotype tumours were significantly associated with a higher incidence of differentiated-type tumours mixed with undifferentiated-type component, compared with GI- and I-phenotype tumours (88.9 vs 33.3%, $P=0.0498$ and 88.9 vs 42.9%, $P=0.0397$; respectively). HGM-positive tumours were significantly associated with a higher incidence of tumours with abnormal expression of E-cadherin, compared with HGM-negative tumours (66.7 vs 21.1%, $P=0.0135$). GI-phenotype tumours were significantly associated with a higher incidence of tumours with abnormal expression of E-cadherin, compared with I-phenotype tumours (77.8 vs 21.4%, $P=0.0131$). HGM-negative tumours were significantly associated with higher frequencies of the gains of 19q13.2 and 19q13.3, compared with HGM-positive tumours (57.9 vs 20.0%, $P=0.0382$ and 63.2 vs 13.3%, $P=0.0051$; respectively). MUC6-positive tumours were significantly associated with higher frequencies of the gains of 20q13.2, compared with MUC6-negative tumours (71.4 vs 30.0%, $P=0.0349$). MUC2-positive tumours were significantly associated with the gain of 19p13.3, compared with MUC2-negative tumours (41.2 vs 5.9%, $P=0.0391$). I-phenotype tumours were significantly associated with higher frequencies of gains of 5p15.2 and 13q33-34, compared with G-phenotype tumours (66.7 vs 0%, $P=0.0481$, each) and also associated with higher frequencies of gain of 7p21, compared with GI-phenotype tumours (66.7 vs 0%, $P=0.0481$). Our present results show that gastric differentiated-type carcinomas have different characteristics according to the phenotypic marker expression of the tumour in terms of histological findings, E-cadherin expression and pattern of chromosomal changes.

Keywords Gastric carcinoma · Human gastric mucin · MUC6 · MUC2 · CD10 · E-cadherin · β-catenin · Comparative genomic hybridization

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1 Introduction

Gastric carcinoma is histologically classified into two types, intestinal and diffuse, differentiated and undifferentiated, based on the gland formation tendency

(Lauren 1965; Nakamura et al. 1968). With respect to the histogenesis of these two types of gastric carcinoma, differentiated-type tumours have generally been considered to arise from the gastric mucosa with intestinal metaplasia and undifferentiated-type tumours from the ordinary gastric mucosa without intestinal metaplasia, and the two are considered to follow different genetic pathways during carcinogenesis (Nakamura et al. 1968; Lauren 1965; Saito and Shimada 1986; Tahara 1995). However, immunohistochemical examinations have recently demonstrated that gastric and intestinal phenotypic cell markers are widely expressed in gastric carcinomas, irrespective of their histological type (Tatematsu et al. 1986, 1990, 2003; Egashira et al. 1999; Sasaki et al. 1999; Endoh et al. 1999a, 1999b; Machado et al. 2000; Koseki et al. 2000). Several authors have reported that gastric carcinomas can be classified as having either a gastric- (G-), gastric and intestinal mixed-(GI-) or intestinal- (I-) phenotype, depending on the immunopositivity to staining with human gastric mucin (HGM), MUC6, MUC2 and CD10 (Tajima et al. 2001; Kabashima et al. 2002). Human gastric mucin, MUC6, MUC2 and CD10 are specifically expressed in gastric surface mucous cells, pyloric gland cells, intestinal goblet cells of the mature gastrointestinal tract and brush border of intestinal epithelial cells, respectively. Previous reports showed that G-phenotype tumours accounted for 27.7% of differentiated tumours, often referred to as intestinal-type tumours, according to Lauren (1965), while I-phenotype tumours accounted for 10.1% of undifferentiated tumours (Tajima et al. 2001). The phenotypic marker expression of the tumour is conventionally thought to imitate that of the tissue of origin. It is thus suggested that gastric carcinomas can occur in various types of gastric mucosa. With respect to the clinicopathological significance of the phenotypic marker expression of the tumour, differentiated-type gastric carcinomas of the G-phenotype are more likely to transform into the undifferentiated-type carcinoma and show infiltrative growth to deeper layers of the mucosa or invasion of the surrounding structures through loss of E-cadherin function as compared with those of the I-phenotype (Endoh et al. 1999b; Saito et al. 2001; Tajima et al. 2001). This histological transformation was suggested to occur relatively in the early stage of gastric carcinogenesis. Recently, G-phenotype tumours have been associated with a poorer outcome and greater malignant potential in the incipient phase of invasion and metastasis, compared with tumours of other phenotype (Endoh et al. 1999b; Koseki et al. 2000; Saito et al. 2001; Tajima et al. 2001; Kabashima et al. 2002; Shibata et al. 2003). Phenotypic marker expression in gastric neoplasms has also been suggested to depend on genetic changes (Endoh et al. 2000). However, there are conflicting opinions regarding the relations between the phenotypic marker expression of the tumour and genetic alterations in gastric differentiated-type carcinomas (Shibata et al. 2003). (Fig. 1).

Comparative Genomic Hybridization (CGH) is an exceptionally useful method that can simultaneously detect gains or losses in the genetic copy number in tumour DNA with one round of hybridization, and can map those regions on to all the chromosomes (Kallioniemi et al. 1992). With CGH, it is possible to detect chromosomal gains and losses in tumours, including those that were hitherto unknown. Since it was first developed, CGH has been applied to study genetic alterations in many solid tumours (Kokkola et al. 1997; Nessling et al. 1998; Sakakura et al. 1999). However, there are only a few reports about chromosomal changes according to the phenotypic

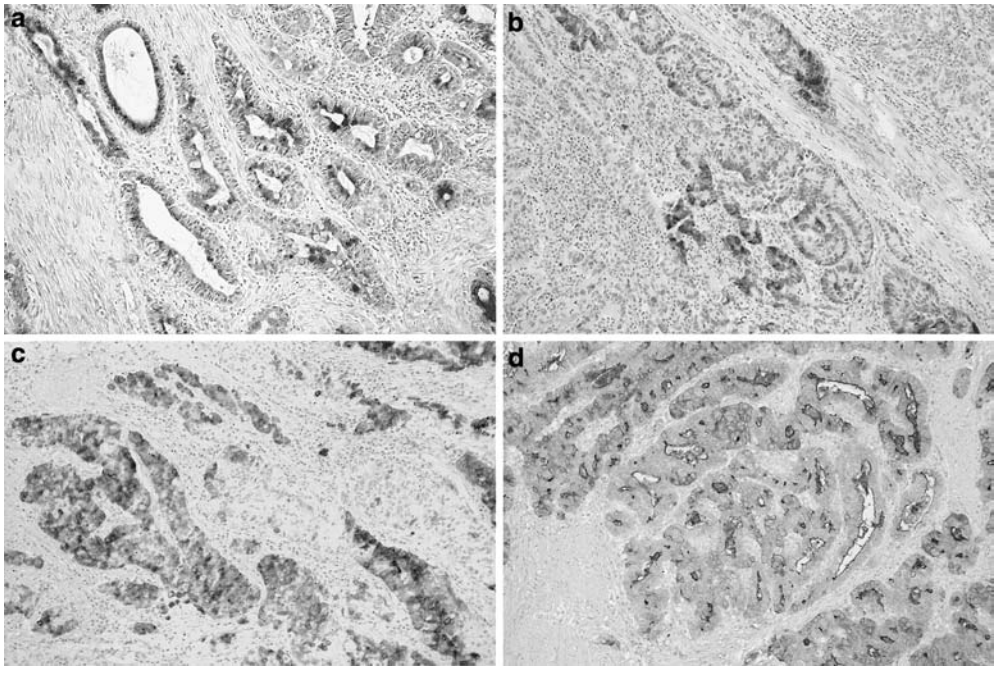

Fig. 1 **a** Human gastric mucin is expressed in the cancer cell cytoplasm (45MI, original magnification ×100). **b** MUC6 glycoprotein is also expressed in the cancer cell cytoplasm (CLH5, original magnification ×100). **c** MUC2 glycoprotein is expressed in the cancer cell cytoplasm (Ccp58, original magnification ×100). **d** CD10 glycoprotein is also expressed on the luminal surfaces of cancerous glands (56C6, original magnification ×100)

