Title

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Original contributions

Genomic alterations in primary gastric adenocarcinomas correlate with clinicopathological characteristics and survival

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Abstract. *Background & aims*: Pathogenesis of gastric cancer is driven by an accumulation of genetic changes that to a large extent occur at the chromosomal level. In order to investigate the patterns of chromosomal aberrations in gastric carcinomas, we performed genome-wide microarray based comparative genomic hybridisation (microarray CGH). With this recently developed technique chromosomal aberrations can be studied with high resolution and sensitivity.

Methods: Array CGH was applied to a series of 35 gastric adenocarcinomas using a genome-wide scanning array with 2275 BAC and P1 clones spotted in triplicate. Each clone contains at least one STS for linkage to the sequence of the human genome. These arrays provide an average resolution of 1.4 Mb across the genome. DNA copy number changes were correlated with clinicopathological tumour characteristics as well as survival.

Results: All thirty-five cancers showed chromosomal aberrations and 16 of the 35 tumours showed one or more amplifications. The most frequent aberrations are gains of 8q24.2, 8q24.1, 20q13.12, 20q13.2, 7p11.2, 1q32.3, 8p23.1–p23.3, losses of 5q14.1, 18q22.1, 19p13.12–p13.3, 9p21.3–p24.3, 17p13.1–p13.3, 13q31.1, 16q22.1, 21q21.3, and amplifications of 7q21–q22, and 12q14.1–q21.1. These aberrations were correlated to clinicopathological characteristics and survival. Gain of 1q32.3 was significantly correlated with lymph node status ($p = 0.007$). Tumours with loss of 18q22.1, as well as tumours with amplifications were associated with poor survival ($p = 0.02$, both).

Conclusions: Microarray CGH has revealed several chromosomal regions that have not been described before in gastric cancer at this frequency and resolution, such as amplification of at 7q21–q22 and 12q14.1–q21.1, as well gains at 1q32.3, 7p11.2, and losses at 13q13.1. Interestingly, gain of 1q32.3 and loss of 18q22.1 are associated with a bad prognosis indicating that these regions could harbour gene(s) that may determine aggressive tumour behaviour and poor clinical outcome.

Keywords: Microarray comparative genomic hybridisation, gastric cancer, survival, lymph node, 1q gain, 18q loss, amplification, 7q21–22, 12q

1. Introduction

Gastric adenocarcinoma is a common cause of cancer death, and it is the fourth most common cancer worldwide [33]. In The Netherlands it ranks fifth, with approximately 2200 new cases annually [45]. *Heli-*

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cobacter pylori (*H. pylori*) is a major etiologic factor probably interacting with dietary factors, and possibly *Epstein Barr* virus also plays a role in a subset of cases [5,12,40]. In general, for an epithelial cell to transform to a cancer cell, it must acquire capabilities of autonomous proliferation, destruction and remodelling of basal membrane and intercellular matrix, motility, stroma induction and angiogenesis [10]. Ultimately it must escape from the immune system. Tumour cells acquire these features in part by an accumulation of genomic changes. Thus, pathogenesis of gastric cancer is driven by an accumulation of genetic changes, and these occur to a large extent at the chromosomal level. Despite the high incidence, knowledge of the genetic events leading to gastric adenocarcinomas is still limited.

Analysis of chromosomal imbalances by comparative genomic hybridisation (CGH) has greatly contributed to the current knowledge of genomic alterations associated with gastric cancer and number of chromosomal regions with frequent gains or losses have been identified [12,22–24,44,49]. However, the information obtained is limited to a resolution of approximately 3–10 Mb. Furthermore, chromosome CGH data are less suitable for quantitative analyses like genomic tumour profiling. Microarray based CGH overcomes these limitations to a large extent. It has a much higher resolution and produces quantitative data. Indeed we could previously demonstrate superior sensitivity and resolution of microarray based CGH in the analysis of a region on chromosome 20q that is frequently gained in gastric cancer [48]. In order to obtain a detailed overview of the chromosomal gains, losses and amplifications in gastric adenocarcinomas with a higher resolution than has been possible so far, we applied this new technique.

Previously we demonstrated that unsupervised cluster analysis of microarray CGH data in gastric cancer yielded subgroups that differed with respect to survival and risk of lymph node metastasis [47]. In the present study we have investigated the occurrence of specific chromosomal imbalances in gastric cancer, and their correlation to clinicopathological characteristics.

2. Material and methods

2.1. Material

Thirty-five patients with primary gastric adenocarcinoma who had undergone (partial) gastrectomy, and of which fresh frozen material was available, were included in the study, as has been described previously [47]. Twenty-six patients were male, and nine were female, with a mean age of 67.4 years (range 32–90). Twenty-five carcinomas were of the intestinal type according to the Laurèn classification [26], five were of the diffuse type, and five showed a mixed histology (components were not analysed separately). Tumours with cohesive tumour cell proliferations were classified as intestinal. In all these cases (remnants of) glandular differentiation was present, and in most cases intestinal metaplasia could be observed in the adjacent mucosa. Tumours with a diffusely infiltrating proliferation of individual tumour cells, frequently with signet cell features, were classified as diffuse type carcinomas. Occasionally in these tumours a relation with the glandular layer of the gastric mucosa was observed. Tumours that showed both cohesive parts and diffusely infiltrating solitary tumour cells were classified as mixed types. Eleven tumours were moderately differentiated and 24 poorly differentiated. Two tumours were stage I, 2 were stage II, 23 were stage III, and 8 were stage IV. Twelve patients were lymph node negative and 23 lymph node positive. Follow-up data were available in 33 of 35 (94%) of cases (mean 21.2 months; range 2–104).

