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Genome-wide Search for Chromosomal Aberrations in Colorectal Cancer: Implications for Pathogenesis and Clinical Management

Georgia Bardi[1], Sverre Heim[2]

SUMMARY

In the present review, we discuss the cytogenetic findings we have made in karyotyping studies of colorectal cancer examined during a 10-year-period at two Scandinavian institutions (Lund University Hospital, Sweden and Odense University Hospital, Denmark) as well as relevant findings by comparative genome hybridization (CGH) since this method too provides a global overview of genomic alterations. Our total series consists of 280 cytogenetically investigated colorectal tumors, of which 82 were benign lesions (mostly adenomas), 162 were primary carcinomas, and 36 were metastases. The cytogenetic studies of these tumors enabled us to: 1. identify early, possibly initiating, genetic events in colorectal tumorigenesis; 2. determine the clonal relationship among synchronously growing, macroscopically distinct colorectal adenomas as well as between carcinomas and polyps growing in the same patients; and 3. describe the cytogenetic make-up of metastatic lesions by comparing karyotypically primary tumors and their local and distal metastases in individual patients. The correlation of tumor karyotypes with clinicopathologic parameters in the series, enabled us to demonstrate considerable genetic heterogeneity in colorectal tumors with distinct cytogenetic subgroups corresponding to at least two oncogenetic pathways in sporadic large bowel cancer, as is known to be the case also in hereditary colorectal tumors. Finally, the karyotypic pattern could provide valuable and in some instances unique information about the prognosis of colorectal cancer patients and, hence, clues as to how they should best be treated.

[1]Department of Genetics, "G. Papanikolaou" Research Institute, "Saint Savas Oncological Hospital of Athens, Greece, and [2]Department of Cancer Genetics, The Norwegian Radium Hospital, Oslo, Norway

Author for correspondence:

Georgia Bardi, 171 Alexandras ave. 11522 Athens, Greece
Tel: +30 210.6409168, e-mail: gbardi@hotmail.com

INTRODUCTION

In spite of the more detailed understanding of the cellular and molecular events underlying colorectal carcinogenesis obtained in recent years, the average 5-year survival rate of patients suffering from this disease remains about 40%[1]. It is therefore clear that the extensive new knowledge about the structure, function, and interaction of key genes in large bowel tumourigenesis has not yet brought about corresponding improvements in the clinical handling of patients, especially as regards the sporadic type that constitutes more than 85% of all colorectal cancers. The reasons for this are undoubtedly complex, but one important aspect of it is, in our opinion, a failure to comprehend fully the complexity and genetic heterogeneity that characterize colorectal malignant tumours. Evidence now exists[2] that the standard, unitarian model of colorectal carcinogenesis, depicting the process as a linear, stepwise accumulation of mutational events, beginning with the APC (adenomatous polyposis coli) mutation is unable to account fully for the initiation and progressional stages of tumourigenesis in many non-APC types of colorectal cancer. Furthermore, the vast majority of studies seeking to unravel the acquired genomic changes of large bowel tumour cells were essentially reductionist[3], focusing exclusively on gene-level alterations without taking into account the numerous coexisting genomic abnormalities at higher organizational levels, in particular numerical and structural chromosomal abnormalities, and without due recognition of the extensive cell-to-cell variability seen in

the neoplastic parenchyma. In only very few studies[4,5] have genetic methodologies been combined to provide simultaneously detailed information about changes at the gene level and a global overview of the genomic profile in individual tumours, let alone in individual cells within that tumour. In addition, most series of genetically characterized colorectal cancers are insufficiently accurate when it comes to the clinical and histopathological description of the tumours. Finally, very few attempts have been made to prepare for metaanalysis genetic information about colorectal tumours obtained by different groups of researchers, even when they utilized the same methodological approach.

In the present review, we discuss the cytogenetic findings we have made in karyotyping studies of colorectal cancer examined during a 10-year-period at two Scandinavian institutions (Lund University Hospital, Sweden and Odense University Hospital, Denmark) and using the same investigative techniques. We also discuss relevant findings published by other groups, including data obtained by comparative genome hybridization (CGH) since this method, too, although, in several ways it differs both principally and in practice from karyotypic analysis, has screening qualities and provides a global overview of genomic alterations.

