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Original Paper

Barrett's adenocarcinomas resemble adenocarcinomas of the gastric cardia in terms of chromosomal copy number changes, but relate to squamous cell carcinomas of the distal oesophagus with respect to the presence of high-level amplifications

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Abstract

Three different cancers predominantly occur at the gastro-oesophageal junction: squamous cell carcinomas of the distal oesophagus, adenocarcinomas of the distal oesophagus (Barrett's carcinomas), and adenocarcinomas of the gastric cardia. The aim of the present study was to investigate how, and to what extent, Barrett's carcinoma differs from adenocarcinoma of the gastric cardia on the one hand and squamous cell carcinoma of the distal oesophagus on the other, with respect to chromosomal aberrations and related gene expression. The present study analysed 14 squamous cell carcinomas, 24 Barrett's carcinomas, and 16 carcinomas of the gastric cardia. Comparative genomic hybridization revealed chromosomal abnormalities in all cases. Typical chromosomal aberrations for the squamous cell carcinoma type were gains at 3q and 11q13, and losses at 3p, 4q, 9p, 11q, and 13q. In contrast, typical copy number changes for both cardiac and Barrett's adenocarcinomas were gains at 2q, 7p, and 13q, and losses at 17p. High-level amplification occurred in all three groups, but its frequency in the cardiac carcinomas was lower than in the other two groups. In conclusion, squamous cell carcinomas are characterized by chromosomal aberrations which are distinct from those seen in carcinomas of the gastric cardia and in Barrett's adenocarcinomas. With respect to Barrett's cancer, the chromosomal aberrations more closely reflect the adenocarcinoma phenotype than the squamous origin of the epithelium.

Keywords: comparative genomic hybridization; chromosomal aberrations; gastric cancer; Barrett's carcinoma; squamous cell carcinoma; adenocarcinoma; *HER2/neu*; *cyclin D1*; immunohistochemistry

Introduction

Adenocarcinoma of the stomach has been the leading cause of cancer death worldwide through most of the 20th century. It now ranks second only to lung cancer, with an estimated 755 500 new cases diagnosed annually around the world. The incidence of this disease has gradually decreased in many parts of the world, principally because of changes in *H. pylori* prevalence, diet, food preparation, and other environmental factors. In contrast, the number of patients with Barrett's cancer (BC) has increased markedly since the mid-1980s [1]. A third type of carcinoma occurring in this area is the squamous cell carcinoma of the distal oesophagus (eSCC). Although these three types of malignancies occur at the same anatomical site, they differ greatly with respect to their epidemiology and risk factors. The incidence of oesophageal adenocarcinoma has recently risen dramatically in Western populations, whereas the incidence of squamous cell carcinomas has remained relatively steady [2]. Reflux disease has been suggested as an aetiological factor not only in oesophageal adenocarcinoma, but also in cancer of the gastric cardia (CC) [3]. Other risk factors include smoking [4], increased fat intake [5], and obesity [6], whereas colonization with *H. pylori* has been suggested to protect against gastro-oesophageal reflux and Barrett's oesophagus [7]. For squamous cell carcinoma of the distal oesophagus, smoking and alcohol consumption are aetiological factors. The histology of adenocarcinomas of the gastric cardia and squamous cell carcinomas of the distal oesophagus matches with the original epithelium of the stomach

and oesophagus, respectively, which are distinctly different. For Barrett's carcinomas, another situation exists. These tumours show histology that differs from that of the original epithelium, since it is preceded by a process of metaplasia, in which the squamous epithelium is replaced with metaplastic columnar epithelium.

The aim of the present study was to investigate how, and to what extent, Barrett's cancer compares with adenocarcinoma of the gastric cardia on the one hand and squamous cell carcinoma of the distal oesophagus on the other, in terms of chromosomal aberrations and related gene expression. To this end, we analysed 16 adenocarcinomas of the gastric cardia, 24 Barrett's carcinomas, and 14 squamous cell carcinomas of the distal oesophagus by comparative genomic hybridization (CGH). This technique allows the detection of chromosomal gains and losses throughout the whole genome in a single experiment, using DNA isolated from either frozen or formaldehyde-fixed, paraffin-embedded tissue [8]. In order to analyse the effect of amplifications at chromosomes 11q13 and 17q12–21 on the expression of genes in these regions, immunohistochemistry on tissue sections was performed with antibodies against *cyclin D1* and *Her-2/Neu*, respectively.