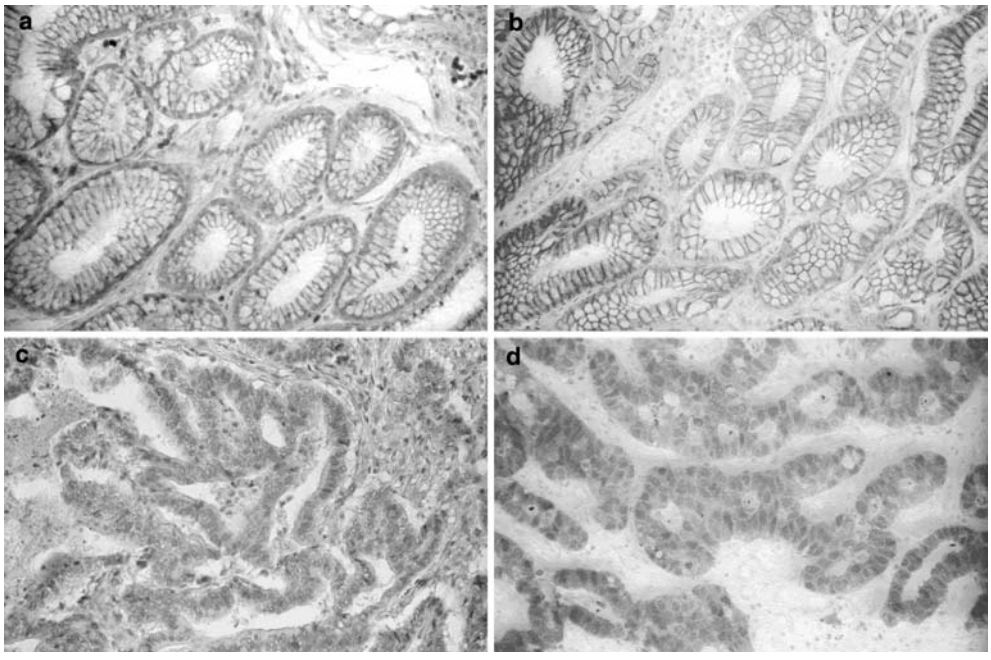

Fig. 2 The preserved expression of E-cadherin (**a**) and β-catenin (**b**), and reduced expression of E-cadherin (**c**) and β-catenin (**d**) (original magnification ×200)

marker expression of tumours using comparative genomic hybridization (Kushima et al. 2003).

In the present study, the relations between the phenotypic tumour cell marker expression, histological findings, expression of cell adhesion molecule, such as E-cadherin and β-catenin, and chromosomal changes in gastric differentiated-type carcinomas were examined (Fig. 2).

2 Materials and methods

2.1 Patients

The materials consisted of clinical specimens obtained from 34 cases of gastric differentiated-type carcinoma who had undergone surgery between January 2000 and March 2004 at the Department of Surgery, Division of General and Gastroenterological Surgery, Showa University, School of Medicine. Of the 34 patients, 26 were men and 8 were women. The mean age of the patients was 71 years (range, 52–88 years). No preoperative radio- and/or chemotherapy had been administered.

2.2 Clinicopathological review

Serial 5-mm-thick tissue sections of the entire tumour were prepared from the resected specimens fixed with 10% buffered formalin, embedded in paraffin and then 4°μm consecutive sections were used for histologic examination by haematoxylin and eosin (H&E) staining and immunohistochemical staining. All the tumours were classified according to the criteria established by The Japanese Classification of Gastric Carcinoma (1998). In the present study, the tumours were classified histologically into 16 pure differentiated-type carcinomas and 18 mixed (with undifferentiated-type component)-type carcinomas. The clinical data of the patients are summarized in Table 1.

2.3 Immunohistochemical staining for analysis of the tumour differentiation phenotype and adhesion molecules

Sections in which the tumour showed the greatest depth of cancer invasion were subjected to immunohistochemical staining. The following mouse monoclonal antibodies were used: 45M1 (Novocastra Laboratories Ltd, UK), diluted 1:50, to detect HGM; CLH5 (Novocastra Laboratories Ltd), diluted 1:50, to detect MUC6 glycoprotein; Ccp58 (Novocastra Laboratories Ltd), diluted 1:100, to detect MUC2 glycoprotein; and 56C6 (Novocastra Laboratories Ltd), diluted 1:40, to detect CD10 glycoprotein expression. 45M1 and CLH5 were examined as G-phenotype markers, and Ccp58 and 56C6 were examined as I-phenotype markers. 45M1 recognizes the mucin epitope located in the peptide core of HGM, which is synonymous with MUC5AC. This antibody is known to react with surface foveolar cells in the stomach (Bara et al. 1998; Nollet et al. 2002). MUC6 glycoprotein is expressed in mucous cells of the neck zone of the oxyntic mucosa and in antral glands (De Bolos et al. 1995; Reis et al. 1999; Machado et al. 2000). MUC2 glycoprotein, also known as the 'intestinal-mucin-related protein antigen', is an intestinal apomucin and also known to be expressed in the supranuclear area

Table 1 Clinicopathological characteristics of the patients

Case No.	Age	Sex	Site	Macroscopic type	Depth	Histologic type
1	88	M	L	Type3	T2(MP)	Differentiated
2	61	F	L	Type2	T2(SS)	Differentiated
3	68	M	M	Type2	T2(SS)	Differentiated
4	52	M	MUL	Type2	T2(SS)	Differentiated
5	85	M	MU	Type2	T4(SI)	Differentiated
6	70	M	LM	Type2	T2(SS)	Differentiated
7	79	M	L	Type3	T2(SS)	Differentiated
8	72	F	ML	Type3	T3(SE)	Differentiated
9	69	M	L	Type2	T2(SS)	Differentiated
10	59	M	LMD	Type2	T3(SE)	Differentiated
11	71	M	M	Type2	T3(SE)	Differentiated
12	67	M	U	Type3	T2(SS)	Differentiated
13	75	M	ML	Type1	T2(SS)	Differentiated
14	70	F	M	Type3	T2(SS)	Differentiated
15	80	F	UE+	Type2	T2(SS)	Differentiated
16	67	M	M	Type3	T3(SE)	Differentiated
17	70	F	L	Type3	T3(SE)	Mixed
18	62	M	MU	Type4	T3(SE)	Mixed
19	88	M	LM	Type3	T2(SS)	Mixed
20	80	F	LD	Type3	T2(SS)	Mixed
21	61	F	MUL	Type4	T3(SE)	Mixed
22	75	M	ML	Type3	T2(SS)	Mixed
23	76	M	L	Type2	T2(MP)	Mixed
24	87	F	ML	Type2	T2(SS)	Mixed
25	53	M	LM	Type3	T3(SE)	Mixed
26	71	M	MLU	Type4	T3(SE)	Mixed
27	73	M	LMU	Type5	T3(SE)	Mixed
28	79	M	ML	Type2	T3(SE)	Mixed
29	53	M	MUL	Type3	T4(SI)	Mixed
30	63	M	LM	Type4	T3(SE)	Mixed
31	82	M	UME	Type3	T3(SE)	Mixed
32	71	M	LM	Type2	T3(SE)	Mixed
33	71	M	LM	Type2	T3(SE)	Mixed
34	69	M	UML	Type3	T3(SE)	Mixed

All tumours were staged according to The Japanese Classification of Gastric Cancer

of the goblet cells in regions showing intestinal metaplasia in the stomach (Kim and Gum 1995; Sakamoto et al. 1997; Baldus et al. 1998; Utsunomiya et al. 1998; Reis et al. 1999; Machado et al. 2000). CD10 glycoprotein is a 100-kDa cell metalloendopeptidase that inactivates a variety of biologically active peptides and is known to be expressed on the brush border of intestinal epithelial cells as well as in the germinal centres of lymphoid follicles and the microvilli of the kidney (Ronco et al. 1984; Trejdosiewicz et al. 1985). The avidin-biotinyl-peroxidase complex immunohistochemical method was used for all immunohistochemical studies, according to a previously described protocol (Hsu et al. 1981).

