Progression from colorectal adenoma to carcinoma is associated with non-random chromosomal gains as detected by comparative genomic hybridisation

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Abstract

***Aims*—Chromosomal gains and losses were surveyed by comparative genomic hybridisation (CGH) in a series of colorectal adenomas and carcinomas, in search of high risk genomic changes involved in colorectal carcinogenesis.**

***Methods*—Nine colorectal adenomas and 14 carcinomas were analysed by CGH, and DNA ploidy was assessed with both flow and image cytometry.**

***Results*—In the nine adenomas analysed, an average of 6.6 (range 1 to 11) chromosomal aberrations were identified. In the 14 carcinomas an average of 11.9 (range 5 to 17) events were found per tumour. In the adenomas the number of gains and losses was in balance (3.6 *v* 3.0) while in carcinomas gains occurred more often than losses (8.2 *v* 3.7). Frequent gains involved 13q, 7p, 7q, 8q, and 20q, whereas losses most often occurred at 18q, 4q, and 8p. Gains of 13q, 8q, and 20q, and loss of 18q occurred more often in carcinomas than in adenomas (p = 0.005, p = 0.05, p = 0.05, and p = 0.02, respectively). Aneuploid tumours showed more gains than losses (mean 9.3 *v* 4.9, p = 0.02), in contrast to diploid tumours where gains and losses were nearly balanced (mean 3.1 *v* 4.1, p = 0.5).**

***Conclusions*—The most striking difference between chromosomal aberrations in colorectal adenomas and carcinomas, as detected by CGH, is an increased number of chromosomal gains that show a non-random distribution. Gains of 13q and also of 20q and 8q seem especially to be involved in the progression of adenomas to carcinomas, possibly owing to low level overexpression of oncogenes at these loci.**
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Keywords: colon; tumour; comparative genomic hybridisation

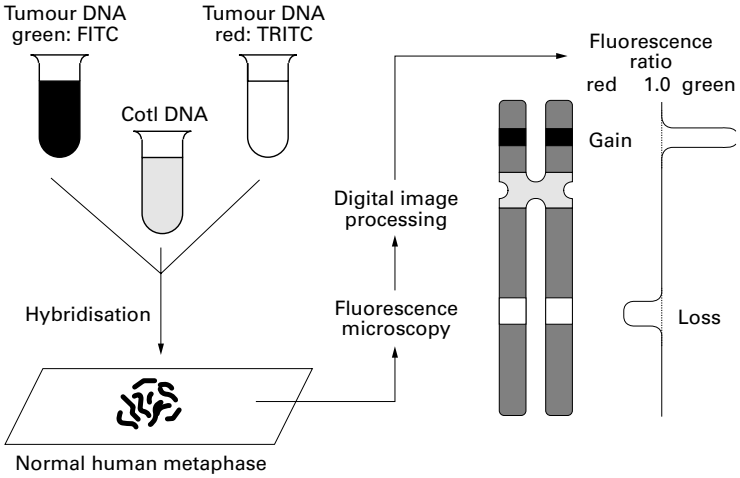

Figure 1 Schematic overview of the comparative genomic hybridisation (CGH) technique. Tumour and reference DNA are labelled with a green and red fluorochrome, respectively, and hybridised to normal metaphase spreads. Images of the fluorescent signals are captured and the green to red signal ratios are digitally quantified along the chromosomal axis for each chromosome. (Reprinted with permission from Hermsen et al, Human Pathology 1996;27:342–9.)

Colorectal cancer is the second leading cause of cancer death in the Western world. Intensive scientific efforts have been made attempting to resolve the genetic changes underlying colorectal cancer.[1] Nevertheless, these changes are still not completely understood. Progress in this area would, however, benefit strategies for both primary and secondary prevention of colorectal cancer. In addition, analysis of genetic changes in cancers is becoming increasingly important for both classification and prognosis assessment.

Because of the research methods available—including mutation analysis and polymerase chain reaction (PCR) based loss of heterozygosity analysis—most attention in the past decade has focused on genetic changes at the subchromosomal level, such as mutations and deletions leading to inactivation of tumour suppressor genes. More recently there has been a reappraisal of changes at the chromosomal level. This reappraisal is a result of the growing awareness that lack of genome integrity, or genomic instability, is in itself a hallmark of the development and progression of neoplasia. However, the term genomic instability is ill defined and may refer to inherently different features such as aneuploidy, microsatellite instability, or p53 mutation.

We would like to adopt the definition of genomic instability as a cellular state characterised by a deficiency in one or more "caretaker" genes, as a result of which structural and numerical genomic alterations accumulate in subsequent generations of daughter cells. Some of these changes may affect genes that play a key role in critical pathways controlling, for example, proliferation or apoptosis—"gatekeeper" genes.[2] Two major patterns of genomic instability have recently been described to fit this definition[3]: first, instability caused by failing DNA repair at the nucleotide level, as