DNA was isolated from snap-frozen tumour samples taken from the gastrectomy specimens. The samples were obtained from the archives of the Department of Pathology of the VU University Medical Centre. Only cases were included that had a tumour content of $>75\%$ in the (sandwich) H&E stained sections taken before and after the sections used for DNA isolation. Normal human male genome DNA was isolated from lymphocytes obtained from a blood bank. DNA isolation was performed following the manufacturers instructions (Qiamp Tissue Kit – QIAgen Inc., Valencia, CA, USA), with some modifications as described before [46].

2.2. Microarrays

Microarrays were produced as described previously [41]. In short, DNA isolated from BAC clones was amplified using ligation-mediated PCR to generate representations of these human BAC DNAs. The DNAs were spotted on chromium coated microscope slides using a custom built arrayer. Genome wide scanning arrays were used as described before [1,34,41]. The scanning array comprised DNA from 2275 BAC and P1 clones spotted in triplicate, evenly spread across

the whole genome at an average resolution of 1.4 Mb. Chromosome X-clones ($n = 61$) were discarded from further analysis since all tumour samples were hybridised to male reference DNA, leaving 2214 clones per array to be evaluated. Each clone contains at least one STS for linkage to the sequence of the human genome.

2.3. Comparative hybridisation

Test and reference genomic DNA (300–500 ng of each) were labelled by random priming (BioPrime DNA labelling system, Gibco BRL) in a 100 μl reaction with Cy3 dCTP (Amersham Pharmacia Biotech) and fluorescein dCTP (DuPont NEN NEL424), respectively, as described previously [41]. Non-incorporated nucleotides were removed using a Sephadex G-50 spin column. Labelled DNA ($\sim$600 ng of input DNA) was mixed with Cot-1 DNA (80–100 μg; Gibco BRL) and ethanol precipitated. The precipitated DNA was dissolved in hybridisation mix (50 μl) to achieve a final composition of 50% formamide, 10% dextran sulfate, 2$\times$SSC, 4% SDS and 500 μg yeast tRNA. The hybridisation solution was heated to 70°C for 10–15 minutes to denature the DNA, and subsequently continued incubation at 37°C for approximately 60 minutes to allow blocking of the repetitive sequences. A ring of rubber cement closely around the array was applied to form a well, into which we added 50 μl of slide blocking solution containing 500 μg salmon sperm DNA. After a 30 min incubation at room temperature, approximately three-quarters of the blocking solution was removed, and the denatured and re-annealed hybridisation mixture was added. The arrays were placed on a slowly rocking table ($\sim$1 rpm) at 37°C to allow hybridisation to occur over 48–72 hours. After hybridisation, slides were washed once in 50% formamide, 2 $\times$ SSC, pH 7, at 45°C for 15 minutes, and once in PN buffer (PN: 0.1 M sodium phosphate, 0.1% nonidet P40, pH 8) at room temperature for 15 minutes. Excess liquid was drained from the slides and the array was mounted in an antifade solution containing DAPI (1 μg/ml) to counterstain the DNA targets.

2.4. Image acquisition

Image acquisition and analysis, and data extraction were performed as described previously [34]. In short, DAPI, Cy3, and fluorescein images were captured using a CCD based imaging system and stored as TIF files in a 1024 $\times$ 1024 resolution.

2.5. Data analysis and statistical analysis

Image analysis and feature extraction were done with dedicated software: UCSF SPOT software [17] (www.jainlab.org) was used to automatically segment the spots based on the DAPI images, perform local background correction and to calculate various measurement parameters, including $\log_2$ ratios of the total integrated Cy3 and Cy5 intensities for each spot. A second custom program, SPROC (www.jainlab.org), was used to associate clone identities and a mapping information file with each spot so that the data could be plotted relative to the position of the BACs on the September 2000 freeze of the draft human genome sequence (http://genome.ucsc.edu/). The precise location of the amplicons and common region of overlaps of gains and losses was confirmed by comparing to the April 2002 freeze draft human genome sequence (http://genome.ucsc.edu/). The SPROC output consists of $\log_2$ transformed averaged fluorescence ratios of the triplicate spots for each clone, standard deviations of the triplicates and plotting position for each clone on the array. Ratios of clones for which only one of the triplicates remained after SPROC analysis were excluded from further analysis.