With regard to the evaluations of HGM, MUC6, MUC2 and CD10 staining, distinct staining in more than 5% of the tumour cells was recorded as positive immunoreactivity for the relevant marker. These immunohistochemical methods were used to classify the tumours into four different phenotypes: tumours with G-phenotypic cells accounting for more than 5% of their cell population were classified as G-phenotype carcinomas; those with I-phenotypic cells accounting for more than 5% of their cell population were classified as I-phenotype carcinomas; those with both gastric and intestinal phenotypic cells accounting for more than 5% of their cell population were classified as GI-phenotype carcinomas; and those with both gastric and intestinal phenotypic cells accounting for less than 5% of their cell population were regarded as carcinomas of the UC-phenotype (Tajima et al. 2004).

The monoclonal antihuman E-cadherin antibody (HECD-1) (Takara Shuzo, Kyoto, Japan), diluted 1:10,000, monoclonal anti-β-catenin antibody (BD Biosciences, NJ, USA), diluted 1:500, and DAKO envision kits (Dako Cytomation, Copenhagen, Denmark) were also used. The avidin-biotinyl-peroxidase complex immunohistochemical method was used for all immunohistochemical studies, according to a previously described protocol (Hsu et al. 1981). The HECD-1 and anti-β-catenin staining was scored in a semiquantitative fashion from 0 to 3, with 0 denoting absent staining, 1 representing cytoplasmic distribution, 2 representing heterogeneous staining (i.e. when tumours were composed of both normal and abnormally stained areas), and 3 representing the normal membranous pattern of staining. Tumours with more than 10% variation were rated as showing heterogeneous staining. For the purpose of data analysis, all tumours showing loss of the membranous pattern of staining were classified as abnormal, including those with absent, heterogeneous

and cytoplasmic patterns of staining (i.e. those with scores 0, 1 and 2) (Jawhari et al. 1997).

The histopathological and immunohistochemical examinations were independently performed by two observers (Morohara and Tajima). The results were then compared, and any discrepancies were resolved by consensus after further histopathological review.

2.4 CGH

The method used for the CGH analysis is described below (Nakao et al. 2001).

2.4.1 Laser microdissection and DNA extraction

The tumour samples obtained from the invasive area of the tumour were embedded in OCT and snap-frozen at −80°C. The samples were sectioned into 7 μm-thick sections and fixed in 100% methanol for 3 min, followed by toluidine blue staining. Then, after being air-dried, the sections were laser-microdissected using the PALM. Laser Microdissection System (PALM Microlaser Technologies AG, Germany) for procuring the cancer cells.

The microdissected tumour tissues were homogenized in digestion buffer [100 mM Tris, 15 mM $MgCl_2$, 500 mM KCl, 0.5% tween 20, proteinase K (1 mg/ml; WAKO, Japan) and incubated overnight at 50°C, with shaking. DNA was extracted using the phenol-chloroform-isoamyl alcohol method. After centrifuging the samples at 14,000 rpm for 5 min, the DNA in the aqueous phase was collected and precipitated with 2.5 volumes of 100% ethanol after the addition of 7.5 M ammonium acetate at half the volume. The DNA was pelleted by centrifugation at 14,000 rpm for 30 min. The pellet was then washed in 70% ethanol and centrifuged at 14,000 rpm for 5 min to separate the supernatant. The DNA was then dissolved in TE buffer (10 mM Tris, 1 mM ethylenediamine tetraacetic acid) and weighed.

2.4.2 GenomiPhi

The GenomiPhi DNA Amplification Kit (Amersham Biosciences Corp., New Jersey, USA), which amplifies linear genomic DNA in a novel fashion, was developed for in vitro cloning, library construction, and other molecular biological applications (Dean et al. 2001). The method utilizes bacteriophage Phi29 DNA polymerase to exponentially amplify single- or double-stranded linear DNA templates during isothermal conditions (30°C); no strand displacement reaction thermal cycling is required. DNA is generated in microgram quantities after overnight incubation of nanogram amounts of starting material. DNA replication is extremely accurate because of the proofreading activity of Phi29 DNA polymerase. In brief, 1 μl of a template to be amplified is added to 9 μl of sample buffer and the mixture is heated to 95°C for 3 min to denature the template DNA. The sample is then cooled and mixed with 9 μl of reaction buffer and 1 μl of enzyme mix, before overnight (18 h) incubation at 30°C. After amplification, Phi29 DNA polymerase is heat-inactivated by 10 min incubation at 65°C (Morohara et al. 2005).

2.4.3 Labelled DNA and hybridization

DNA from tumour samples was labelled with Spectrum-Green (Vysis Inc., Chicago, IL, USA), and normal male DNA was labelled with Spectrum-Red (Vysis Inc.) using the nick-translation method. Labelled tumour DNA (400 ng) and normal DNA (200 ng), together with 10 μg of unlabelled Cot-1 DNA (Vysis Inc.) were collected and precipitated with 2.5 volumes of 100% ethanol after the addition of 3 M sodium acetate at 1/10 the volume. The DNA was pelleted by centrifugation at 14,000 rpm for 30 min. The pellet was then washed in 70% ethanol and centrifuged at 14,000 rpm for 5 min to separate the supernatant. The pellet was resuspended with 7 μL of CGH hybridization buffer and 3 μL of purified H_2O, denatured at 73°C for 5 min, and then applied to normal lymphocyte metaphase preparations. Prior to hybrid-

Table 2 Relations between the histological type and the phenotypic marker expression of the tumour

	HGM expression			MUC6 expression			MUC2 expression			CD10 expression		
	Negative ($n=19$)	Positive ($n=15$)	*P* value	Negative ($n=20$)	Positive ($n=14$)	*P* value	Negative ($n=17$)	Positive ($n=17$)	*P* value	Negative ($n=19$)	Positive ($n=15$)	*P* value
Differentiated	11 (57.9%)	5 (33.3%)	NS	11 (55.0%)	5 (35.7%)	NS	7 (41.2%)	9 (52.9%)	NS	8 (42.1%)	8 (53.3%)	NS
Mixed	8 (42.1%)	10 (66.7%)		9 (45.0%)	9 (64.3%)		10 (58.8%)	8 (47.1%)		11 (57.9%)	7 (46.7%)	

	Phenotypic marker expression pattern of the tumour			
	G-phenotype ($n=9$)	GI-phenotype ($n=9$)	I-phenotype ($n=14$)	UC-phenotype ($n=2$)
Differentiated	1 (11.1%)	6 (66.7%)	8 (57.1%)	1 (50.0%)
Mixed*	8 (88.9%)	3 (33.3%)	6 (42.9%)	1 (50.0%)

*$P=0.0397$ (G-phenotype vs I-phenotype) and $P=0.0498$ (G-phenotype vs GI phenotype)
NS Not significant

ization, the metaphase preparations were denatured at 73°C for 5 min in a denaturation solution (70% formamide and 2× standard saline citrate [SSC], pH7), dehydrated in a graded series of ethanol (70, 85 and 100%), and placed on a 37°C slide warmer. The hybridization was conducted at 37°C for 48 h. After hybridization, the slides were washed thrice in 50% formamide/2 × SSC, pH7, once in 2 × SSC at 45°C followed by PN buffer [0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4, 0.1% Nonidet P-40], and finally in distilled water at room temperature, each for 5 min. Then, after being air-dried, the slides were counterstained with 4′,6-diamidino-2-phenylindole in an antifade solution (Vysis Inc.), and a coverslip was placed on each hybridization location.