Material and methods

Material

A total of 54 patients with CC ($n = 16$), BC ($n = 24$) or eSCC ($n = 14$), diagnosed by endoscopy with biopsy sampling and subsequently treated by partial or total gastrectomy or oesophagectomy, respectively, were included in the study. The Barrett's cancers were obtained from the Departments of Pathology of the VU University Medical Centre and the Academic Medical Centre, both in Amsterdam. All cases were selected according to strict criteria, based on macroscopic [by means of photographic images (Figure 1)] and microscopic re-evaluation. The tumours from the Academic Medical Centre were all macroscopically adenocarcinomas of the distal oesophagus, in which surrounding Barrett's mucosa could be identified on light microscopy, by the presence of specialized columnar epithelium containing goblet cells (intestinal metaplasia). In the samples from the Department of Pathology at the VU University Medical Centre, a background of intestinal metaplasia could not be demonstrated in every case on review of the slides. However, the study only included cases in which careful re-examination of the high-quality macroscopic photographs, taken of each resection specimen as a standard procedure, allowed an unambiguous classification. In this way, only cases with at least 75% of the tumour situated in either the cardia or the oesophagus were included. Clinical and patient characteristics are summarized in Table 1.

Both frozen and formaldehyde-fixed, paraffin-embedded material was used in this study. Tissue blocks with the highest concentration of tumour cells were selected for DNA isolation. For each case, a tumour area of approximately 5×5 mm containing more than 75% tumour cells was outlined on a haematoxylin and eosin (H&E) slide, avoiding areas with a high concentration of inflammatory or other non-tumour cells. In 10–15 consecutive haematoxylin-stained tissue sections of 10 μm thickness, the corresponding area was scraped off with a surgical

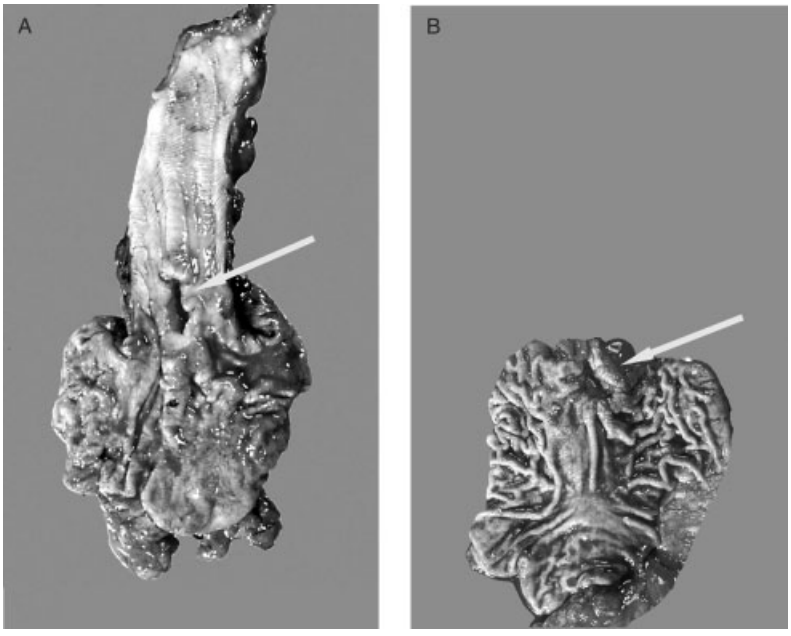

Figure 1. (A) Macroscopic image of a partial oesophagogastrectomy specimen showing a tumour in the distal part of the oesophagus above the Z-line, classified as a Barrett's adenocarcinoma. (B) Macroscopic image of a partial gastrectomy specimen (without oesophagus) showing a tumour in the upper part of the stomach, classified as an adenocarcinoma of the gastric cardia