Chromosomal aberrations were classified as a gain when the normalized $\log_2$ transformed fluorescence ratio was higher than 0.2; as a loss when this ratio was below -0.2. Neighbouring clones with a similar $\log_2$ transformed fluorescence ratio exceeding these borders were regarded to belong to the same chromosomal gain or loss, respectively. Events were considered real when at least 4 neighbouring clones had $\log_2$ ratios below or above the thresholds. Steep copy number changes with the graph showing a peak rather than a plateau, with a minimal normalized $\log_2$ transformed fluorescence ratio of 1.0 or larger, were classified as amplifications. Multiple gains, losses and amplifications were counted as separate events.

2.6. Statistical analyses

For comparing means of continuous variables between two or more groups, Student's t-test and one-way analysis of variance (ANOVA) were used, respectively. For testing significance of differences in distribution of categorical variables, cross tables were analysed with the two-sided Fisher Exact's test and Pearson's test, depending on the number of categories. Univariate survival analysis was carried out by Kaplan–Meier survival analysis and log-rank testing.

P-values < 0.05 were considered significant, unless otherwise specified. All statistical analyses were carried out with SPSS software version 10 (SPSS Inc., Chicago, IL, USA).

3. Results

All thirty-five cancers showed chromosomal aberrations. The mean number of chromosomal events (gains, losses, and amplifications) per case was 16.0 (range 3–53), with on average 7.9 gains (range 1–28), 7.1 losses (range 0–23), and 1.0 amplification (range 0–4). The most frequent aberrations are gains of 8q24.2, 8q24.1, 20q13.12, 20q13.2, 7p11.2, 1q32.3, 8p23.1–p23.3, losses of 5q14.1, 18q22.1, 19p13.12–p13.3, 9p21.3–p24.3, 17p13.1–p13.3, 13q31.1, 16q22.1, 21q21.3 (Table 1), and amplifications of 7q21.2–q22.3 and 12q14.1–q21.1 (Table 2). Examples of array CGH data of a few of these frequent aberrations are shown in Fig. 1. An overview of all chromosomal aberrations is displayed in Fig. 2. The total number of chromosomal aberrations ('events') was significantly correlated to the number of amplifications ($r = 0.7, p < 0.0001$).

All amplifications are summarized in Table 2. The most frequent amplification was found at the long arm of chromosome 7 ($7/35 = 20\%$). In this region, three

Table 1

Most frequent common regions of overlap detected by microarray comparative genomic hybridisation in 35 gastric adenocarcinomas, together with candidate genes

Chromosomal aberration	Number of tumours	Candidate genes
1q32.3+	10 (29%)	*NEK2*
7p11.2+	11 (31%)	*EGFR*
8p23.1–23.3+	10 (29%)	*GATA4*; *MASL1*
8q24.1+	19 (54%)	?
8q24.2+	23 (66%)	*C-Myc*
20q13.12+	19 (54%)	?
20q13.2+	18 (51%)	*CYP24; ZNF217*
5q14.1−	14 (40%)	*MSH3*
9p21.3–24.3−	10 (29%)	*INF*
13q31.1−	9 (26%)	?
16q22.1−	9 (26%)	*CDH 1* (=*E-Cadherin*), *CDH3, CDH5, CDH16*
17p13.1–13.3−	10 (29%)	*P53*
18q22.1−	13 (37%)	*CDH 7 and CDH 19*
19p13.12–13.3−	11 (31%)	?
21q21.3−	8 (23%)	?

Table 2

Genomic amplifications and candidate genes detected by microarray comparative genomic hybridisation in 35 gastric adenocarcinomas, together with candidate genes

Region of amplification	Tumor	Amplicon size	Candidate genes
1q21.1–q21.3	#5	5.3 Mb	Phosphatidylinositol-4-kinase, catalytic, β (PIK4CB)
1q24.1–24.3	#8	6.0 Mb	Flavin-containing monooxygenase 1, 2, 3 and 4 (FMO1, FMO2, FMO3, FMO4) Myoc
3q26.1–q26.31	#8	10.2 Mb	?
4p11–q13.2	#12	12.0	PDGFRA
7p22.3–p21.3	#12	7.0 Mb	?
7p11.2	#10	0.56 Mb	EGFR
7q21.2–q22.3	#6	3.7 Mb	CDK6
	#1	12.9 Mb	MDR1
	#10	16.4 Mb	GNG11
	#2	13.2 Mb	
	#7	22.3 Mb	
	#3	32.2 Mb	
7q22.2–q31.1	#7	4.4 Mb	PRKAR2B
7q22.2–q22.3	#2	2.8 Mb	
7q31.1–q32.1	#11	18.1 Mb	C-met; TFEC
	#2	14.8 Mb	
8p23.3–p23.2	#9	6.3 Mb	GNB2L1
8p23.1	#14	3.4 Mb	Cathepsin B
	#9	2.9 Mb	GATA-4
			farnesyl-diphosphate farnesyltransferase (FDFT1)
8q24.13–q24.21	#10	6.3 Mb	C-myc
10q22.1	#3	5.1 Mb	?
11p11.2–p12	#2	7.0 Mb	diacylglycerol kinase (DGKZ)
			MDK
			DDB2
			novel death domain (MADD)
			PTPRJ
11q13.3–q14.1	#2	18.8 Mb	Cyclin D1
		peak of 4.6 Mb	FGF3, 4, and 19
			EMS1
12p12	#14	4.5 Mb	K-ras
	#15		
12q14.1–q21.1	#4	7.7 Mb	MDM2
	#6	7.7 Mb	interferon gamma (IFNG)
	#7	13.5 Mb	MDM1