2.4.4 Digital image analysis

Three single-colour images (matching 4′,6-diamidino-2-phenylindole, fluorescein isothiocyanate, and Texas red fluorescence) were collected from each metaphase spread using a cooled charge-coupled device (CCD) camera (KX85, APOGEE, USA). Relative DNA sequence copy number changes were detected by analysing the hybridization intensities of tumour DNA and normal DNA along the length of all the chromosomes in the metaphase spread, as described previously. Each chromosome was analysed using FISH-CGH for Optimas (Optimas Corporation). The absolute fluorescence intensities were normalized so that the average ratio of green to red fluorescence for all chromosome objects in each metaphase was 1:0. The green-to-red ratio was calculated in each image. A ratio <0.8 was evaluated as a loss, whereas a ratio >1.2 was regarded as a gain in the tumour genome. Amplification were defined by a ratio >1.4.

2.5 Statistical analysis

The data were analysed with Student's t test, and the χ^2 test or Fisher's exact test. Differences with a value of $P<0.05$ were considered to be significant.

3 Results

3.1 Relations between histological-type and the phenotypic marker expression of the tumour

The relations between histological-type and the phenotypic marker expression of the tumour are shown in Table 2. HGM-negative, HGM-positive, MUC6-negative, MUC6-positive, MUC2-negative, MUC2-positive, CD10-negative and CD10-positive tumours composed 57.9, 33.3, 55.0, 35.7, 41.2, 52.9, 42.1 and 53.3% of differentiated-type tumours, respectively; and 42.1, 66.7, 45.0, 64.3, 58.8, 47.1, 57.9 and 46.7% of mixed-type tumours, respectively. There was no significant association between the histological-type tumours and the expressions of HGM, MUC6, MUC2 or CD10. The G-, GI-, I-, and UC-phenotype tumours composed 11.1, 66.7, 57.1 and 50.0% of differentiated-type tumours, respectively; and 88.9, 33.3, 42.9 and 50.0% of mixed-type tumours, respectively. G-phenotype tumours were significantly associated with a higher incidence of mixed-type tumours, compared with the GI- and I-phenotype ($P=0.0498$ and $P=0.0397$).

3.2 Relations between expressions of E-cadherin and β-catenin and the phenotypic marker expression of the tumour

The relations between expressions of E-cadherin and β-catenin and the phenotypic marker expression of the tumour are shown in Table 3. Abnormal expressions of E-cadherin in HGM-negative, HGM-positive, MUC6-negative, MUC6-positive, MUC2-negative, MUC2-po sitive, CD10-negative and CD10-positive tumours were observed in 21.1, 66.7, 35.0, 50.0, 29.4, 52.9, 42.1 and 40.0%, respectively. The expression of HGM was significantly associated with a higher incidence of tumours with abnormal expressions of E-cadherin ($P=0.0135$). Abnormal expression of β-catenin in HGM-negative, HGM-positive, MUC6-negative,

Table 3 Relations between abnormal expressions of E-cadherin and β-catenin and the phenotypic marker expression of the tumour

	HGM expression			MUC6 expression			MUC2 expression			CD10 expression		
	Negative ($n=19$)	Positive ($n=15$)	P value	Negative ($n=20$)	Positive ($n=14$)	P value	Negative ($n=17$)	Positive ($n=17$)	P value	Negative ($n=19$)	Positive ($n=15$)	P value
E-cadherin	4 (21.1%)	10 (66.7%)	0.0135	7 (35.0%)	7 (50.0%)	NS	5 (29.4%)	9 (52.9%)	NS	8 (42.1%)	6 (40.0%)	NS
β-catenin	9 (47.4%)	4 (26.7%)	NS	9 (45.0%)	4 (28.6%)	NS	6 (35.3%)	7 (41.2%)	NS	5 (26.3%)	8 (53.3%)	NS

	Phenotypic marker expression pattern of the tumour			
	G-phenotype ($n=9$)	GI-phenotype ($n=9$)	I-phenotype ($n=14$)	UC-phenotype ($n=2$)
E-cadherin*	3 (33.3%)	7 (77.8%)	3 (21.4%)	1 (50.0%)
β-catenin	2 (22.2%)	4 (44.4%)	6 (42.9%)	1 (50.0%)

*$P=0.0131$(GI-phenotype vs I-phenotype)