Table 1. Clinical and pathological patient characteristics

Type	n	Sex: Male (*n*)	Sex: Female (*n*)	Mean age (range), years	Stage*: T1–T2	Stage*: T3–T4	Histological differentiation: Well	Histological differentiation: Moderate	Histological differentiation: Poor	Laurèn type†
SCC	14	6	8	64 (40–75)	*n* = 6	*n* = 7	*n* = 0	*n* = 3	*n* = 11	—
BC	24	20	4	62 (28–73)	*n* = 12	*n* = 14	*n* = 4	*n* = 8	*n* = 12	—
CC	16	13	3	63 (34–85)	*n* = 4	*n* = 12	*n* = 0	*n* = 7	*n* = 9	15 intestinal 1 diffuse

* For one SCC case, the stage was not possible to judge, since this was a mucosectomy sample.
† For cancers of gastric cardia only.

blade. As a control, two H&E slides were taken following the 'sandwich' method. DNA isolation with the Qiamp Tissue Kit (Qiagen GmbH, Hilden, Germany) was performed as described previously [8].

Methods

Comparative genomic hybridisation (CGH)

CGH was performed as described before [8]. Eight to 12 metaphases were recorded and analysed. This yielded relative copy number karyotypes with mean fluorescence ratios and their 95% confidence intervals (CIs) plotted along the ideograms of the corresponding chromosomes. Deviations from normal were interpreted as gains or losses when the mean fluorescence ratios were above 1.2 or below 0.8, respectively, provided that the 95% confidence interval around the mean fluorescence ratio did not include 1.0. An event was defined as gain or loss of (part of) a chromosomal arm. Two or more distinct sub-regional copy number changes affecting the chromosomal arm were counted as separate events. Chromosomal aberrations with frequencies of 35% or higher were considered non-random changes associated with oesophageal or gastric cancer (Table 2).

Immunohistochemical staining

In order to analyse the effect of amplifications at chromosomes 11q13 and 17q12–21 on the expression of genes in these regions, immunohistochemistry on tissue sections was performed with antibodies against *cyclin D1* and *Her-2/Neu*, respectively. One CC could not be analysed immunohistochemically because of lack of material. Immunohistochemistry for *cyclin D1* was performed as described before, with minor modifications [9]. In short, 4 μm sections were cut from paraffin-embedded tissue and mounted on Superfrost® Plus slides (Menzel-Gläser, Germany). The sections were deparaffinized, blocked for endogenous peroxidase activity, and antigen retrieval was performed by heating the slides in an autoclave in citrate buffer. The primary antibody against *cyclin D1* (DCS-6, Neomarkers, Labvision, Fremont, CA, USA) was used in a 1 : 400 dilution. Slides were incubated

Table 2. Statistical evaluation of the differences of the most frequent aberrations between the squamous cell carcinomas, Barrett's adenocarcinomas, and cardiac adenocarcinomas

CGH event	eSCC (*n* = 14)	BC (*n* = 24)	CC (*n* = 16)	*p* value: eSCC vs CC	*p* value: eSCC vs BC	*p* value: SCC vs adeno
1q+	43%	33%	63%	NS	NS	NS
2q+	7%	42%	38%	0.05	0.02	0.02
3p−	64%	4%	19%	0.01	<0.001	<0.001
3q+	79%	38%	50%	NS	0.01	0.02
4q−	43%	8%	19%	NS	0.01	0.02
6q+	36%	33%	44%	NS	NS	NS
7p+	14%	46%	50%	0.04	0.05	0.03
7q+	36%	54%	50%	NS	NS	NS
8q+	50%	54%	56%	NS	NS	NS
9p−	50%	17%	19%	NS	0.03	0.02
11q−	50%	13%	31%	NS	0.01	0.03
11q13+	57%	17%	31%	NS	0.01	0.02
13q−	50%	4%	0%	0.001	0.001	<0.001
13q+	14%	42%	44%	NS	NS	0.06
17p−	14%	58%	38%	NS	0.008	0.02
18q−	36%	46%	25%	NS	NS	NS
20q+	21%	38%	44%	NS	NS	NS

p values (two-sided Pearson chi-square test) are given pairwise. The *p* values in the last column give the differences between the squamous cell carcinomas versus the adenocarcinomas (BC and CC).