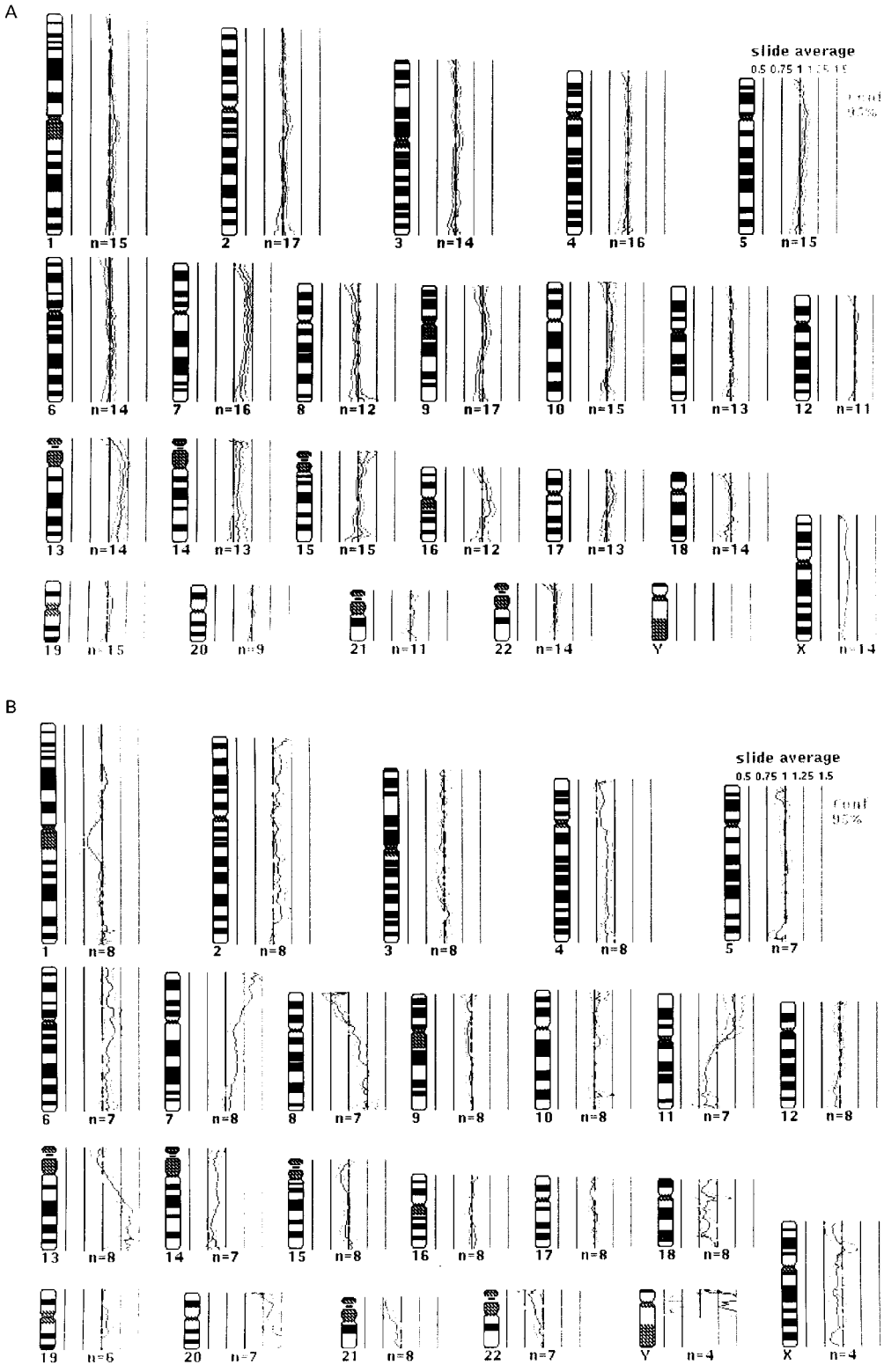

Figure 2 Relative copy number karyotypes of a colorectal adenoma (A) and carcinoma (B) showing a graphical display of the mean and 95% confidence interval of the green to red fluorescence ratio along all individual chromosomes, obtained by analysing multiple metaphases. When at a locus the complete 95% confidence interval (CI) of the mean fluorescence ratio is right of the 1.0 line this is interpreted as a gain. On the other hand, when the complete 95% CI is left of the 1.0 line, this is regarded as a loss. The adenoma (A, case 7) shows gains at 5p, 5q, 7p, 7q, 10q, and 13q. The fluorescence ratio for chromosome X is smaller than 1.0 because male tumour DNA was compared to female normal DNA. In the carcinoma (B, case 14), clear gains can be seen for 7p, 7q, 8q, 11p, 13q, 20p, and 20q, and losses for 4p, 4q, 8p, 11q, 14q, 18p, and 18q. Smaller aberrations are seen at 5q, 6p, and 6q.

Table 1 Summary of clinical and pathological data, DNA ploidy, and CGH results in nine colorectal adenomas and 14 carcinomas