Table 4 Summary of CGH results in 34 gastric cancers

Chromosome	Frequency											
	Total ($n=34$)	HGM		MUC6		MUC2		CD10		G-phenotype	GI-phenotype	I-phenotype	UC-phenotype
		Negative ($n=19$)	Positive ($n=15$)	Negative ($n=20$)	Positive ($n=14$)	Negative ($n=17$)	Positive ($n=17$)	Negative ($n=19$)	Positive ($n=15$)	($n=9$)	($n=9$)	($n=14$)	($n=2$)
Gains													
1p	12 (34.3%)	7 (36.8%)	6 (40.0%)	6 (30.0%)	6 (42.9%)	4 (23.5%)	7 (41.2%)	8 (42.1%)	4 (26.7%)	3 (33.3%)	4 (44.4%)	4 (28.6%)	1 (50.0%)
1q	10 (29.4%)	6 (31.6%)	5 (33.3%)	5 (25.0%)	5 (35.7%)	4 (23.5%)	7 (41.2%)	5 (26.3%)	5 (33.3%)	2 (22.2%)	3 (33.3%)	5 (35.7%)	0 (0.0%)
2p	4 (11.8%)	2 (10.5%)	3 (20.0%)	2 (10.0%)	2 (14.3%)	1 (5.9%)	3 (17.6%)	2 (10.5%)	2 (13.3%)	0 (0.0%)	3 (33.3%)	1 (7.1%)	0 (0.0%)
2q	7 (20.6%)	6 (31.6%)	2 (13.3%)	5 (25.0%)	2 (14.3%)	3 (17.6%)	4 (23.5%)	2 (10.5%)	5 (33.3%)	1 (11.1%)	1 (11.1%)	5 (35.7%)	0 (0.0%)
3p	7 (20.6%)	3 (15.8%)	4 (26.7%)	4 (20.0%)	3 (21.4%)	1 (5.9%)	6 (35.3%)	3 (15.8%)	4 (26.7%)	1 (11.1%)	3 (33.3%)	3 (21.4%)	0 (0.0%)
3q	11 (32.4%)	5 (26.3%)	6 (40.0%)	7 (35.0%)	4 (28.6%)	6 (35.3%)	5 (29.4%)	7 (36.8%)	4 (26.7%)	5 (55.6%)	2 (22.2%)	4 (28.6%)	0 (0.0%)
4p	3 (8.8%)	2 (10.5%)	3 (20.0%)	1 (5.0%)	1 (7.1%)	1 (5.9%)	3 (17.6%)	4 (21.1%)	0 (0.0%)	1 (11.1%)	1 (11.1%)	1 (7.1%)	0 (0.0%)
4q	5 (14.7%)	2 (10.5%)	3 (20.0%)	2 (10.0%)	3 (21.4%)	2 (11.8%)	3 (17.6%)	2 (10.5%)	3 (20.0%)	1 (11.1%)	2 (22.2%)	2 (14.3%)	0 (0.0%)
5p	13 (38.7%)	9 (47.4%)	5 (33.3%)	7 (35.0%)	6 (42.9%)	3 (17.6%)	9 (52.9%)	6 (31.6%)	6 (40.0%)	2 (22.2%)	4 (44.4%)	7 (50.0%)	0 (0.0%)
5q	7 (20.6%)	5 (26.3%)	2 (13.3%)	6 (30.0%)	1 (7.1%)	2 (11.8%)	5 (29.4%)	1 (5.3%)	6 (40.0%) *	1 (11.1%)	1 (11.1%)	5 (35.7%)	0 (0.0%)
6p	18 (52.9%)	12 (63.2%)	7 (46.7%)	9 (45.0%)	9 (64.3%)	8 (47.1%)	10 (58.8%)	9 (47.4%)	8 (53.3%)	4 (44.4%)	5 (55.6%)	9 (64.3%)	0 (0.0%)
6q	5 (14.7%)	3 (15.8%)	3 (20.0%)	2 (10.0%)	3 (21.4%)	2 (11.8%)	3 (17.6%)	1 (5.3%)	4 (26.7%)	0 (0.0%)	3 (33.3%)	2 (14.3%)	0 (0.0%)
7p	19 (55.9%)	11 (57.9%)	8 (53.3%)	12 (60.0%)	7 (50.0%)	9 (52.9%)	10 (58.8%)	12 (63.2%)	8 (53.3%)	5 (55.6%)	4 (44.4%)	8 (57.1%)	2 (100%)
7q	19 (55.9%)	10 (52.6%)	10 (66.7%)	10 (50.0%)	9 (64.3%)	7 (41.2%)	12 (70.6%)	11 (57.9%)	8 (53.3%)	5 (55.6%)	6 (66.7%)	8 (57.1%)	0 (0.0%)
8p	12 (34.3%)	5 (26.3%)	7 (46.7%)	7 (35.0%)	5 (35.7%)	6 (35.3%)	6 (35.3%)	6 (31.6%)	6 (40.0%)	4 (44.4%)	3 (33.3%)	5 (35.7%)	0 (0.0%)
8q	21 (61.8%)	11 (57.9%)	11 (73.3%)	10 (50.0%)	11 (78.6%)	10 (58.8%)	11 (64.7%)	12 (63.2%)	9 (60.0%)	5 (55.6%)	9 (100%)	8 (57.1%)	0 (0.0%)
9p	8 (23.5%)	6 (31.6%)	2 (13.3%)	6 (30.0%)	2 (14.3%)	3 (17.6%)	5 (29.4%)	2 (10.5%)	6 (40.0%)	0 (0.0%)	2 (22.2%)	5 (35.7%)	1 (50.0%)
9q	9 (26.5%)	5 (26.3%)	5 (33.3%)	4 (20.0%)	5 (35.7%)	5 (29.4%)	4 (23.5%)	3 (15.8%)	6 (40.0%)	2 (22.2%)	4 (44.4%)	3 (21.4%)	0 (0.0%)
10p	8 (23.5%)	5 (26.3%)	4 (26.7%)	5 (25.0%)	3 (21.4%)	2 (11.8%)	6 (35.3%)	3 (15.8%)	5 (33.3%)	1 (11.1%)	3 (33.3%)	4 (28.6%)	0 (0.0%)
10q	12 (34.3%)	9 (47.4%)	5 (33.3%)	5 (25.0%)	7 (50.0%)	6 (35.3%)	6 (35.3%)	5 (26.3%)	7 (46.7%)	3 (33.3%)	4 (44.4%)	5 (35.7%)	0 (0.0%)
11p	7 (20.6%)	6 (31.6%)	1 (6.7%)	5 (25.0%)	2 (14.3%)	2 (11.8%)	5 (29.4%)	2 (10.5%)	5 (33.3%)	1 (11.1%)	1 (11.1%)	5 (35.7%)	0 (0.0%)
11q	12 (34.3%)	9 (47.4%)	4 (26.7%)	7 (35.0%)	5 (35.7%)	8 (47.1%)	4 (23.5%)	5 (26.3%)	7 (46.7%)	2 (22.2%)	3 (33.3%)	5 (35.7%)	2 (100%)
12p	12 (34.3%)	7 (36.8%)	6 (40.0%)	6 (30.0%)	6 (42.9%)	4 (23.5%)	8 (47.1%)	7 (36.8%)	5 (33.3%)	3 (33.3%)	4 (44.4%)	4 (28.6%)	0 (0.0%)
12q	15 (44.1%)	11 (57.9%)	5 (33.3%)	9 (45.0%)	6 (42.9%)	6 (35.3%)	9 (52.9%)	7 (36.8%)	8 (53.3%)	3 (33.3%)	4 (44.4%)	8 (57.1%)	0 (0.0%)
13q	16 (47.1%)	11 (57.9%)	5 (33.3%)	10 (50.0%)	6 (42.9%)	7 (41.2%)	9 (52.9%)	6 (31.6%)	10 (66.7%)	2 (22.2%)	3 (33.3%)	9 (64.3%)	0 (0.0%)
14q	11 (32.4%)	8 (42.1%)	3 (20.0%)	8 (40.0%)	3 (21.4%)	5 (29.4%)	6 (35.3%)	5 (26.3%)	6 (40.0%)	1 (11.1%)	2 (22.2%)	7 (50.0%)	1 (50.0%)
15q	12 (34.3%)	7 (36.8%)	6 (40.0%)	6 (30.0%)	6 (42.9%)	7 (41.2%)	5 (29.4%)	7 (36.8%)	5 (33.3%)	3 (33.3%)	4 (44.4%)	3 (21.4%)	1 (50.0%)
16p	15 (44.1%)	8 (42.1%)	8 (53.3%)	8 (40.0%)	7 (50.0%)	6 (35.3%)	9 (52.9%)	11 (57.9%)	4 (26.7%)	3 (33.3%)	6 (66.7%)	4 (28.6%)	2 (100%)
16q	11 (32.4%)	6 (31.6%)	6 (40.0%)	5 (25.0%)	6 (42.9%)	7 (41.2%)	4 (23.5%)	7 (36.8%)	4 (26.7%)	3 (33.3%)	3 (33.3%)	3 (21.4%)	2 (100%)
17p	6 (17.6%)	4 (21.1%)	3 (20.0%)	3 (15.0%)	3 (21.4%)	3 (17.6%)	3 (17.6%)	4 (21.1%)	2 (13.3%)	3 (33.3%)	1 (11.1%)	2 (14.3%)	0 (0.0%)
17q	17 (50.0%)	11 (57.9%)	7 (46.7%)	10 (50.0%)	7 (50.0%)	7 (41.2%)	10 (58.8%)	10 (52.6%)	7 (46.7%)	2 (22.2%)	6 (66.7%)	8 (57.1%)	1 (50.0%)
18p	1 (2.9%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)
18q	8 (23.5%)	4 (21.1%)	4 (26.7%)	6 (30.0%)	2 (14.3%)	4 (23.5%)	4 (23.5%)	1 (5.3%)	7 (46.7%) **	1 (11.1%)	3 (33.3%)	4 (28.6%)	0 (0.0%)
19p	10 (29.4%)	7 (36.8%)	4 (26.7%)	8 (40.0%)	2 (14.3%)	2 (11.8%)	8 (47.1%)	5 (26.3%)	5 (33.3%)	0 (0.0%)	4 (44.4%)	5 (35.7%)	1 (50.0%)
19q	22 (64.7%)	13 (68.4%)	9 (60.0%)	10 (50.0%)	11 (78.6%)	12 (70.6%)	9 (52.9%)	13 (68.4%)	8 (53.3%)	6 (66.7%)	6 (66.7%)	7 (50.0%)	2 (100%)
20p	15 (44.1%)	10 (52.6%)	6 (40.0%)	11 (55.0%)	4 (28.6%)	3 (17.6%)	12 (70.6%)	7 (36.8%)	8 (53.3%)	1 (11.1%)	5 (55.6%)	9 (64.3%) ***	0 (0.0%)
20q	24 (70.6%)	15 (78.9%)	10 (66.7%)	13 (65.0%)	11 (78.6%)	10 (58.8%)	14 (82.4%)	14 (73.7%)	10 (66.7%)	5 (55.6%)	8 (88.9%)	10 (71.4%)	1 (50.0%)
21q	7 (20.6%)	4 (21.1%)	3 (20.0%)	4 (20.0%)	3 (21.4%)	2 (11.8%)	5 (29.4%)	3 (15.8%)	4 (26.7%)	1 (11.1%)	3 (33.3%)	3 (21.4%)	0 (0.0%)
22q	12 (34.3%)	7 (36.8%)	6 (40.0%)	6 (30.0%)	6 (42.9%)	4 (23.5%)	8 (47.1%)	6 (31.6%)	6 (40.0%)	2 (22.2%)	6 (66.7%)	4 (28.6%)	0 (0.0%)

Table 4 (Contd.)