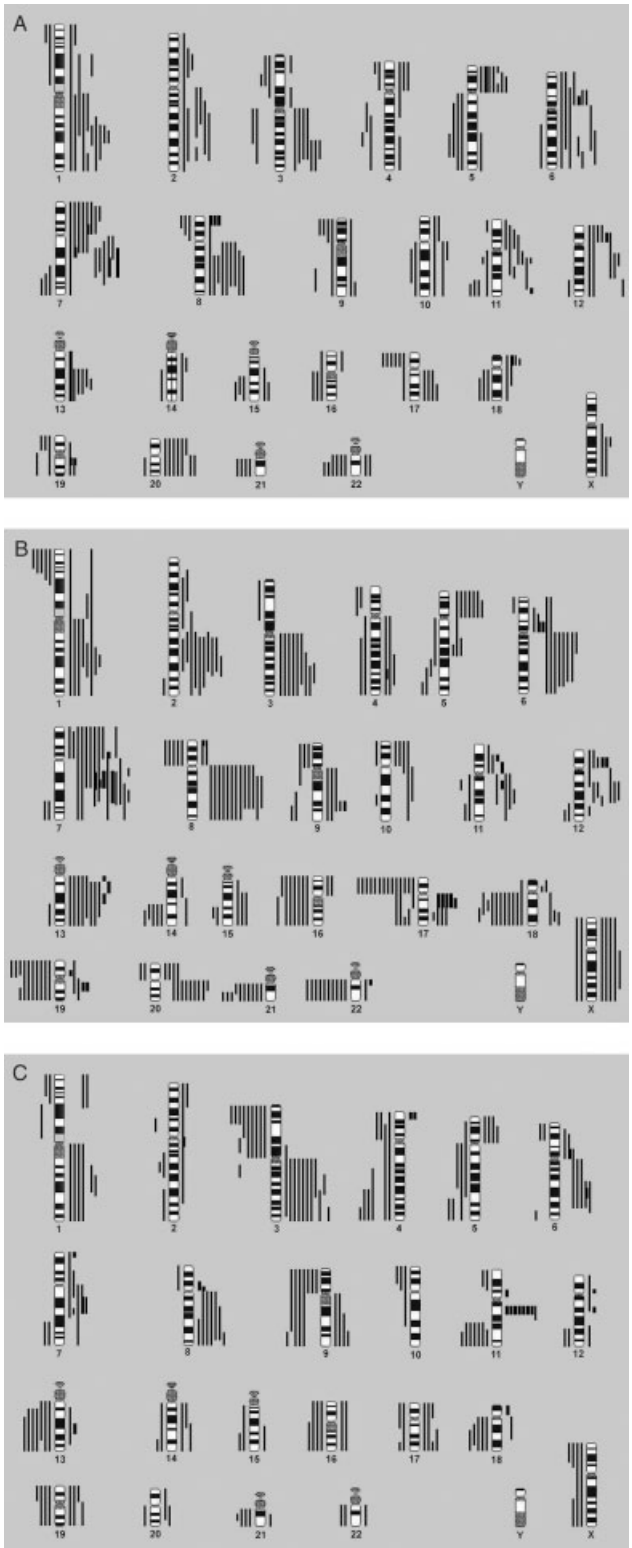

Figure 2. Overview of the genetic changes detected by CGH in 16 adenocarcinomas of the gastric cardia (A), 24 Barrett's carcinomas (B), and 14 squamous cell carcinomas of the distal oesophagus (C). Bars on the left of each chromosomal ideogram represent a loss, and on the right a gain of the corresponding part of the chromosome in a particular tumour. Thick lines represent amplifications

overnight with the primary antibodies at 4 °C. Subsequently, slides were incubated with biotinylated rabbit anti-mouse antibody (1 : 200), followed by incubation with 1 : 100 streptavidin-biotinylated horseradish-peroxidase complex. 3,3′-Diaminobenzidine was used as the chromogen and haematoxylin as the counterstain.