Table 2
(Continued)

Region of amplification	Tumor	Amplicon size	Candidate genes
			IL22
			protein tyrosine phosphatase, receptor type, B (PTPRB)
13q13.1–q13.3	#1	5.6 Mb	Cyclin A1
17q21.1–q21.3	#16	5.4 Mb	Her2/neu
			BRCA1
			Topoisomerase II
19q12–q13.1	#5	4.2 Mb	Cyclin E1
20p11.2	#8	1.5 Mb	?
20q13.11–q13.12	#13	1.9 Mb	?
20q13.2	#8	1.5 Mb	CYP24
	#12	15.0 Mb	ZNF217

separate amplifications could be identified; 7q21.1–q22.3, 7q22.2–q31.1, and 7q31.1–q32.1 (Fig. 3). The second most frequent amplification, occurring in 3 tumours ($3/35 = 9\%$), was found at chromosome 12q14.1–q21.1 in. Other amplifications occurred in lower frequencies.

Array CGH data (all events in Table 1) were correlated to lymph node status (negative or positive), histological type (intestinal, diffuse, mixed), differentiation grade (moderate or poor), tumour stage (T1+T2 versus T3 + T4), gender (male or female), and age (Table 3).

Of the individual gains and losses, gain of 1q32.3 was significantly correlated with lymph node status ($p = 0.007$); all ten tumours harbouring gain of this chromosomal locus were lymph node positive. Furthermore, significant correlations were found between loss of 17p13.1–p13.3 and histological type ($p = 0.01$; 4 of 5 diffuse type tumours and only 6 of 25 intestinal type tumours showed loss of 17p), and loss of 18q22.1 with gender ($p = 0.007$; all 13 tumours showing 18q22.1 loss were from male patients). None of the other frequently occurring chromosomal common regions of overlap were correlated with either lymph node status, histological type, grade, stage, or gender. Interestingly, loss of 18q22.1 and gain of 20q13.2 were significantly correlated ($p = 0.01$). These events co-occurred in 11 tumours and 13 tumours showed neither 18q22.1− nor 20q13.2+.

No correlations were found between any of the above mentioned clinicopathological characteristics and the presence or absence of amplifications.

In univariate analysis, patient survival was not correlated with the total number of gains, losses or events.

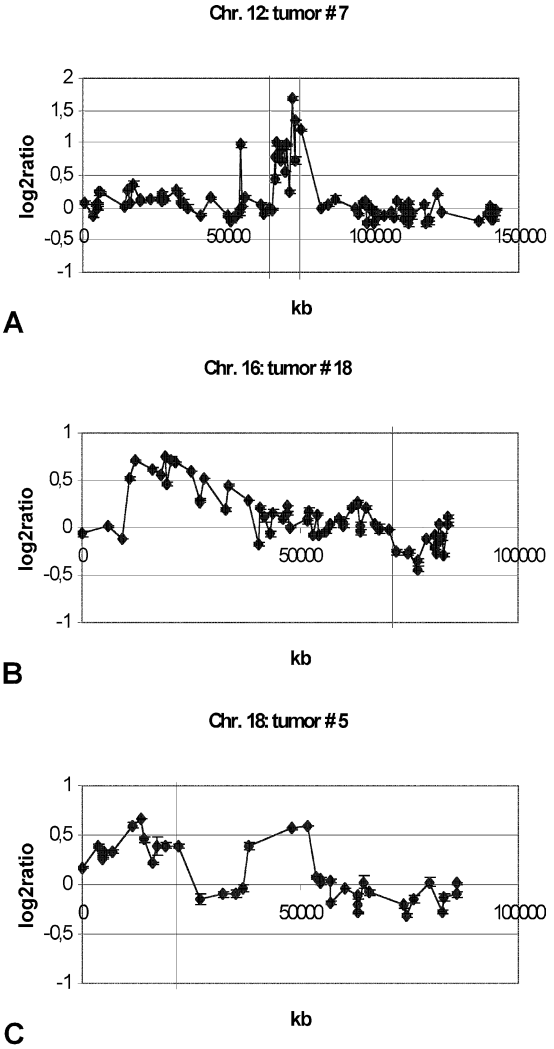

Fig. 1. Examples of microarray CGH results for three of the most frequent common regions of overlap (boundaries are indicated by the vertical lines). Clones are ordered by their position from pter (left) to qter (right). The $\log_2$ fluorescence ratios of every clone in this specific case are plotted as a line graphs. Multiple clear copy number changes (gains, losses and amplifications) can be recognized.