Tumour	*Age (years)*	*Sex*	*Site*	*Histological type*	*Grade of dysplasia*	*Size (mm)*	*DNA index*	*Gains*	*Losses*	*Events*
1	66	F	Sigmoid	T	Mild	11	1.0	18q, 20q	2p, 2q, 7q, 14q	6
2	67	M	Sigmoid	V	Moderate	20	1.0	1p, 1q, 6q, 7p, 12q, 19p, 19q	9p, 9q, Xp, Xq	11
3	69	F	Rectum	V	Moderate	10	1.0	2p, 2q, 4q, 5q, 13q	17p, 20p	7
4	48	F	Sigmoid	T	Moderate	3	1.0	6q, 15q	10p	3
5	84	F	Sigmoid	T	Moderate	10	1.0	3q, 7p, 7q, 8q, 10q, 20q	10p, 10q, 16p, 19p, 19q	11
6	66	M	Transverse	T	Moderate	9	1.0	-	7q	1
7	66	M	Ascending	T	Mild	5	1.0	5p, 5q, 7p, 7q, 10q, 13q	-	6
8	66	M	Transverse	T	Mild	6	1.0	5q, 7p, 7q	5q, 22q	5
9	83	M	Caecum	T	Moderate	5	1.0	8q	1p, 2q, 9q, 10q, 11q, 12q, 17q, 20q	9
10	72	F	Rectum	B2	Moderate	47	1.6	6p, 6q, 7p, 9p, 9q, 10q, 11p, 12p, 12q, 13q, 20p, 20q	22q	13
11	29	F	Rectum	B2	Poor	40	1.0	4q, 5q, 6p, 6q, 8q, 13q, 18q	22q	8
12	57	F	Rectum	C2	Moderate	55	1.0	1q, 7p, 7q, 13q, 20p, 20q	2q, 4q	8
13	66	F	Sigmoid	C2	Moderate	55	1.7	8q, 13q	4q, 5p, 6q, 10q, 14q, 16q	8
14	67	M	Sigmoid	B1	Moderate	40	1.8	6p, 6q, 7p, 7q, 8q, 11p, 13q, 20p, 20q	4p, 4q, 5q, 8p, 11q, 14q, 18p, 18q	17
15	80	F	Sigmoid	C2	Moderate	80	1.2	1q, 7p, 7q, 13q	22q	5
16	47	M	Rectum	B1	Moderate	20	1.0	4q, 5q, 7q, 9q, 10q, 13q	6p, 21q, Xq	9
17	65	M	Rectum	C2	Moderate	60	1.4	7p, 7q, 8q, 9p, 9q, 12q, 13q, 16p, 20q	3q, 8p, 17p, 18p, 18q, 19p, 21q	16
18	67	F	Caecum	D	Moderate	50	1.5	2p, 2q, 3q, 7p, 7q, 8q, 9p, 9q, 11q, 13q, 20q	1q, 12p, 18q	14
19	58	M	Caecum	C2	Moderate	90	2.0	1p, 1q, 2q, 4q, 6p, 6q, 7p, 7q, 8q, 11q, 14q, 16p, 16q, 17q, 20q, Xq	6q	17
20	80	F	Rectum	C2	Moderate	35	1.0	7p, 7q, 8q, 13q, 20p, 20q	15q, 17p, 18p, 18q, Xq	11
21	63	M	Liver	D	Moderate	80	1.3	1q, 7p, 7q, 8q, 9p, 9q, 12p, 12q, 13q, 20q, 21q	3p, 4p, 4q, 8p, 11q, 18q	17
22	84	F	Sigmoid	B2	Moderate	35	1.0	1q, 3p, 7p, 7q, 8q, 11q, 13q	10p, 19p, 19q	10
23	91	F	Rectum	B1	Moderate	52	1.0	1q, 7p, 13q, 20p, 20q, 21q, 22q, Xp, Xq	1p, 2p, 6p, 16p, 18q	14

Histological type (for adenomas): T, tubular; V, villous. Stage (for carcinomas): Astler-Coller stage. Grade of dysplasia (adenomas): mild, moderate, severe. Grade of differentiation (carcinomas): well, moderate, poor.

found in hereditary non-polyposis colorectal cancer where a failing DNA mismatch repair system gives rise to replication errors (RER) which can be visualised as microsatellite instability (MIN); second, genomic instability caused by chromosomal copy number and structural changes affecting large parts and often complete arms of chromosomes. The latter process, which eventually gives rise to DNA aneuploidy, appears to be the cause of genomic instability in the vast majority of colorectal cancers and other solid tumours.[3 4] Various hereditary syndromes exist in which an increased sensitivity to agents causing chromosomal breakage is associated with an increased tumour incidence. Nevertheless, no clear mechanism has yet been found to explain chromosomal instability in the majority of sporadic tumours in general, or colorectal cancer in particular.[5 6]

The opportunities of analysing the magnitude and patterns of chromosomal instability in colorectal carcinogenesis have been greatly expanded by the recent development of comparative genomic hybridisation (CGH). This technique uniquely allows the detection of chromosomal gains (for example, trisomies and gene amplifications) and losses (monosomies and deletions) using only a small amount of tumour material, without the need for cell culturing, and it provides an overview of genomic changes at the chromosomal level in a tumour.[7 8] In this procedure (fig 1), DNA isolated from tumour specimens is labelled with a green fluorochrome and mixed with red labelled DNA obtained from cells with a normal diploid chromosome complement. The mixture is hybridised to normal metaphase preparations. Hybridised test and control DNA sequences compete in the annealing process for their complementary sites on the individual chromosomes, and are recognised by the different fluorochromes. The ratio of green to red fluorescence for each chromosomal region is a measure of the under- or overrepresentation of genetic material for that region in the tumour studied. The relative copy number karyotype of a tumour, based on the analysis of multiple metaphases, gives an overview of chromosomal gains and losses in that tumour (fig 2). In this study, chromosomal gains and losses were surveyed by comparative genomic hybridisation in a series of colorectal adenomas and carcinomas, in search of high risk genomic changes involved in colorectal carcinogenesis.