Chromosome	Frequency												
	Total ($n=34$)	HGM		MUC6		MUC2		CD10		G-phenotype	GI-phenotype	I-phenotype	UC-phenotype
		Negative ($n=19$)	Positive ($n=15$)	Negative ($n=20$)	Positive ($n=14$)	Negative ($n=17$)	Positive ($n=17$)	Negative ($n=19$)	Positive ($n=15$)	($n=9$)	($n=9$)	($n=14$)	($n=2$)
Losses													
1p	1 (2.9%)	1 (5.3%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
3p	4 (11.8%)	3 (15.8%)	2 (13.3%)	2 (10.0%)	2 (14.3%)	3 (17.6%)	1 (5.9%)	3 (15.8%)	1 (6.7%)	1 (11.1%)	1 (11.1%)	1 (7.1%)	1 (50.0%)
4p	4 (11.8%)	3 (15.8%)	3 (20.0%)	2 (10.0%)	1 (7.1%)	1 (5.9%)	2 (11.8%)	2 (10.5%)	2 (13.3%)	1 (11.1%)	1 (11.1%)	2 (14.3%)	0 (0.0%)
4q	8 (23.5%)	5 (26.3%)	4 (26.7%)	4 (20.0%)	4 (28.6%)	4 (23.5%)	5 (29.4%)	4 (21.1%)	4 (26.7%)	2 (22.2%)	2 (22.2%)	4 (28.6%)	0 (0.0%)
5p	2 (5.9%)	1 (5.3%)	1 (6.7%)	1 (5.0%)	1 (7.1%)	1 (5.9%)	1 (5.9%)	1 (5.3%)	1 (6.7%)	0 (0.0%)	1 (11.1%)	1 (7.1%)	0 (0.0%)
5q	5 (14.7%)	4 (21.1%)	1 (6.7%)	3 (15.0%)	1 (7.1%)	1 (5.9%)	3 (17.6%)	2 (10.5%)	3 (20.0%)	0 (0.0%)	1 (11.1%)	4 (28.6%)	0 (0.0%)
6q	3 (8.8%)	3 (15.8%)	0 (0.0%)	3 (15.0%)	0 (0.0%)	1 (5.9%)	2 (11.8%)	1 (5.3%)	2 (13.3%)	0 (0.0%)	0 (0.0%)	3 (21.4%)	0 (0.0%)
7q	1 (2.9%)	1 (5.3%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
8p	3 (8.8%)	2 (10.5%)	1 (6.7%)	2 (10.0%)	1 (7.1%)	0 (0.0%)	3 (17.6%)	2 (10.5%)	1 (6.7%)	0 (0.0%)	1 (11.1%)	2 (14.3%)	0 (0.0%)
8q	1 (2.9%)	1 (5.3%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
9p	1 (2.9%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	1 (7.1%)	1 (5.9%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
9q	3 (8.8%)	1 (5.3%)	2 (13.3%)	1 (5.0%)	2 (14.3%)	2 (11.8%)	0 (0.0%)	2 (10.5%)	1 (6.7%)	2 (22.2%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
10p	1 (2.9%)	1 (5.3%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
11p	3 (8.8%)	2 (10.5%)	1 (6.7%)	2 (10.0%)	1 (7.1%)	1 (5.9%)	2 (11.8%)	1 (5.3%)	2 (13.3%)	0 (0.0%)	1 (11.1%)	2 (14.3%)	0 (0.0%)
11q	1 (2.9%)	1 (5.3%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
12q	2 (5.9%)	1 (5.3%)	1 (6.7%)	1 (5.0%)	1 (7.1%)	1 (5.9%)	1 (5.9%)	1 (5.3%)	1 (6.7%)	0 (0.0%)	1 (11.1%)	1 (7.1%)	0 (0.0%)
13q	1 (2.9%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	1 (5.9%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)
14q	2 (5.9%)	1 (5.3%)	1 (6.7%)	1 (5.0%)	1 (7.1%)	2 (11.8%)	0 (0.0%)	1 (5.3%)	1 (6.7%)	1 (11.1%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
15q	2 (5.9%)	2 (10.5%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	2 (11.8%)	0 (0.0%)	0 (0.0%)	2 (13.3%)	0 (0.0%)	0 (0.0%)	2 (14.3%)	0 (0.0%)
16p	3 (8.8%)	3 (15.8%)	0 (0.0%)	3 (15.0%)	0 (0.0%)	0 (0.0%)	3 (17.6%)	0 (0.0%)	3 (20.0%)	0 (0.0%)	0 (0.0%)	3 (21.4%)	0 (0.0%)
16q	4 (11.8%)	2 (10.5%)	2 (13.3%)	3 (15.0%)	1 (7.1%)	1 (5.9%)	3 (17.6%)	1 (5.3%)	3 (20.0%)	0 (0.0%)	2 (22.2%)	2 (14.3%)	0 (0.0%)
17p	2 (5.9%)	2 (10.5%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	1 (5.9%)	1 (5.9%)	0 (0.0%)	2 (13.3%)	0 (0.0%)	0 (0.0%)	2 (14.3%)	0 (0.0%)
17q	1 (2.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
18p	6 (17.6%)	5 (26.3%)	1 (6.7%)	5 (25.0%)	1 (7.1%)	4 (23.5%)	3 (17.6%)	3 (15.8%)	3 (20.0%)	1 (11.1%)	1 (11.1%)	4 (28.6%)	1 (50.0%)
18q	12 (34.3%)	8 (42.1%)	5 (33.3%)	6 (30.0%)	0 (0.0%) ****	6 (35.3%)	5 (29.4%)	8 (42.1%)	4 (26.7%)	3 (33.3%)	2 (22.2%)	5 (35.7%)	1 (50.0%)
19p	1 (2.9%)	1 (5.3%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
21q	3 (8.8%)	2 (10.5%)	1 (6.7%)	1 (5.0%)	2 (14.3%)	1 (5.9%)	2 (11.8%)	1 (5.3%)	2 (13.3%)	0 (0.0%)	2 (22.2%)	1 (7.1%)	0 (0.0%)
22q	4 (11.8%)	3 (15.8%)	1 (6.7%)	3 (15.0%)	1 (7.1%)	1 (5.9%)	3 (17.6%)	2 (10.5%)	2 (13.3%)	1 (11.1%)	0 (0.0%)	3 (21.4%)	0 (0.0%)

*$P=0.0282$, **$P=0.0113$, ***$P=0.0288$ (G-phenotype vs I-phenotype), ****$P=0.0311$

Table 5 Relations between minimal overlapping regions of recurrent DNA copy number changes and phenotypic marker expression of the tumour