Immunohistochemistry (IHC) for *Her-2/Neu* was performed in the Ventana ES, using a monoclonal antibody [anti-C-erbB-2 (*Her-2/Neu*), Ventana Medical

Systems, Inc, Tucson, AZ, USA] to *Her-2/Neu*, an oncoprotein that is localized on the cell membrane, and occasionally in the cytoplasm of some neoplastic cells. The Ventana ES Immunohistochemistry Staining System is a microprocessor-controlled system for performing routine IHC. Paraffin-embedded tissue sections of 4 μm were cut and deparaffinized. Antigen retrieval was done by heating the slides in an autoclave in citrate buffer. The incubation time for the primary antibody was 32 min. A known positive ductal breast carcinoma was used as a positive control, while a negative control was obtained by omitting the primary antibodies from the staining procedure.

The intensity of nuclear and/or membranous staining, for *cyclin D1* or *Her-2/Neu*, respectively, was scored as negative or positive on microscopic examination by two observers.

Statistical analysis

The differences in the incidence of chromosomal aberrations per diagnostic category were pairwise tested for significance using the two-sided Pearson chi-square test. p values less than 0.05 were considered significant. Significance of differences in the means of the total number of CGH events, gains, losses, and high-level gains per tumour in three diagnostic categories was tested pairwise by Student's t-test.

Results

Comparative genomic hybridization revealed chromosomal abnormalities in all cases. The mean number and standard deviation of events in eSSC, BC, and CC was 14 (8.3), 12 (5.8), and 14 (8.0), respectively (NS). In addition, the numbers of gains, losses, and high-level amplifications were similar between the three groups. An overview of the CGH results in all three categories ($n = 54$) is shown in Figures 2A, 2B, and 2C. The most frequent aberrations in eSCC are, in descending order, gains at 3q (79%), 11q13 (57%), 8q (50%), 1q (43%), 6q (36%), and 7q (36%), and losses at 3p (64%), 9p (50%), 11q (50%), 13q (50%), 4q (43%), and 18q (36%). In BC, frequent gains were found at 7q (54%), 8q (54%), 7p (46%), 2q (42%), 13q (42%), 3q (38%), and 20q (38%), and losses at 17p (58%), and 18q (46%). In CC, the most frequent gains were at 1q (63%), 8q (56%), 3q (50%), 7p (50%), 7q (50%), 6q (44%), 13q (44%), 20q (44%), and 2q (38%), and loss at 17p (38%) (Table 2).

The main differences in the patterns of chromosomal aberrations were found between eSCC on the one hand and the adenocarcinomas (BC and CC) on the other. Typical chromosomal aberrations for the squamous cell carcinoma type were gains at 3q and 11q13, and losses at 3p, 4q, 9p, 11q, and 13q. In contrast, typical copy number changes for (BC and CC) adenocarcinomas were gains at 2q, 7p, and 13q, and losses at 17p (Table 2). One chromosomal aberration did show a significant difference between BC and CC (gain of 4p), but this gain occurred at a rather low frequency (three times in CC; none in BC).

High-level amplification occurred in all three groups, but its frequency in CC was lower than in the other two groups. From a biological perspective there was a high incidence of recurrent amplifications, although the absolute numbers were too low for reliable statistical analysis. Nevertheless, a certain specificity for diagnostic categories could be noted here (Table 3); for example, in the eSCC group, amplification of 11q13 occurred in 50% of the cases, compared with only a single case in BC and none in CC. In addition, amplification of 7q21–22 and 17q12–21 occurred preferentially in BC (25% and 21%, respectively), versus 0% for these regions in CC and 7% and 0%, respectively, in eSCC.

Overexpression of *cyclin D1* is shown in 11/14 eSCCs (79%), 7/24 BCs (29%), and 6/15 CCs (40%) by immunohistochemistry (Table 4). All but one of the cases with amplification of 11q13 showed overexpression at the protein level, while in one eSCC case we detected amplification by CGH without protein overexpression. *Her-2/Neu* overexpression was seen in only 1/11 (7%) eSCCs, 10/24 (42%) BCs, and 3/15 (20%) CCs. Of BC with protein overexpression, four cases were seen with and six without amplification. One BC with 17q12–21 amplification did not show protein overexpression. Finally, in all three CCs showing *Her-2/Neu* protein overexpression, we could not detect amplification of the chromosomal locus.