Methods

TISSUE SAMPLES

Nine consecutive colorectal adenomas and 14 colorectal carcinomas (13 primary tumours and one liver metastasis) were used in the study. Patient and tumour characteristics are given in table 1. For CGH, special care was taken to avoid admixture of stromal, blood, and non-tumour epithelial cells by brushing the surface of the tumour with cyto-brushes. Fresh tumour specimens were sampled within one hour of surgical resection. The brushes containing tumour cells were collected in phosphate buffered saline (PBS). The cells were removed from the brushes by pipetting with PBS using a Pasteur pipette, transferred to a new tube, and washed in PBS. The yield was estimated using a Buchner cell counter. In all cases adequate numbers of cells for CGH analysis (200 000 to 5 000 000) were obtained from the brushings. In addition to the cytological samples obtained from primary tumours, in one case material from a liver metastasis of a colorectal cancer was used. DNA was extracted using a QiAmp isolation kit (Qiagen GmbH).

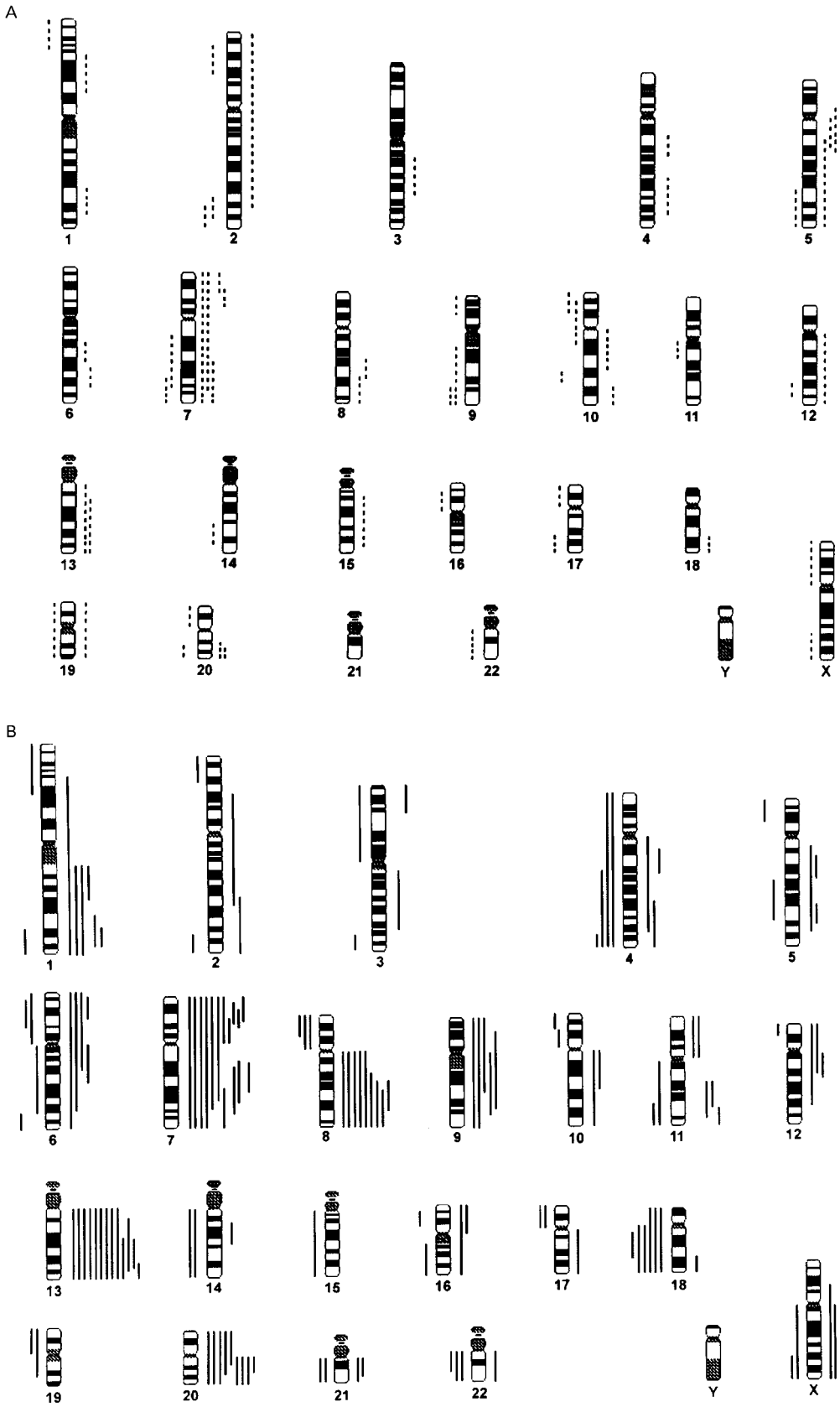

Figure 3 Frequency distribution of gains and losses of chromosomal material detected by comparative genomic hybridisation (CGH) in nine adenomas (A) and 14 carcinomas (B) of the colorectum. Lines left of each chromosome represent losses, and lines on the right represent gains.