The original data on which this review is based were published between 1991 and 2001.[6-20] Our total series consists of 280 cytogenetically investigated colorectal tumours, of which 82 were benign lesions (mostly adenomas), 162 were primary carcinomas, and 36 were metastases. The cytogenetic studies of these tumours enabled us to: 1. identify early, possibly initiating, genetic events in colorectal tumourigenesis; 2. determine the clonal relationship among synchronously growing, macroscopically distinct colorectal adenomas as well as between carcinomas and polyps growing in the same patients; and 3. describe the cytogenetic make-up of metastatic lesions by comparing karyotypically primary tumours and their local and distal metastases in individual patients. The correlation of tumour karyotypes with clinicopathologic parameters in the series, enabled us to demonstrate considerable genetic heterogeneity in colorectal tumours with distinct cytogenetic subgroups corresponding to at least two oncogenetic pathways in sporadic large bowel cancer, as is known to be the case also in hereditary colorectal tumours. Finally, the karyotypic pattern could provide valuable and, in some instances, unique information about the prognosis of colorectal cancer patients and, hence, clues as to how they should best be treated.

RECURRENT CHROMOSOME ABERRATIONS IN COLORECTAL TUMOURS

In total, 67 references exist in the scientific literature, presenting altogether 534 cytogenetically investigated large intestine epithelial tumours, of which more than half were examined by our group. Of the total published cases, 139 were classified as adenomas, 350 were adenocarcinomas, and 45 were listed as carcinomas not otherwise specified[21]. Clonal chromosome aberrations were detected in up to 37.5% of the non-adenomatous polyps examined, in 80% of the investigated adenomas, and in up to 90% of the primary carcinomas and in all metastatic cancers reported.

The systematic cytogenetic studies behind these numbers have provided important information that in part corroborates and extends the molecular genetic model of colorectal carcinogenesis proposed by Fearon and Vogelstein in 1990. In part, however, the discoveries made have been unexpected and have yielded novel insights into the processes of initiation and progression of colorectal cancer.

The main conclusion reached by karyotyping of colorectal neoplastic cells is that benign and malignant lesions of the large intestine display characteristic patterns of acquired chromosomal abnormalities. Although no single cytogenetic aberration can be said to distinguish colorectal adenomas from carcinomas with absolute certainty, in groupwise comparisons adenomas come across as karyotypically much more simple than their malignant counterparts.

Cytogenetic analysis in the form of karyotyping of banded chromosomes remains the only methodological approach capable of detecting genome-wide aberrations, including both balanced and unbalanced chromosomal changes involving euchromatic as well as heterochromatic regions, and also of detecting cell-to-cell genomic variation within a tumour. However, even this technique has some distinct drawbacks, the principal of which is the inevitable selection bias in favour of cycling tumour cell populations that it entails; cells that do not readily divide cannot be processed for metaphase analysis. Karyotyping, furthermore, only yields information at the microscopic resolution level, it is time-consuming and requires highly skilled and trained investigators, of whom there are not many around. It also requires fresh tumour samples and (mostly) tissue culturing prior to the analysis of tumour cells. The development of a wide array of fluorescence in-situ hybridization-based techniques in recent years has enabled a marriage of conventional

cytogenetics (karyotyping of banded chromosomes) with molecular genetics, and the resulting molecular cytogenetic techniques have greatly facilitated the genetic analysis of solid cancers. In particular, comparative genomic hybridization (CGH) has overcome many of the drawbacks of standard cytogenetics, as it avoids the need for fresh biopsies and tissue culturing. In a single experiment, CGH may screen the entire genome of a tumour for gains and losses of genetic material, even if the sample is from archival material. However, CGH cannot detect rearrangement other than those leading to imbalances of euchromatic chromosomal regions; all balanced aberrations and all minor clones remain undetected. With the aforementioned limitations, CGH has been performed on several series of colorectal tumours consisting of adenomas, primary carcinomas, and metastases. The aberration pattern thus revealed has substantially contributed to our understanding of the cytogenetics of colorectal carcinogenesis, modifying and expanding the picture arrived at on the basis of karyotyping studies alone.