Discussion

Patients with cancer of the cardia more closely resemble those with Barrett's carcinoma than those with cancers of the distal stomach, in terms of sex and racial characteristics, frequency of reflux symptoms, and frequency of associated atrophic gastritis. Disease behaviour and survival in Barrett's and gastric cardiac cancer also appear very similar [10]. However, Barrett's cancer differs from cancers of the gastric cardia with respect to certain epidemiological parameters that could reflect independent carcinogenetic mechanisms. There is a higher proportion of males among the patients with carcinoma of the cardia. In addition, patients with cardiac cancer more frequently have a history of heavy smoking and alcohol intake, whereas those with Barrett's carcinoma are much more likely to have hiatus hernias [11]. Morphologically, Barrett's carcinoma and cardiac cancer are virtually identical, and clearly different from squamous cell carcinoma of the oesophagus, although Barrett's carcinoma arises in squamous epithelium through a phase of metaplasia.

In clinical practice, adenocarcinomas of the gastric cardia and those arising in the distal oesophagus, the Barrett's carcinomas, were in the past often classified under the single heading of adenocarcinoma

Table 3. High-level gains and candidate genes located at these loci

High-level gain	eSCC ($n = 14$)	BC ($n = 24$)	CC ($n = 16$)	Candidate genes
2q11.2–12	1 (7%)	—	—	
4p16	2 (14%)	—	—	Cyclin G-associated kinase (GAK) Fibroblast growth factor receptor 3 (FGFR3) Zinc finger protein 141 (ZNF141) HGF activator (HGFAC)
4q27	—	1 (4%)	—	Cyclin A
6p12–21.2	1 (7%)	2 (8%)	1 (6%)	Vascular endothelial growth factor A (VEGFA) Protein-tyrosine kinase-7 (PTK7)
6q22	1 (7%)	—	—	MYB oncogene, ROS oncogene
7p11.2–13	—	1 (4%)	1 (6%)	Epidermal growth factor receptor (EGFR)
7p22	1 (7%)	—	—	
7q21–22	1 (7%)	6 (25%)	—	Hepatocyte growth factor (HGF) Nerve growth factor-inducible (VGF) MUC 3A, 11, and 12
7q22–31	—	1 (4%)	3 (19%)	C-Met (HGF-receptor)
8p11.2–12	2 (14%)	—	—	Fibroblast growth factor receptor 1 (FGFR1)
8p22–23	—	1 (4%)	3 (19%)	Platelet-derived growth factor receptor-like Cathepsin B
9q31–33	—	1 (4%)	—	
11p14	—	1 (4%)	—	
11p11–13	1 (7%)	1 (4%)	—	Neurite growth-promoting factor-2
11q13	7 (50%)	1 (4%)	—	Fibroblast growth factor 3 (FGF3) Fibroblast growth factor 4 (FGF4) Cyclin D1 EMS1 oncogene PAK1
11q23–24	—	—	1 (6%)	
12p11.2–12	1 (7%)	2 (8)%	—	Retinoblastoma-binding protein-2
12q14	1 (7%)	—	—	MDM2
13q12–13	—	1 (4%)	—	Fibroblast growth factor 9 (FGF9) BRCA2
13q13–14	—	1 (4%)	—	Rb
13q21–22	1 (7%)	1 (4%)	—	
17q12–21	—	5 (21%)	—	HER-2-Neu (ERBB2) Nerve growth factor receptor (NGFR) Fibroblast growth factor 11 (FGF11)
18p	1 (7%)	—	1 (6%)	Yes1
19p13.1	—	1 (4%)	—	
19q13.1	—	2 (8%)	2 (13%)	Transforming growth factor beta 1 (TGFB1) Cyclin E
22q11.2	—	1 (4%)	—	

of the gastro-oesophageal junction. However, international classifications do discriminate between cardiac and oesophageal adenocarcinomas. This discrimination appears to be of relevance since these tumours differ in time with respect to changes in incidence and may also differ with respect to their underlying aetiology. In clinical practice, differentiation can in most cases be made on parameters such as the location of