Table 2 Mean number (and range) of chromosomal gains, losses and total number of events per tumour in nine colorectal adenomas and 14 colorectal carcinomas, as detected by comparative genomic hybridisation, and the p values from the Mann-Whitney U test for the significance of differences between the groups

	Adenomas (n = 9)		*Carcinomas (n = 14)*		
	Mean	*Range*	*Mean*	*Range*	*p Value*
Gains	3.6	0 to 7	8.2	2 to 16	0.003
Losses	3.0	0 to 8	3.7	1 to 8	NS
Events	6.6	1 to 11	11.9	5 to 17	0.008

All samples were analysed by CGH, and DNA ploidy was assessed in all cases using flow cytometry. Additional automated image cytometry was applied to cases that appeared diploid by flow cytometry, because the former is more sensitive for detecting small aneuploid cell populations. For the purpose of DNA cytometry, formaldehyde fixed, paraffin embedded material was used.

Histological type and grade of dysplasia of the adenomas were classified by one observer (GAM) according to generally accepted criteria.[9 10]

COMPARATIVE GENOMIC HYBRIDISATION

The CGH procedure is summarised in fig 1. One microgram each of tumour and of reference DNA were labelled by nick translation with biotin-16-dUTP and digoxygenin-11-dUTP (Boehringer Mannheim), respectively. Normal metaphase slides were prepared from PHA (phytohaemaglutinin) stimulated peripheral blood lymphocyte cultures from a healthy individual using standard procedures.

A 150 µg sample of both tumour and reference labelled DNA was mixed with 100 times the same amount of human Cot-1 DNA (Boehringer Mannheim), precipitated, and dissolved in 10 µl of hybridisation mix containing 50% formamide and 10% dextran sulphate in 2 × SCC (0.3 M sodium chloride/0.03 M sodium citrate) (pH 7). The Cot-1 DNA was added to block highly polymorphic repeat sequences. The probes and the normal metaphase slides were denatured simultaneously for 22 seconds in a 130 kW Phillips microwave oven at 60% power.[11] The hybridisation took place in a humid incubator at 37°C for at least four days.

After hybridisation, the slides were washed three times for five minutes in 50% formamide/2 × NaCl/sodium citrate (SSC) at 45°C, two times for five minutes in 2 × SSC at 45°C, and once for five minutes in TNT (100 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.05% Tween-20). The slides were then preincubated for 10 minutes at 37°C in blocking solution (0.5% blocking agent (Boehringer) dissolved in TNT), followed by a 60 minute incubation at 37°C with 12.5 µg/ml FITC (fluorescein isothiocyanate) conjugated avidin (Sigma) and 4 µg/ml TRITC (tetraethylrhodamin isothiocyanate) conjugated sheep antidigoxygenin (Boehringer Mannheim) in blocking solution. The slides were washed three times for five minutes in TNT and counterstained with 200 ng/ml DAPI (4,6-di-amino-2-phenylindole) in 2 × SCC. Finally, the slides were washed in 2 × SSC for five minutes, dehydrated in an ethanol series of increasing concentration, and sealed in 10 µl antifade solution (Vectashield, Vector Laboratories).

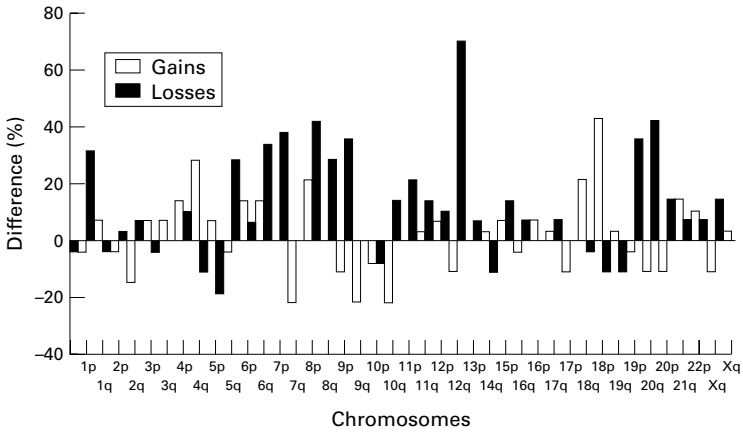

Figure 4 Differences in incidence of chromosomal gains and losses (%) between nine colorectal adenomas and 14 carcinomas. For example, a gain of 13q occurred in 22% of the adenomas and in 93% of the carcinomas, so a difference of 71% is displayed in the graph. Negative figures refer to events with a higher incidence in adenomas than carcinomas. This never exceeds ~15% and thus may mark the level of background noise.

IMAGE ACQUISITION AND ANALYSIS

Using a Zeiss Axioskop fluorescence microscope equipped with three separate band pass filters (for DAPI, FITC and TRITC, respectively) and a CCD (charge coupled device) camera (Cohu 4913, Cohu Inc), separate DAPI, TRITC, and FITC digital images were recorded from each metaphase. Interactive karyotyping of the chromosomes and calculation of the green to red fluorescence ratio of each chromosome was performed with the Cytovision CGH software package (Applied Imaging). For the chromosomes of 5–10 well selected metaphases, the averaged fluorescence ratios and their 95% confidence intervals (CI) were plotted along ideograms of the corresponding chromosomes in a so called relative copy number karyotype. Deviations from normal were interpreted as gains or losses when the 95% CI of the fluorescence ratio did not contain 1.0. An event was defined as gain or loss of (part of) a chromosomal arm. Results were validated by fluorescence in situ hybridisation with centromere specific probes on cytological preparations of the tumours.