Colorectal carcinomas

In our experience, as well as in all other major published series of cytogenetically investigated colorectal carcinomas[22-24], clonal aberrations were found in the vast majority of cases. However, a small fraction of tumours, perhaps 10%, do appear to have a normal chromosome complement. Some of these tumours may have only submicroscopic genomic rearrangements or the examined cells may have been of stromal origin; once a cell enters mitosis and is taken through the steps necessary for chromosome analysis, it is no longer possible to determine its phenotype. That stromal or normal epithelial admixture in primary cultures of colorectal carcinomas represents a real problem is evident from the fact that the growth fraction of tumour parenchyma cells may be as low as 2%.[25] However, at least to some extent, this problem can be overcome, as demonstrated by the increased percentage of cytogenetically abnormal cases seen when the culturing techniques are optimized. Whatever the explanation behind the finding of normal karyotypes in colorectal cancer cultures, these normal karyotypes seem to have some kind of biological importance, inasmuch as patient survival in these cases tends to be longer (see below).The modal chromosome number of colorectal carcinomas displaying clonal chromosome aberrations varies from hypo- or neardiploid (Figure 1) to near-pen-

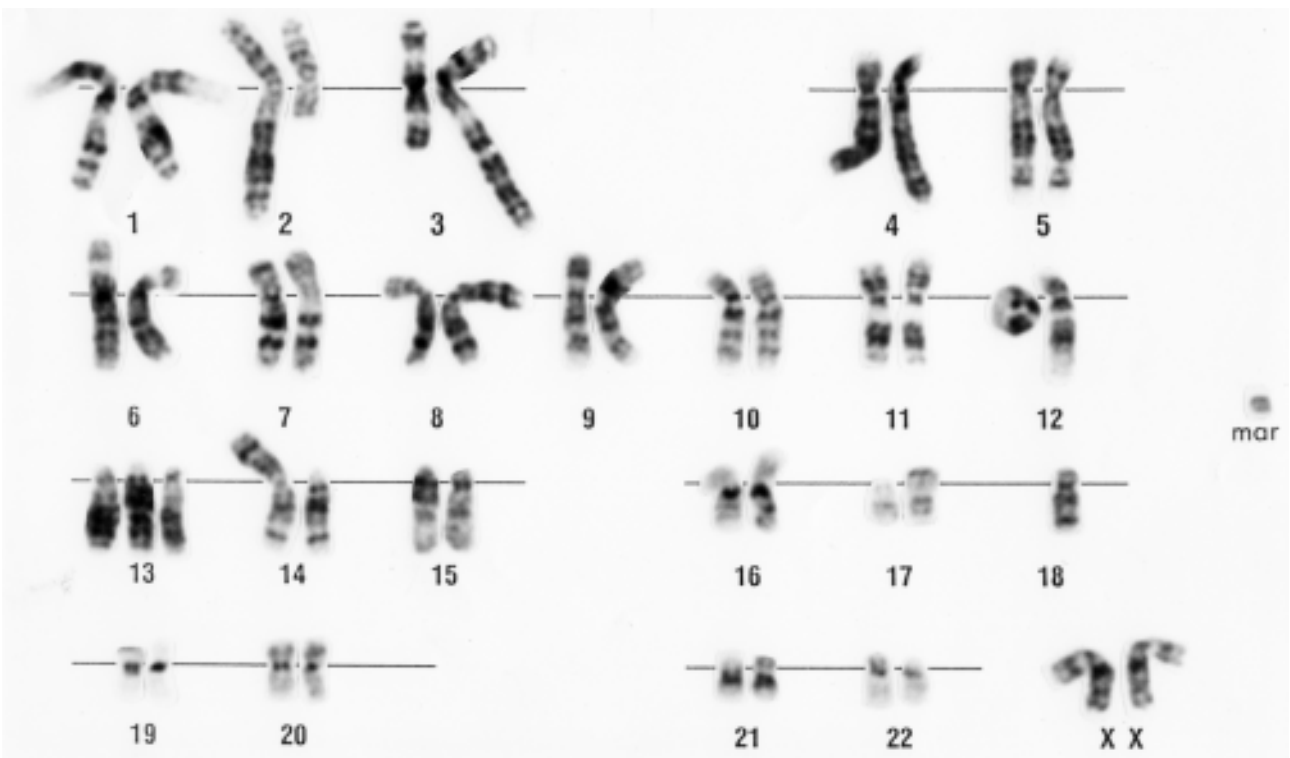

Figure 1. Representative karyogram from a colon adenocarcinoma with a neardiploid karyotype (47 chromosomes) and several numerical and structural chromosome changes

taploid. More than half of the cases have had neardiploid or pseudodiploid karyotypes, which is consistent with the finding of diploidy in several DNA flow cytometry studies. Evidently, among flow cytometrically normal cases may not only be tumours with normal karyotypes or one or few numerical and/or structural changes, but even tumours with extensive chromosomal rearrangements leading to no or only small changes in the total DNA content. Carcinomas with a near-triploid chromosome number usually have both numerical and structural rearrangements, although a few such tumours with numerical abnormalities only have been described.

The most common numerical aberrations in our material have been loss of chromosome 18, gain of chromosome 7, gain of chromosome 20, loss of chromosome 17, gain of chromosome 13, and loss of chromosomes 14 and 22. When all reported cases with clonal aberrations were pooled, it turned out that the most common numerical aberrations remained the same but a certain difference in the reported frequencies was apparent; in decreasing order of frequency the most common numerical changes were monosomy 18 and trisomy 7, followed by trisomy 13, monosomy 14, trisomy 20, and monosomy 22 (Figure 2).

With the exception of the Y, all chromosomes have been seen to be involved in structural rearrangements in colorectal carcinomas. The breakpoints recurrently involved in such rearrangements were mapped to no less than 160 chromosome bands, but at highly variable fre-