Changes	HGM expression		
	Negative ($n=19$)	Positive ($n=15$)	P value
19q13.2 (gain)	11 (57.9%)	3 (20.0%)	0.0382
19q13.3 (gain)	12 (63.2%)	2 (13.3%)	0.0051
Changes	MUC6 expression		
	Negative ($n=20$)	Positive ($n=14$)	P value
20q13.2 (gain)	6 (30.0%)	10 (71.4%)	0.0349
Changes	MUC2 expression		
	Negative ($n=17$)	Positive ($n=17$)	P value
19p13.3 (gain)	1 (5.9%)	7 (41.2%)	0.0391

MUC6-positive, MUC2-negative, MUC2-positive, CD10-negative and CD10-positive tumours were observed in 47.4, 26.7, 45.0, 28.6, 35.3, 41.2, 26.3 and 53.3%, respectively. There was no significant association between the abnormal expression of β-catenin and expressions of HGM, MUC6, MUC2 or CD10. Abnormal expressions of E-cadherin in G-, GI-, I-, and UC-phenotype tumours were observed in 33.3, 77.8, 21.4 and 50.0%, respectively. GI-phenotype tumours were significantly associated with a higher incidence of tumours with abnormal expression of E-cadherin, compared with I-phenotype tumours ($P=0.0131$). Abnormal expressions of β-catenin in G-, GI-, I-, and UC-phenotype tumours were observed in 22.2, 44.4, 42.9 and 50.0%, respectively. There was no significant association between the abnormal expression of β-catenin and the phenotypic marker expression pattern of the tumour.

3.3 Chromosomal changes in 34 gastric carcinomas

The summary of CGH results in 34 gastric cancers is shown in Table 4. The most frequently gained chromosomal regions in 34 gastric cancers (> 40% of all tumours) were 6p (52.9%), 7p (55.9%), 7q (55.9%), 8q (61.8%), 12q (44.1%), 13q (47.1%), 16p (44.1%), 17q (50.0%), 19q (64.7%), 20p (44.1%) and 20q (70.6%); The most frequently lost (> 20% of all tumours) chromosomal regions were 4q (23.5%) and 18q (34.3%). With respect to the relations between chromosomal changes and the phenotypic marker expression of the tumour, significant differences were found in gains of 5q (CD10-negative vs CD10-positive, $P=0.0282$), 18q (CD10-negative vs CD10-positive, $P=0.0113$) and loss of 18q (MUC6-negative vs MUC6-positive, $P=0.0311$). With respect to the relations between chromosomal changes and the phenotypic marker expression pattern of the tumour, I-phenotype tumours were significantly associated with the gain of 20p, compared with G-phenotype tumours ($P=0.0288$).

3.4 Relations between minimal overlapping regions of recurrent DNA copy number changes and the phenotypic marker expression of the tumour

The relations between the minimal overlapping regions of recurrent DNA copy number changes and the expression of HGM, MUC6, MUC2 and CD10 are shown in Table 5. HGM-negative tumours were significantly associated with higher frequencies of the gains of 19q13.2 and 19q13.3, compared with HGM-positive tumours ($P=0.0382$ and $P=0.0051$). MUC6-positive tumours were significantly associated with a higher frequency of the gain of 20q13.2, compared with MUC6-negative tumours ($P=0.0349$). MUC2-positive tumours were significantly associated with a higher frequency of the gain of 19p13.3, compared with MUC2-negative tumours ($P=0.0391$). There was no significant difference between the expression of CD10 and DNA

Table 6 Relations between minimal overlapping regions of recurrent DNA copy number changes and phenotypic marker expression pattern of the tumour

Changes	Phenotypic marker expression pattern of the tumour			
	G-phenotype ($n=9$)	GI-phenotype ($n=9$)	I-phenotype ($n=14$)	UC-phenotype ($n=2$)
5p15.2 (gain)*	0 (0.0%)	1 (7.1%)	6 (66.7%)	0 (0.0%)
7p21 (gain)**	3 (33.3%)	0 (0.0%)	6 (66.7%)	1 (50.0%)
13q33–34 (gain)***	0 (0.0%)	2 (14.3%)	6 (66.7%)	0 (0.0%)

*$P=0.0481$ (G-phenotype vs I-phenotype), **$P=0.0481$ (GI-phenotype vs I-phenotype),
***$P=0.0481$ (G-phenotype vs I-phenotype)

copy number changes. The relations between minimal overlapping regions of recurrent DNA copy number changes and the phenotypic marker expression pattern of the tumour are shown in Table 6. I-phenotype tumours were significantly associated with higher frequencies of gains of 5p15.2 and 13q33-34, compared with G-phenotype tumours ($P = 0.0481$, each) and also associated with higher frequencies of gain of 7p21, compared with GI-phenotype tumours ($P = 0.0481$) (Fig. 3).

4 Discussion

E-cadherin is the major cadherin molecule expressed by epithelial cells (Takeichi 1991). Cadherin forms complexes with cytoplasmic proteins, α-catenin, β-catenin and plakoglobin. Structural abnormalities of E-cadherin and catenin caused by the loss of gene function have been shown to disrupt E-cadherin-mediated intercellular adhesion, causing loose cell-to-cell adhesion in tumour cells (Oda et al. 1994; Oyama et al. 1994). In fact, previous reports showed that the loss or reduced expression of E-cadherin and β-catenin were frequently found in undifferentiated-type gastric carcinomas (Ochiai et al. 1994). However, the abnormal expression of E-cadherin has also been seen in differentiated-type gastric carcinomas. Koseki et al. (2000) detected the abnormal expression of E-cadherin in 26.7% of differentiated-type gastric carcinomas in the early stage. They found abnormal E-cadherin expression more frequently in G-phenotype tumours than in I-phenotype tumours. Furthermore, Endoh et al. (1999b) detected an E-cadherin gene mutation in 21% of early differentiated-type carcinomas of the G-phenotype. It has been suggested that differentiated-type G-phenotype carcinomas are more likely to transform into the undifferentiated-type carcinoma in the relatively early stage of gastric carcinogenesis with progression through loss of E-cadherin function as compared with those of the I-phenotype (Endoh et al. 1999b). In the present study of advanced stage carcinomas, G-phenotype tumours were significantly associated with a higher incidence of mixed-type tumours, as compared with GI- and I-phenotype tumours. HGM-expression was significantly associated with abnormal expression of E-cadherin. GI-phenotype tumours were also significantly associated with a higher incidence of tumours with abnormal expression of E-cadherin, as compared with I-phenotype tumours. Mixed-type tumours have been considered to represent a progressive loss of glandular structure and a histological transformation from differentiated- to undifferentiated-type carcinoma during the progression of the tumour. Therefore, our present results support the previous findings that the phenotypic marker expression of the tumour is associated with histological transformation from differentiated- to undifferentiated-type carcinoma and abnormal expression of E-cadherin in gastric differentiated-type carcinomas.