However, presence of one or more amplifications was significantly correlated with poor survival (log rank = 5.2, $p = 0.02$, hazard ratio 3.3 (95% confidence interval 1.1–9.6)). When investigating individual events, tumours with loss of 18q22.1 were associated with a poorer survival (log rank = 5.9, $p = 0.02$, hazard ratio 3.3 (95% confidence interval 1.2–10.0)). A similar trend was seen for gain of 20q13.2, but this did not reach statistical significance (log rank = 1.92, $p = 0.17$). None of the other individual events did correlate with survival, including gain of 1q32.3 (log rank = 2.28, $p = 0.13$) (Table 3).

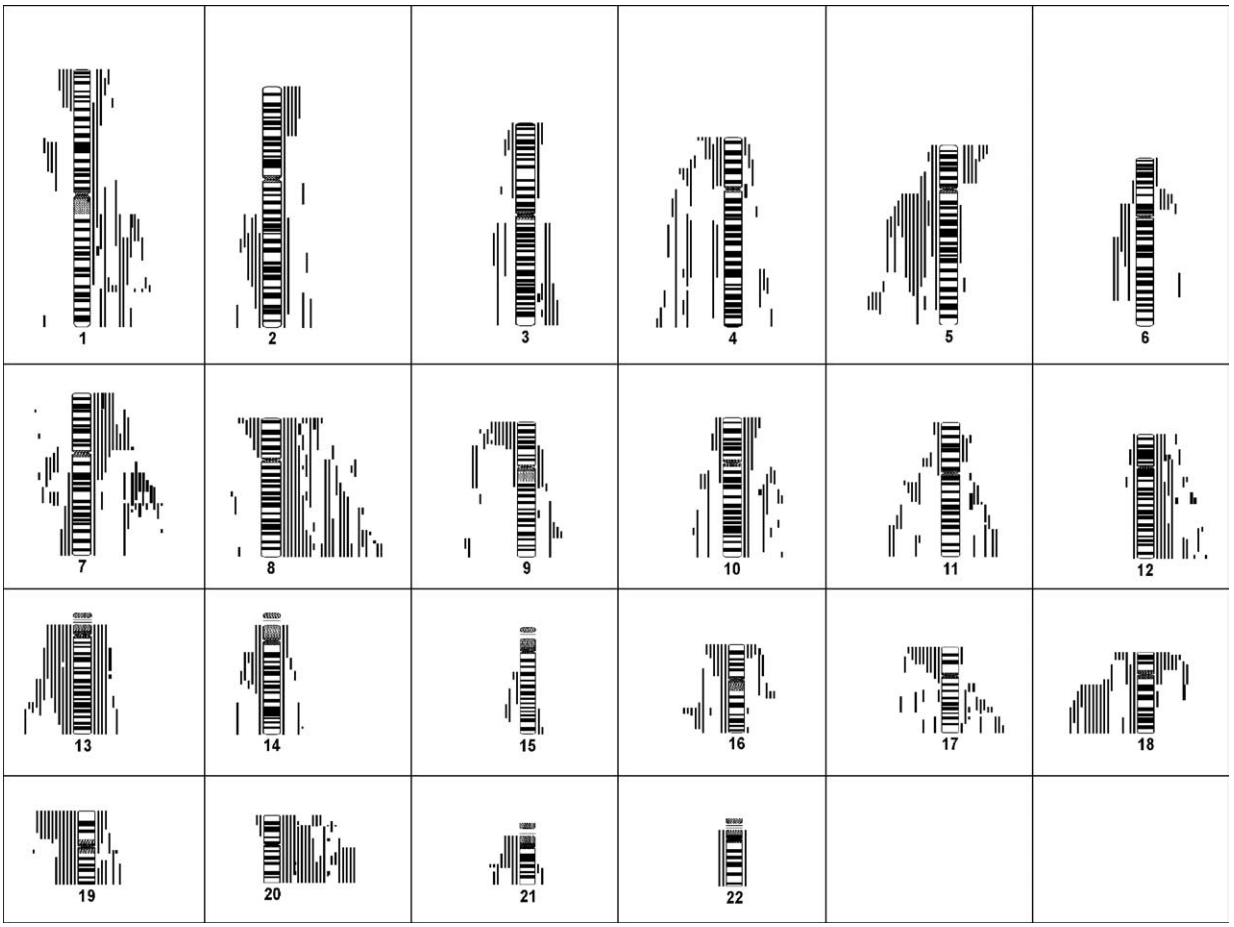

Fig. 2. Overview of chromosomal aberrations in 35 primary gastric carcinomas detected by microarray comparative genomic hybridisation. Each bar represents a chromosomal aberration in an individual tumour. On the right side of the ideograms the gains (thin lines) and amplifications (thick lines) are displayed, and on the left side the losses (thin lines).