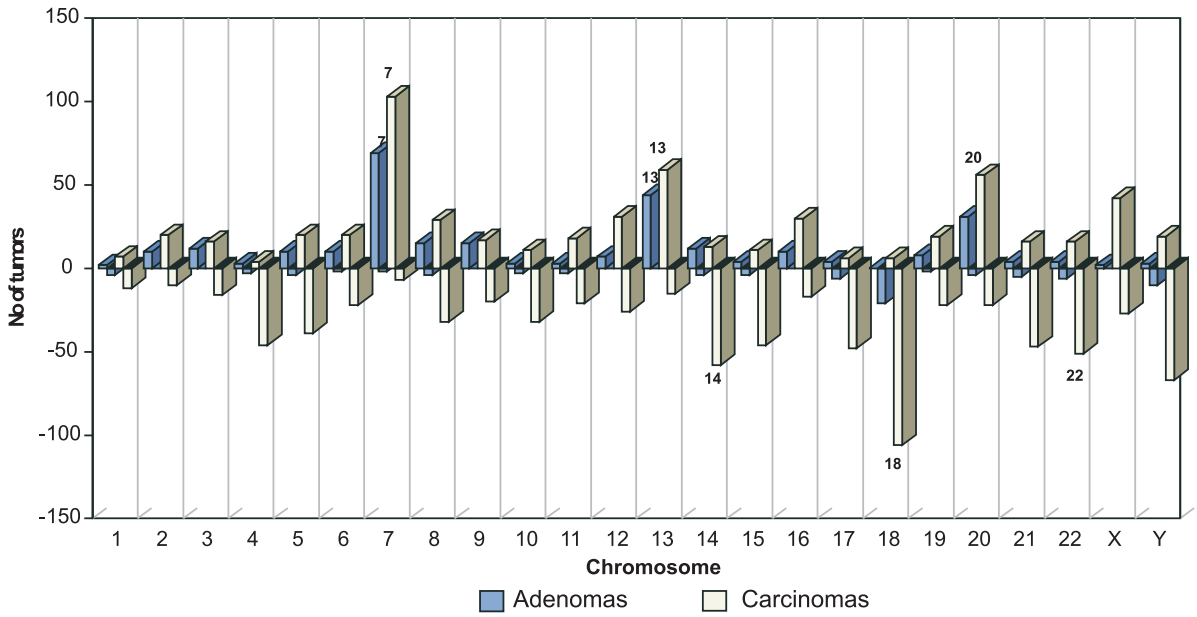

Figure 2. Whole-chromosome imbalances in colorectal adenomas and carcinomas

quencies in different studies. In our series of tumours, breakpoint clusters were observed at the centromeric regions of chromosomes 1, 8, 13, and 17 as well as at bands 1p22, 1q12, 3q13, 7p22, 7q11, 7q32, 11q13, 12q13, 12q24, 16p13, 19p13, and 20p13. When all data available in the literature are considered, the most marked clustering of breakpoints is seen at 8q10, 13q10, 17q10, and 18q21, followed by 1q10 and 5p10 (Figure 3). The most frequently rearranged are 8q10 and 17q10 (12% and 11% of all karyotypically abnormal tumours, respectively) followed by 13q10 (7%), 18q21 (5%), 1q10 (4%), 5p10 (in 3%), and 1p22, 11q23, 16p12, 5q31, and 6q13 (2% each). Several other chromosomal breakpoints were recurrently involved in 1.5% or less of the total number of cytogenetically examined colorectal carcinomas.