In the present study, we confirmed previous reports of chromosomal gains of 7q, 8q, 17q, 20p and 20q, and losses of 4q and 18q as frequent events in gastric carcinomas (Kokkola et al. 1997; El-Rifai et al. 1998; Sakakura et al. 1999; van Dekken et al. 1999; Nessling et al. 1998; Vidgren et al. 1999; Knuutila et al. 2000; Noguchi et al. 2001; Wu et al. 2002; Peng et al. 2003). Koizumi et al. (1997) reported that gains of 3q27, 6q23-q25, and 7cen-p14 and losses of 1p34.2-35 and 17p12 were predominantly observed in the differentiated-type, and gains of 2p23-pter, 9p22-pter, and 13q31-qter and a loss of 6p21.3 were predominantly observed in the undifferentiated-type. They suggested that these changes, predominantly observed for their histological type, might be associated with the cell growth and differentiation of gastric carcinomas (Koizumi et al. 1997). Kokkola et al. (1997) reported that the most common gains in the differentiated-type gastric carcinomas were found at 20pq, 8q and 17q, with the minimal

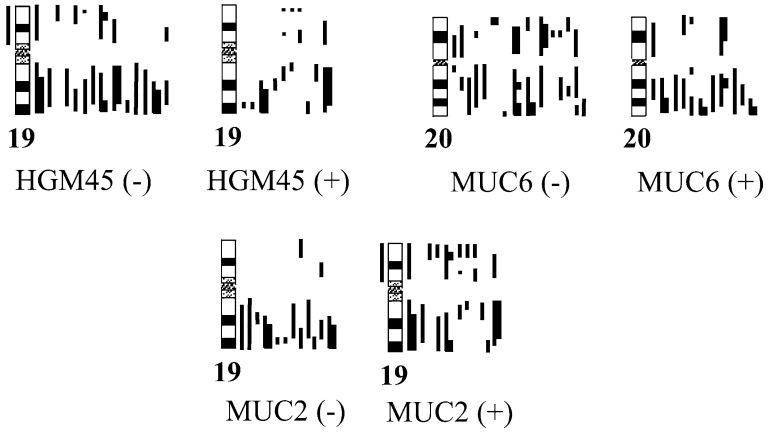

Fig. 3 DNA copy number alterations in chromosomes 19 and 20 analysed by CGH in 34 gastric carcinomas. The regions of DNA copy number gains and losses are shown as the bars on the right and left sides, respectively. The *thick bars* indicate amplifications. HGM-negative tumours are significantly associated with higher frequencies of the gains of 19q13.2 and 19q13.3, compared with HGM-positive tumours. MUC6-positive tumours are significantly associated with a higher frequency of the gain of 20q13.2, compared with MUC6-negative tumours. MUC2-positive tumours are significantly associated with a higher frequency of the gain of 19p13.3, compared with MUC2-negative tumours

common regions at 20q, 8q21.3-23 and 17q12-21, and the most common losses involved 18q and 4q. They also showed that the most frequent gains were at 20q and 6p in the undifferentiated-type of gastric carcinomas. Furthermore, high-level amplifications were detected at 2p, 17q, 18q and 20q in the differentiated type and on 13q in the undifferentiated type. However, the relation between chromosomal changes and phenotypic marker expression of the tumour in gastric differentiated-type carcinomas remains unclear. In the present study, HGM-negative tumours were significantly associated with higher frequencies of gains of 19q13.2 and 19q13.3, compared with HGM-positive tumours. MUC6-positive tumours were significantly associated with a higher frequency of gain of 20q13.2, compared with MUC6-negative tumours. MUC2-positive tumours were significantly associated with a higher frequency of gain of 19p13.3, compared with MUC2-negative tumours. Furthermore, I-phenotype tumours were significantly associated with higher frequencies of gains of 5p15.2, 7p21 and 13q33-34, compared with G- and GI-phenotype tumours. Therefore, the findings in our present study suggest that the phenotypic marker expression of the tumour is related to chromosomal changes detected by CGH in gastric differentiated-type carcinomas.

Obvious differences in the biological behaviour of tumours with different phenotypic marker expressions have been reported. Kabashima et al. (2002) reported that G-phenotype tumours could potentially degrade the extracellular matrix through the overexpression of matrix metalloproteinases, compared with I-phenotype tumours. Shibata et al. (2003) reported that the apoptotic index/proliferative index ratio was significantly lower in G-phenotype tumours than in I-phenotype tumours. Tajima et al. (2001) previously reported that patients with G-phenotype tumours have a poorer prognosis than those with I-phenotype tumours among patients with advanced gastric carcinoma. We recently reported that the majority of peritoneal recurrences after surgery for gastric carcinoma occurred in G-phenotype tumours, especially HGM-positive tumours, while haematogenous recurrence occurred more frequently in MUC2-negative tumours and CD10-positive tumours (Tajima et al. 2004). These differences in the biological behaviour of tumours with different phenotypic marker expressions suggest the action of different genetic alterations, depending on the phenotypic marker expression of the tumour. Several studies have shown significant correlation between microsatellite instability and gastric foveolar phenotypic expression in the stomach cancer (Endoh et al. 2000a, 2000b; Takahashi et al. 2002). Tamura et al. (1995) have shown that the majority of differentiated adenocarcinomas of the stomach may develop through a de novo pathway from the viewpoint of the microsatellite alterations. In the present study, we demonstrated that gastric differentiated-type carcinomas had different characteristics according to the phenotypic marker expression in terms of not only histological findings and E-cadherin expression but also in the pattern of chromosomal changes detected by CGH. Therefore, there could be a possibility of unknown gene groups being present in the regions of these genes associated with the phenotypic marker expression of the tumour. Previous molecular genetic studies have shown

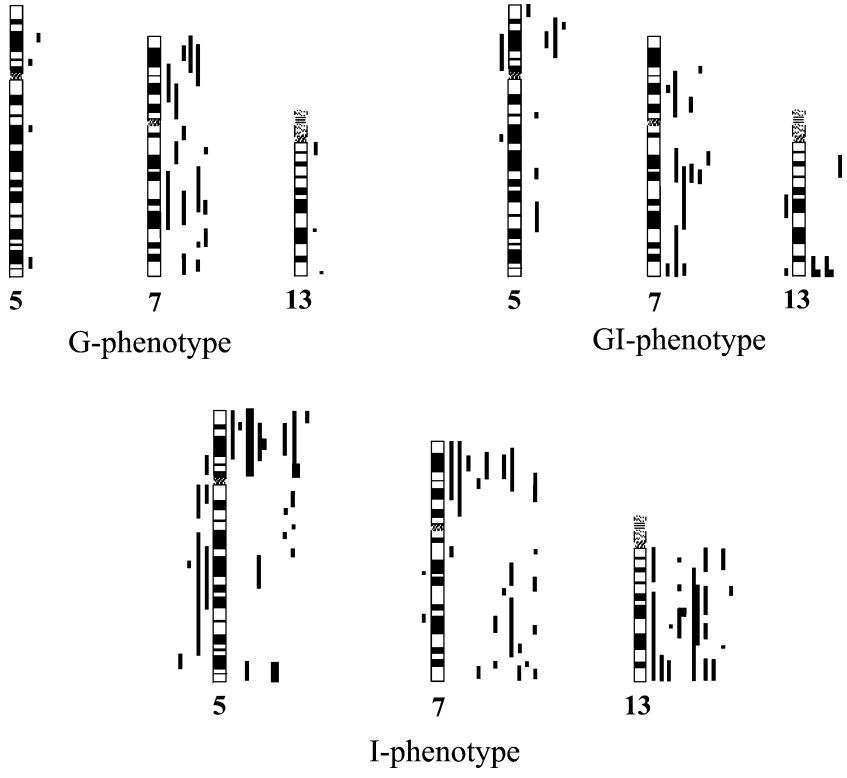

Fig. 4 DNA copy number alterations in chromosomes 5, 7 and 13 analysed by CGH in 34 gastric carcinomas. The regions of DNA copy number gains and losses are shown as the *bars* on the *right and left sides*, respectively. The *thick bars* indicate amplifications. I-phenotype tumours are significantly associated with higher frequencies of gains of 5p15.2 and 13q33-34, compared with G-phenotype tumours and also associated with higher frequencies of gain of 7p21, compared with GI-phenotype tumours

that gastric tumourigenesis is a multistep process with an accumulation of genetic alterations (Stadtlander and Waterbor 1999; Tahara et al. 1996). Therefore, the findings in our present study suggest that different genetic pathways, according to the phenotypic marker expression of the tumour, could exist in the tumourigenesis of the gastric differentiated-type carcinoma, leading to their different biological behaviour (Fig. 4).

In conclusion, our present results show that gastric differentiated-type carcinomas have different characteristics according to the phenotypic marker expression of the tumour in terms of histological findings, E-cadherin expression and pattern of chromosomal changes.

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