DNA CYTOMETRY

Single cell suspensions for DNA ploidy analysis were prepared from all tumours using 50 µm thick sections cut from the routinely processed, 4% buffered, formaldehyde fixed and paraffin embedded tissue.[12] Tissue blocks with the highest concentrations of tumour were selected. The cell suspensions were divided for the purpose of both DNA flow cytometry and DNA automated image cytometry. The single cell suspensions for DNA flow cytometry were stained with DAPI and analysed with a PAS II (Partec Instruments) mercury lamp flow cytometer.

Cytospin preparations for DNA automated image cytometry were prepared from the cell suspensions by cytocentrifugation of 1 ml of suspension onto a glass slide for five minutes at

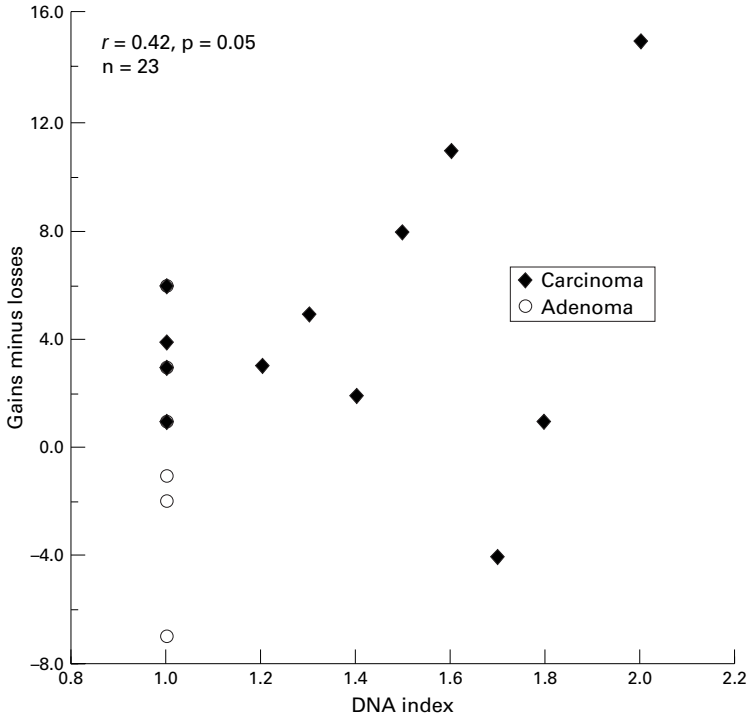

Figure 5 Correlation between the DNA index, assessed by DNA cytometry, and the number of gains minus the number of losses per tumour, as detected by comparative genomic hybridisation, in nine colorectal adenomas and 14 carcinomas.

1000 rpm and stained according to the (para-rosaniline) Feulgen procedure.[13] For DNA automated image cytometry, the pathology image processing environment (PIPE) system was used.[14] The PIPE system consists of a Zeiss Axioplan microscope equipped with an autofocus device, automated scanning stage, a Sony XC-77-CE CCD camera, and laboratory made dedicated software based on the SCIL Image (DIFA) image processing software. After shading correction and automatic segmentation, DNA content of at least 1000 (up to 4000) nuclei was automatically measured, based on the integrated optical density.[14] DNA histograms were analysed using the MultiCycle (Phoenix Flow Systems) cell cycle analysis software. The DNA index was determined as the ratio of the modal DNA value of the second G0/G1 phase peak to the modal DNA value of the first G0/G1 phase peak in the DNA histogram. If only one G0/G1 phase peak was present, a DNA index of 1.0 was assigned.

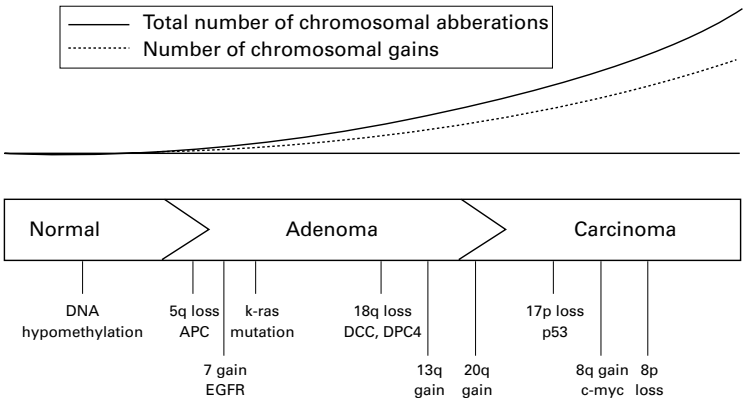

Figure 6 A model of chromosomal aberrations and possible candidate genes involved in the pathogenesis of colorectal cancer. In addition to the classical events (DNA hypomethylation, loss of 5q/APC, k-ras mutation, loss of 18q/DCC, DPC4, and 17p/p53), comparative genomic hybridisation and cytogenetic studies have pointed at the role of a gain of chromosome 7/EGFR, 13q, 20, and 8q/c-myc and a loss of 8p. Furthermore, the number of chromosomal aberrations increases with progression of neoplasia, and the number of gains is consistently higher than the number of losses.

STATISTICS

For continuous variables, means and ranges were computed. Means of continuous variables in different groups were compared using the Mann–Whitney U test. Correlations were analysed with Pearson's coefficient of correlation. Differences in incidences of gains or losses between adenomas and carcinomas were analysed using Pearson's χ^2. Probability (p) values < 0.05 were regarded as significant. All analyses were performed using the SPSS statistical software (SPSS Inc).