The most common structural aberrations in our series (198 tumours) were i(8)(q10), i(17)(q10), del(17)(p11), i(13)(q10), i(1)(q10), and del(1)(p13). When all reported data on colorectal carcinomas are considered, giving a total of 395 tumours, i(8)(q10) and i(17)(q10) remain the most common rearrangements found in about 10% of the carcinomas published to date, followed by del(17)(p11or p12) (8%) and i(13)(q10) and del(18)(q21) (5% each). The net outcome of these rearrangements is loss of 8p and gain of 8q, gain of 13q, loss

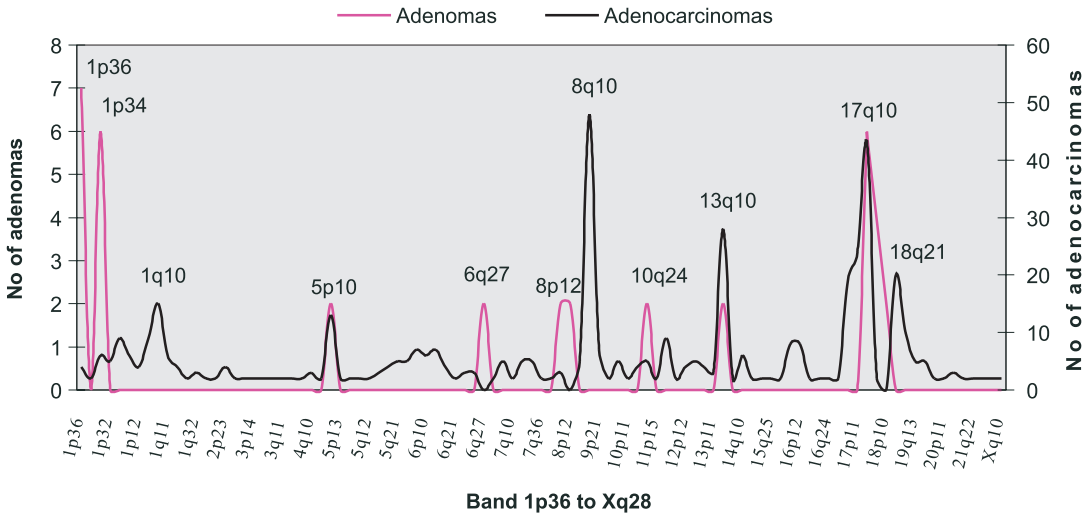

Figure 3. Distribution of recurrent imbalances in colorectal adenomas and adenocarcinomas.

of 17p, and gain of 17q. Although the aforementioned anomalies were seen repeatedly in all major published series of cytogenetically characterized colorectal carcinomas, the reported frequencies varied considerably. Technical and stochastic factors undoubtedly play a role in bringing about this variability, but systematic differences related to the composition of the series examined could also be important, in particular with regard to possible etiologic and pathogenetic differences among different groups of patients.