4. Discussion

Chromosomal instability is a major mechanism of genetic damage in gastric cancer as has been demonstrated by classical CGH studies showing chromosome abnormalities in almost all gastric tumours analysed [9,23,44]. Events related to progression of gastric cancer include activating mutations, amplifications and overexpression of various growth factors (e.g. *epidermal growth factor* (*EGFR*), *platelet derived growth factor* (*PDGFR*), *c-Met*), signalling intermediates (e.g. *Ras*, *Protein Kinase C* (*PKC*)), and cell cycle regulatory molecules (e.g. *mouse double minute-2* (*MDM2*), *cyclin-dependent kinase-4* (*CDK4*), and *CDK6*, that positively regulate proliferation and cell cycle progression. Amplifications of some of these oncogenes like *Cyclin D1* on 11q13, *C-Met* on 7q31 and *HER2-neu/C-erbB2* on 17q21–q22 have been described. A number of chromosomal losses, causing loss of tumour suppressor function, have been identified in sporadic gastric cancer, like *p53* on 17p13, and *E-cadherin* on16q22. However, the picture is yet far from complete.

Although chromosome CGH has increased our possibilities to analyse the complete genome of primary tumour samples in a single experiment, it has certain limitations in resolution for chromosomal aberrations. Array-based CGH, as used in this study, has overcome this problem to a large extent [1,34]. The most frequent amplification ($6/35 = 17\%$) was located at 7q21–q22, in the neighbourhood of this chromosomal region two other amplified regions at the long arm of chromosome 7 were detected (Fig. 3). The common region of overlap of the amplifications at 7q21–q22 is 3.7 Mb in size. A putative candidate gene for driving the selection of tumour cells harbouring this amplification

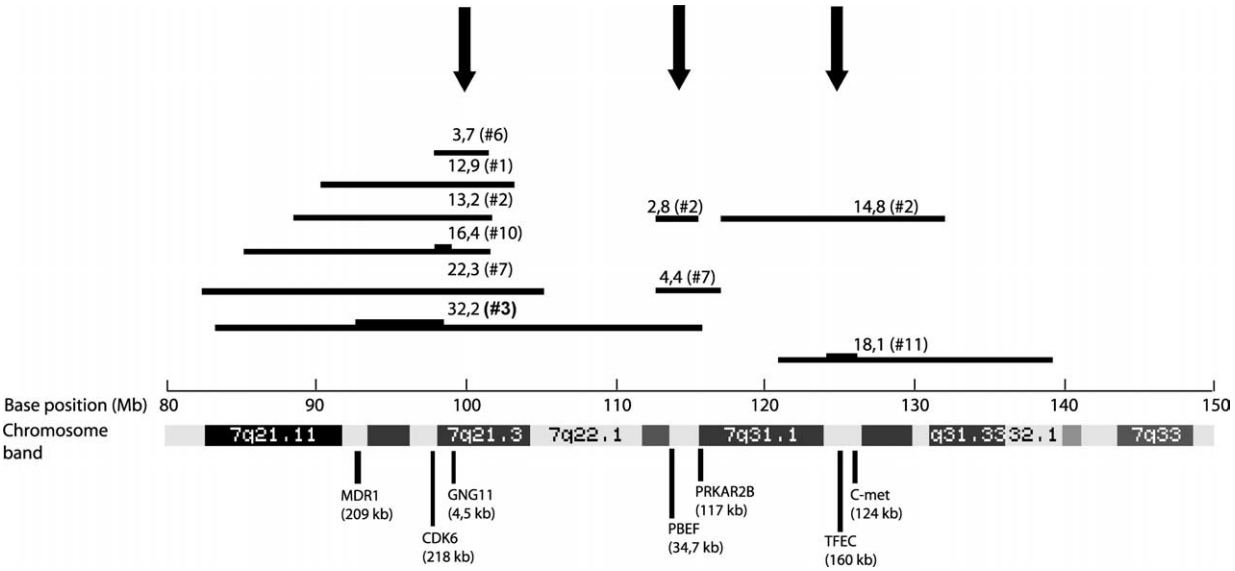

Fig. 3. Detailed mapping of three regions of amplification on the long arm of chromosome 7. Seven tumours showed amplification at one or more regions at 7q. The horizontal bars represent the length and position of the amplified regions. Thick bars represent the peak within the amplified region. On the X-axeis the base position, chromosome band (image derived from http://genome.ucsc.edu/) and candidate genes are plotted.

Table 3
Correlations of DNA copy number changes with clinicopathological characteristics and survival

	Lymph node status		Total	*p*-value
	Negative	Positive		
no 1q32.3+	12	13	25	0.007
1q32.3+	–	10	10	
Total	12	23	35	

	Histological type			Total	*p*-value
	Intestinal	Mixed	Diffuse		
no 17p13−	19	5	1	25	0.013
17p13−	6		4	10	
Total	25	5	5	35	

	Sex		Total	*p*-value
	Female	Male		
no 18q22.1−	9	13	22	0.007
18q22.1−		13	13	
Total	9	26	35	