Results

Results for individual tumours are listed in table 1 and a frequency distribution of chromosomal gains and losses for adenomas and carcinomas is shown in fig 3. Overall, gains appeared more frequent than losses. The level of gains was mostly in the range of one to two extra copies (low level). Both gains and losses often involved large parts of, or even complete chromosome arms (fig 3).

Analysis of the 14 carcinomas revealed a significantly larger number of chromosomal events than in the nine adenomas (11.9 (5 to 17) *v* 6.6 (1 to 11), p = 0.008), as shown in table 2. While the number of losses did not differ significantly between carcinomas and adenomas (3.7 (1 to 8) and 3.0 (0 to 8), respectively), there was an increased number of gains

in the carcinomas compared with the adenomas (8.2 (2 to 16) *v* 3.6 (range 0 to 7), p = 0.003)). In the adenomas, gains occurred most often at 7p, 7q, and 5q, and no chromosome showing more than two losses occurred in this series (fig 3A). In the carcinomas, gains were seen most often at 13q, 7p, 7q, 8q, and 20q. Most frequent losses in the carcinomas occurred at 18q, 4q, 8p, and 18p (fig 3B). The differences between adenomas and carcinomas in incidence of chromosomal gains and losses are shown in fig 4. Most prominent was a 71% higher incidence of 13q gain in carcinomas compared with adenomas (p = 0.0005), while for 8q gain, 20q gain, and 18q loss this difference was 42% (p = 0.05, p = 0.05, and p = 0.02, respectively).

A clear association between the CGH results and DNA ploidy of the tumours was seen. The total number of events per tumour detected by CGH showed a significant positive correlation with DNA index assessed by DNA cytometry (r = 0.61, p = 0.001). Overall, DNA aneuploid tumours showed more gains than losses (mean 9.3 *v* 4.9, p = 0.02), in contrast with DNA diploid tumours (mean 3.1 *v* 4.1, p = 0.5), and DNA index showed a significant positive correlation with the difference between gains and losses in each tumour (fig 5).

Discussion

The most striking difference in chromosomal aberrations between colorectal adenomas and carcinomas, as detected by CGH, is an increase in the number of (low level) gains. The meaning of this observation in terms of tumour biology is not yet clear. The Knudson model of tumour suppressor gene inactivation provides a clue to explain the biological meaning of losses.[15] While high level gains often are associated with overexpression of oncogenes, the role of low level gains is not yet defined. However, this should be no reason to neglect these observations. One might argue that this increased incidence of gains of chromosomal material is merely the reflection of increased genomic instability. However, the fact that these gains show a non-random distribution favours the hypothesis that certain specific events like a gain of 13q especially, and also gains of 8q and 20q, provide a growth advantage to tumour subclones experiencing these events. As these gains are much more prevalent in carcinomas than in adenomas, they could be critical in the progression of premalignant adenomas to invasive carcinomas. Alternatively, the gains might not be important in themselves, but merely reflect a process that has concurrently caused chromosomal loss. Gain of 8q, for example, was often associated with loss of 8p, possibly because of isochromosome formation. Indeed studies on loss of heterozygosity have pointed to chromosome 8p as a site for putative tumour suppressor genes involved in colorectal cancer.[16–18] However, for acrocentric chromosomes like chromosome 13, isochromosome formation of the long arm is not likely to cause loss of tumour suppressor genes since these chromosomes do not have a functional p arm. Another explanation could therefore be that certain oncogenes already show a biologically relevant increase of expression with a gain of only one or two copies.[19] Increased expression in colorectal tumours of both epidermal growth factor receptor (EGFR) on 7p and c-myc on 8q support the importance of chromosomal gains in these tumours, and CGH studies of breast cancers have pointed at 20q13 as the site for an important oncogene.[20 21] Recently, gain of 20q13 was found to be associated with immortalisation in human papillomavirus transformed uroepithelial cells.[22] Less straightforward options must also be considered. For instance, with respect to 13q both amplification and increased expression of the retinoblastoma gene have been reported in colorectal carcinomas, while in general one prefers to think that loss of Rb is the event that counts since it is a well known tumour suppressor gene.[23 24]

Gains may not be sufficient for progression to malignancy. In colorectal cancer loss of 18q, where the DCC gene is located, is also a major event at this stage of tumour progression. In the present study 18q loss was the most prominent loss detected, showing a clear rise in incidence in carcinomas as compared with adenomas. One would also expect loss of p53 function to play a role, and although loss of 17p was seen with CGH in some carcinomas, the incidence was lower than would be expected. This may mean that loss of p53 function occurred by mutations (which cannot be detected by CGH), or by small deletions which are below the detection threshold of CGH.

In the present study, loss of 5q was an infrequent finding. This may seem surprising considering the crucial role that loss of the APC tumour suppressor function on 5q is purported to play early in the adenoma–carcinoma sequence. However, this low frequency of 5q loss is consistently found in several CGH and cytogenetic studies of both colorectal adenomas and carcinomas.[19 25 26] Some studies have not found any loss of 5q at all.[27 28] This could mean that inactivation of APC would mainly occur at a subchromosomal level (for example, mutations or microdeletions), or that the incidence of loss of APC function is lower in some series of colorectal tumours than is currently thought. This issue requires further investigation.