The consistent losses of genomic material in colorectal cancer presumably exert their pathogenetic influence through loss of tumour suppressor genes, but the effect of gains remains elusive, even for genomic loci undergoing amplification. Using combined CGH and DNA microarray expression profiling, Platzer et al[5] recently examined the expression of over 2000 genes situated on chromosome arms 7p, 8q, 13q, and 20q, i.e., genomic areas which, in their study, were consistently found amplified in metastatic colorectal cancers. Interestingly, for 96.2% of the genes located in areas of chromosome amplification did not show up-regulation of expression.

Colorectal adenomatous and hyperplastic polyps

The large bowel offers unique possibilities to evaluate all stages of carcinogenesis inasmuch as the earliest benign precursor lesions, the adenomas, can be identified and sampled with relative ease. Chromosome aberrations have now been reported in 139 such adenomas. The first studies left the impression that gains of whole chromosomes were the only, or at least the predominant, aberrations. As data from new and larger series were added, the consensus picture of the karyotypic characteristics of benign colorectal tumours has undergone considerable refinement. In our experience, based on the cytogenetic examination of 82 colorectal polyps, up to 80% of large bowel adenomas carry clonal chromosome aberrations. Other investigators have reported lower percentages of cytogenetically abnormal lesions, from 30 to 50%. In all series, however, most karyotypically abnormal polyps (90% in our material) had only few cytogenetic abnormalities giving rise to pseudo- or near-diploid karyotypes. The remainder are near-triploid or near-tetraploid with massive chromosome changes, often showing anomalies that are recurrent in colorectal adenocarcinomas as well. At present, therefore, one cannot point to any single karyotypic feature that is capable of distinguishing unequivocally between benign and malignant colorectal tumours. In groupwise comparisons, however, the adenomas come across as karyotypically much more simple than their malignant counterparts.

The most frequent chromosome abnormality in colorectal polyps is +7 (Figure 4), found in approximately 50% of all reported cases (Figure 2), mostly as the sole anomaly. Although the pathogenetic relevance of +7 in both large bowel neoplasms as well as tumours of other tissues has been questioned[6,26], the finding of this trisomy in the epithelial component of adenomatous polyps[11,15-17,19] by chromosome banding analysis of metaphase cells and by in situ hybridization with centromere-specific probes in interphase cells supports the early suggestion that it plays a primary role in some colorectal neoplasms. A gene called DRA, from down regulated in adenomas, has been localized to 7q22-q31.1 and shown to be ex-

pressed specifically in colon mucosa. Overexpression of this gene could be an important outcome of trisomy 7. Among other candidate genes are the MUC3 and MET oncogenes that map to 7q22. The actual pathogenetic involvement of any of these gene loci is still entirely speculative, however, as is indeed the very mechanism by which the presence of an extra copy of any particular chromosome might translate into a shift in growth potential sufficient to precipitate neoplastic transformation.

Gain of chromosome 13 is the second most common numerical abnormality in large bowel adenomas and has been found in about 30% of the cases. Then, in decreasing order of frequency, follow gain of chromosome 20 (22% of the cases), loss of chromosome 18 (15%), and gain of chromosomes 8 and 9, each seen in 10% of the cases. Each of these changes has occasionally been detected as the only anomaly. Whereas loss of chromosome 18 might work through loss of one DCC allele, the important molecular result of the other whole-chromosome imbalances is unknown.