Table 4. Correlation between protein expression of *cyclin D1* and *Her-2/Neu* and amplification of their chromosomal loci

		eSCC ($n = 14$)			Barrett's ($n = 24$)			Cardia ($n = 15$)*			Totals		
		11q13 amplification			11q13 amplification			11q13 amplification			11q13 amplification		
		Yes	No	Totals	Yes	No	Totals	Yes	No	Totals	Yes	No	Totals
Cyclin D1 overexpression	Yes	6	5	11	1	6	7	—	6	6	7	17	24
	No	1	2	3	—	17	17	—	9	9	1	28	29
	Totals	7	7	14	1	23	24	0	15	15	8	45	53
		17q12–21 amplification			17q12–21 amplification			17q12–21 amplification			17q12–21 amplification		
		Yes	No	Totals	Yes	No	Totals	Yes	No	Totals	Yes	No	Totals
Her-2/Neu overexpression	Yes	—	1	1	4	6	10	—	3	3	4	10	14
	No	—	13	13	1	13	14	—	12	12	1	38	39
	Totals	0	14	14	5	19	24	0	15	15	5	48	53

* One CC case was not investigated by immunohistochemistry since tissue was no longer available.

the bulk of the tumour and the presence or absence of surrounding Barrett's mucosa. Discrimination between these two tumour types is a key issue in the present study. We therefore carefully selected Barrett's cancers according to strict criteria, in order to arrive at an unambiguous classification. Only in this way can a sound comparison be made of the chromosomal aberrations in these two types of cancer.

In the past, CGH studies have yielded indications for different patterns of chromosomal aberration between squamous cell carcinoma and adenocarcinoma, as was the case in the present study. Squamous cell carcinomas frequently show gains at 3q, 5p, 8q, 11q13, and17q; losses at 3p, 4p, 5q, 11qter, 13q, and 18q; and high-level gains at 11q13, and 3q24-qter, while adenocarcinomas frequently show gains at 1q, 8q, 13q, and 20, and losses at 1p, 8p, 9p, and 17p [12–24]. This could indicate that different cell biological pathways need to be (de-) activated, or that the same pathway can be deregulated via different genetic changes, for tumour progression in squamous cell epithelium and columnar epithelium. An intriguing question is how tumours would behave that arise through a phase of metaplasia. The present study demonstrates that at the chromosomal level, adenocarcinomas of the gastric cardia and Barrett's carcinomas show close similarities. When comparing the Barrett's cancers to the squamous cell carcinomas of the distal oesophagus, there is little to remind us of a common origin, the epithelial stem cell.

This study is the first to compare squamous cell carcinomas, Barrett's carcinomas, and cardiac carcinomas in a *single series* of experiments. This is important because although CGH in itself is a straightforward technique, comparison of data from different studies may be influenced by different experimental conditions as well as by different strategies for interpreting the results. A sound comparison of chromosomal aberrations in different sets of tumours can therefore only come from a study that has been designed to eliminate these sources of bias.

We found significant differences in chromosomal aberrations between the squamous cell carcinomas and the adenocarcinomas (both Barrett's and cardiac). Gains at 11q13 and 3q, and losses at 3p, 4q, 9p, 11q, and 13q were found more often in the squamous cell carcinomas. Gains at 2q, 7p, and 13q, and loss at 17p seemed to occur predominantly in the adenocarcinomas. Except for 4p gain, we did not detect any relevant differences in gains and losses between the Barrett's carcinomas and the cardiac cancers. Loss of 14q31–32 has been suggested to have some discriminative power between cardiac and Barrett's cancer [25,26], but the data of the present, larger study and the LOH study of Yanagi *et al*. could not confirm this [27].