	Survival	*N*	Hazard ratio	Log rank	*p*-value
no 18q22.1−	Good	19	3.3 (1.2–10.0)	5.8	0.02
18q22.1−	Poor	10			
Total		29			
No amplifications	Good	16	3.3 (1.1–9.6)	5.2	0.02
Amplifications	Poor	13			
Total		29			

at 7q21–q22 could be *CDK6*. The protein encoded by this gene is a member of the CDK family. The D type cyclins complex with *CDK4* and *CDK6* to govern progression through the G1 phase of the cell cycle and later are involved with inactivating phosphorylation of the *Rb* protein which results in release of *Rb*-associated transcription factors that are needed for entry into S phase. The activity of the *CDKs* is in part controlled by inhibitors such as the *INK4* family [32,39]. *INK4a* (at 9p21.3) has been shown to act by competing with *CDK4* and *CDK6* and functions as a tumour suppressor in a variety of cancers. Inhibition of *CDK6* prevents the phosphorylation of *Rb1* and maintains the *Rb1–E2F* complex in its growth-suppressing state [19]. Amplification of *CDK6* has been detected in 6% of high-grade astrocytic tumours [4], and in these tumours it was not co-amplified with either *EGFR* or c-*met,* which map to 7p11.2 and 7q31.2, respectively. In the gastric carcinomas in the present study, amplification of the *CDK6* region was found without co-amplification of other regions at chromosome 7 in three tumours (#1, #3, #6). In one tumour (#10), *CDK6* was co-amplified with the *EGFR* locus, and in 2 tumours (#2, #7) the chromosomal region of *CDK6* was co-amplified with a small region distal of *CDK6* starting at 7q22.2 (Fig. 3). These data suggest that the amplification of 7q21.2–q22.3 containing *CDK6* may be a new specific amplicon in gastric cancer.

One of the other amplified regions on 7q (in two tumours) harbours the *c-met* gene, that has been found

to be frequently overexpressed in gastric cancer (70%), which was not caused by the activation of *tpr*, an upstream regulator of *c-met* [13]. Data on *c-met* protein expression analysed by immunohistochemistry were available for 13 cases of the present study [13]. Two of these 13 cases showed amplification of the *c-met* gene locus by array CGH, and interestingly both these tumours showed overexpression of the *c-met* protein. *C-met* amplification has been correlated with positive lymph-node metastasis but not with other clinicopathological factors [29]. Also, in our set the two tumours with *c-met* amplification are both lymph node positive.

Gains and amplifications of chromosome 7q have been reported before in gastric cancer [6,24,25,30,43], and *c-met* is in most cases suggested as the candidate gene. However, in this study we showed that a region proximal (7q21–q22) of *c-met* is amplified in a higher percentage of gastric cancers analysed, suggesting that another oncogene (e.g. *CDK6*) may be more important in gastric carcinogenesis. These data show the advantage of array CGH compared to chromosome CGH, because of the higher resolution and the possibility to position amplifications more precisely.

Amplification at chromosome 12q14.1–q21.1 is the second most frequent amplification ($3/35 = 9\%$). A well-known candidate oncogene at this chromosomal locus is *MDM2* at 12q15. *MDM2* is a multifunctional protein that acts as a regulator of the tumour suppressor *p53*. It binds to and abrogates the *p53* function by either targeting *p53* for degradation in the cytoplasm by the proteosome, or by repressing *p53*-mediated transcriptional activity in the nucleus [35]. The *MDM2* gene enhances the tumourigenic potential of cells in which it is overexpressed [20]. Furthermore, *MDM2* interacts physically and functionally with the *Rb* protein and can inhibit its growth regulatory capacity. So both *Rb* and *p53* can be subjected to negative regulation by the product of a single cellular protooncogene [50]. Recently, it has been shown that *MDM2* can interact with a large number of other cellular proteins besides p53 [8]. In our series of 35 gastric carcinomas, however, there was no correlation between amplification of the *MDM2* and the *p53* locus (1 tumour with *MDM2* amplification showed 17p loss, and 2 did not show 17p loss). The small number of 12q amplifications, however, does not permit any final conclusions in this respect.

The amplification at 8p23.1 of the short arm of chromosome 8 occurred in 2 tumours (6%). This region has been reported to be frequently amplified in oesophageal (13.6%) and cardia adenocarcinomas (12.5%) [27]. Candidate genes are *GATA-4*, a member of a zinc finger transcription factor family, *farnesyldiphosphate farnesyltransferase* (*FDFT1*), and *cathepsin B* (*CTSB*). Both amplification and overexpression of *GATA-4, FDFT1* and *CTSB* have been reported in oesophageal adenocarcinoma [15,27], indicating an important role for these genes in the carcinogenesis of gastrointestinal tumours. Furthermore, the *MASL1* gene in this amplicon is reported as a candidate oncogene in malignant fibrous histiocytomas [37].

In many types of tumours, amplifications become more prevalent with advanced tumour stage and are rare in premalignant lesions [36]. This could mean that low-level copy number increases (low-level gains) of many genes on a chromosome arm is beneficial to render an initial growth advantage to the cell, whereas more localized high-level gains (or amplifications) could be important at later stages as a response to selective environmental pressure. Frequently, the presence of one or more amplifications is correlated with clinicopathological characteristics that influence survival, like tumour stage, grade, and lymph node status. In the present study, the presence or absence of amplifications was not correlated to any of the clinicopathological characteristics, except for survival ($\log \text{rank} = 5.2$, $p = 0.02$, hazard ratio 3.3 (95% confidence interval 1.1–9.6)) (Table 3).