It is known that CGH has some limitations in detecting aberrations at certain chromosomal regions with a high concentration of repetitive DNA sequences like 1p34-36, 16p, and chromosome 19, the profile rations of which should be interpreted with care.[29] Since 1p34-36 has been reported to be commonly involved in colorectal tumours,[30] additional techniques like in situ hybridisation with target specific probes may be needed to obtain unequivocal information for this region.[31]

In our present study most gains and losses detected appeared to involve major parts of chromosomes and often whole chromosome arms. This could indicate that (peri) centromeric breakage is an important event in the pathogenesis of these tumours, as has previ-

ously been described for head and neck tumours.[4]

The results of our study are not isolated observations. However, comparison of the present data with those of other studies of genomic changes in colorectal cancer should be undertaken only with care. CGH is a powerful tool which provides the possibility of obtaining an overview of changes in chromosomal copy number in tumour material, without the need for cell culturing, thus enabling the analysis of chromosomal instability in a given tumour. This feature distinguishes CGH from other more "single target" molecular genetic techniques like FISH and loss of heterozygosity analysis, and in this respect CGH is only comparable with karyotyping. The latter, however, does require cell culturing, and although it has the additional advantage of detecting structural chromosomal changes, this prerequisite makes it difficult to apply in the "routine" pathology laboratory. As discussed elsewhere in more detail, these techniques should therefore be regarded as complementary and the one cannot be used unconditionally as a control for the other.[32]

CGH results can to some extent be compared with results of karyotyping, although here selection during cell culturing can occur. Probably the best control of CGH results should come from independent studies. Our present study provides the second series of colorectal adenomas and carcinomas analysed by CGH, and when the results are compared with those of the first series of colorectal adenomas and carcinomas analysed by CGH, the agreement in chromosomal aberrations detected indeed is striking.[19] As in the present study, frequent gains were found at 13q, 7p and q, 8q, and 20q, whereas losses most often occurred at 18q, and also at 4q, 8p, and 18p. Another study yielded similar CGH results in colorectal carcinomas, but no adenomas were analysed and the assessment of fluorescence ratios was primarily performed subjectively.[25] In addition, the results in these CGH studies are in overall agreement with cytogenetic studies of large series of colorectal tumours, which found non-random gains at chromosome arms 7p, 7q, 8q, 13q, and 20q, and losses at 8p, 17p, 18p, and 18q.[26 33] This certainly strengthens the impact of these CGH observations.

The results of DNA cytometry are in agreement with the CGH findings, further strengthening their import. In the DNA diploid tumours in this study (including all the adenomas), the number of gains and losses did not differ significantly, whereas DNA aneuploid tumours showed more gains than losses, thus explaining the increased DNA index. The fact that all DNA diploid tumours already showed chromosomal aberrations is in agreement with the observation that genomic instability is not caused by aneuploidy, but most probably precedes it.[3] Still, some of the tumours that were DNA diploid in our present study could have shown DNA aneuploidy if fresh material had been available. Aneuploid adenomas most often show rather low DNA indices, which can more easily be detected in fresh samples than in formaldehyde fixed, paraffin embedded samples, like the ones that had to be used in this study. In addition, most adenomas showed mild or moderate dysplasia only with tubular histology (table 1), while DNA aneuploidy is more prevalent in adenomas with severe dysplasia.[34]

Current ideas on the genomic changes involved in colorectal carcinogenesis have been mainly based on the model proposed by Fearon and Vogelstein.[1] However, CGH studies including the present study, and cytogenetic studies of colorectal tumours, have provided interesting additional information with respect to the genetic changes associated with colorectal cancer. In addition to the events outlined in Vogelstein's model, other chromosomal changes—and especially gains of 7, 8q, 13q, and 20q—consistently appear to play a role in the genesis of colorectal carcinoma. Cytogenetic studies had already suggested this, but CGH has shown that these gains occur at even higher frequencies. These studies have also provided information to map some of these events along the adenoma–carcinoma sequence. Gain of chromosome 7 commonly occurs in adenomas.[19 35] Gain of 13q is also found in a minority of adenomas (22% in the present study), but it becomes the most frequent event in carcinomas (93% in the present study, figs 3 and 4), indicating that it might play an important role in the transition from adenoma to carcinoma. Gain of 8q and 20q also seem to occur with much higher frequency in carcinomas than in adenomas, indicating that they might be late events in the adenoma–carcinoma sequence. In the same way, cytogenetic studies of adenomas reported gain of chromosome 20 in only 17% and 8% of adenomas respectively.[27 28] Gain of 8q was also an infrequent event in cytogenetic studies of adenomas.[27 28]

Combining this information, putative additional genetic events can be mapped to the adenoma–carcinoma sequence model of colorectal carcinogenesis (fig 6). One should keep in mind however, that this model presents a general picture, while differences may occur between individual tumours, and multiple pathways towards colorectal cancer probably exist.[36]

We conclude that the most striking difference between chromosomal aberrations in colorectal adenomas and carcinomas, as detected by CGH, is an increased number of chromosomal gains that show a non-random distribution. Gains, especially of 13q and also of 20q and 8q, seem to be involved in the progression of adenomas to carcinomas, possibly because of low level overexpression of oncogenes at these loci.

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