However, Barrett's carcinomas shared with the squamous cell carcinomas a higher incidence of amplifications than the cardiac cancers. 11q13 is amplified in 50% of the squamous cell carcinomas and almost never in the other two groups. In addition, the frequency of *cyclin D1* overexpression was also highest in the squamous cell carcinomas (79%). Nevertheless, *cyclin D1* protein overexpression was detected in 29% and 40% of the Barrett's and the cardiac carcinomas, respectively. In contrast, 17q12–q21 was only found to be amplified in the Barrett's carcinomas, while *Her-2/Neu* positivity was also highest in the Barrett's carcinomas (42%). Interestingly, the one squamous cell carcinoma with *Her-2/Neu* overexpression appeared on re-examination also to harbour glandular differentiation and was thus classified as an adenosquamous variant. In addition, the relatively high frequency of 7q and 17q amplifications in Barrett's

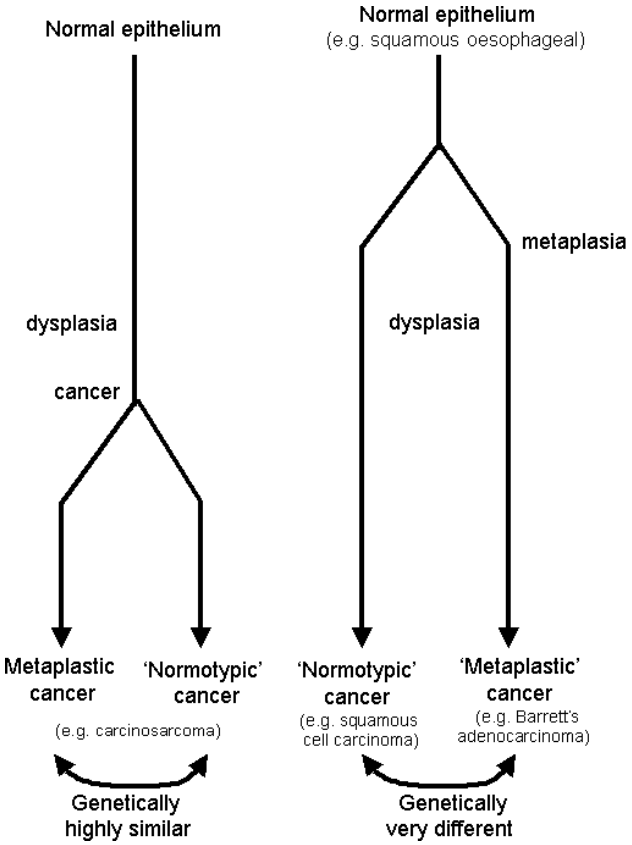

Figure 3. Relationship between genotype and phenotype in carcinogenesis (see text)

cancers compared with cardiac cancers could be an argument in favour of Barrett's cancer being a separate entity from cancers of the gastric cardia.

As to the association between gene expression and amplification, different patterns occurred. Gene amplification status matched with immunohistochemically detected protein expression for *cyclin D1* in 66% (35/53) of cases and for *Her-2/Neu* in 79% (42/53). The finding of one squamous cell cancer showing 11q13 amplification without *cyclin D1* overexpression, as well as one Barrett's cancer showing 17q12–21 amplification without *Her-2/Neu* overexpression, could mean that still other oncogenes at these chromosomal regions are involved in the development of these tumours (Table 3). Conversely, in 32% (17/53) of cases *cyclin D1* overexpression and in 19% (10/53) of cases *Her-2/Neu* overexpression was found without amplification of the corresponding chromosomal regions, indicating that other mechanisms than gene amplification can also disrupt these biological pathways, such as amplification or mutation of upstream regulators.

The relationship between the metaplastic phenotype and genetic alterations remains intriguing from a pathogenetic point of view. When metaplastic change occurs early in the pathogenesis of cancer, as in the case of intestinal metaplasia in Barrett's oesophagus, major differences are found between 'metaplastic' and 'non-metaplastic' cancers, as shown in the present study, although some characteristics remain, such as the higher tendency to high-level amplifications. Similar findings have been made for adenocarcinomas and squamous cell carcinomas of both the uterine cervix [28] and the lung [29–31]. At the other end of the spectrum, a change in phenotype can occur at a stage when a cancer has already developed, such as adenosquamous carcinomas and carcinosarcomas. In such truly metaplastic carcinomas, the distinct components may show huge phenotypic, histological, ultrastructural, and immunohistochemical differences, but genotypically these different tumour components may show marked resemblances (Figure 3) [32]. This indicates that much of the genotype–phenotype relationship still remains to be resolved.

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