In many types of tumours gain of 1q is associated with a poor prognosis; e.g. with adverse overall survival and event-free survival in Ewing tumours [11], with a higher relapse rate in breast cancer [51], and with aggressive behaviour in ependymomas [3]. In the present study, gastric cancer, gain of 1q32.3 was significantly correlated with lymph node status ($p = 0.007$); all ten tumours harbouring gain of this chromosomal locus were lymph node positive. Gain of 1q in gastric cancers has been described as a frequent chromosomal aberration by others [6,31,43], but not with this resolution. In this regions the *NEK2* gene, a member of the *Never In Mitosis A* (*NIMA*) family of cell cycle regulators, is one of the candidates. *NEK2* is a protein kinase that localizes to the centrosome. Overexpression of this protein has two consequences; i.e. centrosome splitting and the gradual disappearance of centrosomes, indicating that *NEK2* could play a role in preparation of the chromosomes for separation prior to the onset of mitosis. This suggests that *NEK2* has a role in the cell cycle, and could be involved in chromosomal instability [7]. Indeed, gain of the chromosomal locus of *NEK2* at 1q was significantly correlated with the total number of events ($p = 0.01$).

While 1q gain was correlated with lymph node status, other chromosomal changes were correlated with survival, indicating that yet other mechanisms than lymph node metastasis determine patient outcome. Loss of 18q22.1 was significantly correlated with poor survival (log rank $= 5.88$, $p = 0.015$, hazard ratio 3.3 (95% confidence interval 1.2–10.0). *Cadherins* (*CDH*) 7 and *19* are located at this chromosomal band. The *cadherins* are a family of cell surface molecules involved in the structural and functional organization of cells in various tissues. These proteins act as mediators of selective cell–cell adhesion, which is a feature of epithelium. Since disturbance of intercellular adhesion is a prerequisite for invasion and metastasis of tumour cells, *cadherins* are considered prime candidates for tumour suppressor genes. Loss of *E-cadherin* (located at 16q22.1) is a characteristic feature of diffuse gastric cancer [28]. Loss of 16q22.1 is a frequent aberration in this set of gastric carcinomas ($9/35 = 26\%$). Mutations occur at a very early non-invasive stage, associating *E-cadherin* mutations with loss of growth control. These mutations mostly occur in combination with loss of heterozygosity (LOH) of the wild-type allele, resulting in loss of *E-cadherin* mediated cell–cell adhesion [2]. In this set of gastric tumours, loss of 16q22.1 is not significantly correlated with histological type. However, there are only 5 (of 35) diffuse type gastric carcinomas included in this study. Two of which show loss of 16q22.1.

No correlation between loss of 18q22.1 and histological type was found in this study (of the 13 tumours with losses of 18q22.1, 11 were of the intestinal, 1 was of the mixed and 1 was of the diffuse type). LOH on chromosome 18q22–23 has been reported to correlate with serosal invasion, haematogenous recurrence and survival in gastric carcinomas [16]. In colon cancer, loss of 18q12–21 is a strong indicator for progression in colorectal carcinogenesis (significant correlation with grade of dysplasia), as well as survival [14,18]. Although the specific gene(s) on 18q in both gastric and colorectal cancer have not yet been identified, the *DCC* and *Smad4* are candidates. Expression of *DCC* in gastric and colon cancer is decreased, and in colon cancer this correlates with the development of nodal metastasis [21]. Using mouse models, it has been suggested that *SMAD*$-/-$ adenomas in the colon are larger and progress quicker [42]. However, the chromosomal region involved in colorectal and gastric carcinogenesis seems to be different but still close to each other (18q12–21 and 18q22.1, respectively). Taken into account the resolution of chromosome CGH, and even array CGH, it may very well be possible that it is indeed the same chromosomal region that harbours the candidate tumour suppressor gene important in gastric and colorectal carcinogenesis. This is in line with the fact that in both gastric and colorectal cancer, loss of 18q was correlated with gain of 20q [14]. In colorectal cancer, this combination of events is associated with a higher grade of dysplasia [14]. The association of loss of 18q22.1 with survival suggests the possibility that the abnormalities of the gene present on chromosome 18q22.1 or nearby may contribute to tumour growth.

Here we report a genome-wide microarray CGH study of gastric cancer and the correlation with clinicopathological characteristics and survival. Microarray CGH has revealed several chromosomal regions that have not described before in gastric cancer at this frequency and resolution, such as amplification of 7q21–q22 and 12q14.1–q21.1, as well gains at 1q32.3, 7p11.2, and losses at 13q13.1.

In particular gain of 1q is associated with a poor prognosis and loss of 18q with poor survival. This suggests that at 1q32.3 oncogene(s) and 18q22.1 tumour supressor gene(s) are located that may determine aggressive tumour behaviour and poor clinical outcome.

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