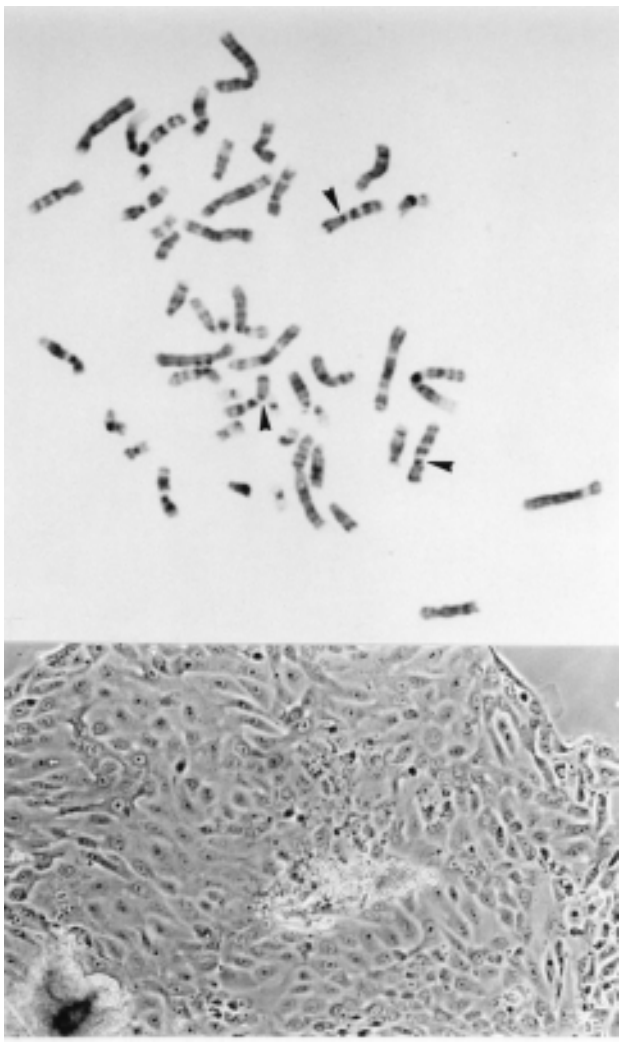

Figure 4. Metaphase fron a colon adenoma with trisomy 7 as the only cytogenetic anomaly (upper part). Arrowheads indicate the three copies of chromosome 7. Inverted microscopic picture the epithelial morphology of an adenoma (lower part) with trisomy 7 as the only chromosomal change.

The necessity to culture the tumour cells prior to G-banding investigation and also the fact that different clones are likely to enter mitosis at different rates, reduce the information value of cytogenetic analysis regarding the presence and relative size in vivo of clones with numerical changes. To better estimate the clonal composition of colorectal adenomas, we therefore performed interphase fluorescence in situ hybridization (FISH) analyses with probes for chromosomes 1, 7, 13, and 20 in a series of previously karyotyped adenomas. Gains of chromosomes 7, 13, and 20 were found in 32-44% of the adenomas, verifying that these trisomies are indeed common and that they occur in vivo. Although gain of chromosome 7 usually preceded the other gains in those instances where this could be assessed, this was not always so. Evidently, the end result of the acquired chromosomal imbalances rather than the sequence in which they occur is of essence in colorectal tumourigenesis.

A deletion of part of the short arm of chromosome 1 (Figure 5) is the most common structural rearrangement in intestinal polyps. We have seen it in 30% of all cases with an abnormal karyotype, often as the only anomaly, which led us to suggest that this is an early, possibly primary, genetic change in the development of large bowel tumours[9]. The finding was later confirmed by a combined cytogenetic and molecular genetic approach[18]. The latter study also showed that a minimal region of overlap seemed to map to between markers D1S199 and D1S234 in band 1p35-36, suggesting that this may be the site of the hypothetical tumour suppressor gene presumed to be lost in the deletion. This genomic area contains the human homologue of the tumour modifier gene Mom1 (1p35-36.1) which, in mice, modifies the number of intestinal tumours in multiple intestinal neoplasia (Min)-mutated animals. Using FISH, we could also show that the deletions in 1p were interstitial[18]. Corroborative evidence that this area in 1p is important in colorectal tumourigenesis came from the study by Tanaka et al.[27], who showed that the introduction of a normal 1p36 segment into a colorectal carcinoma cell line rendered it non-tumourigenic.

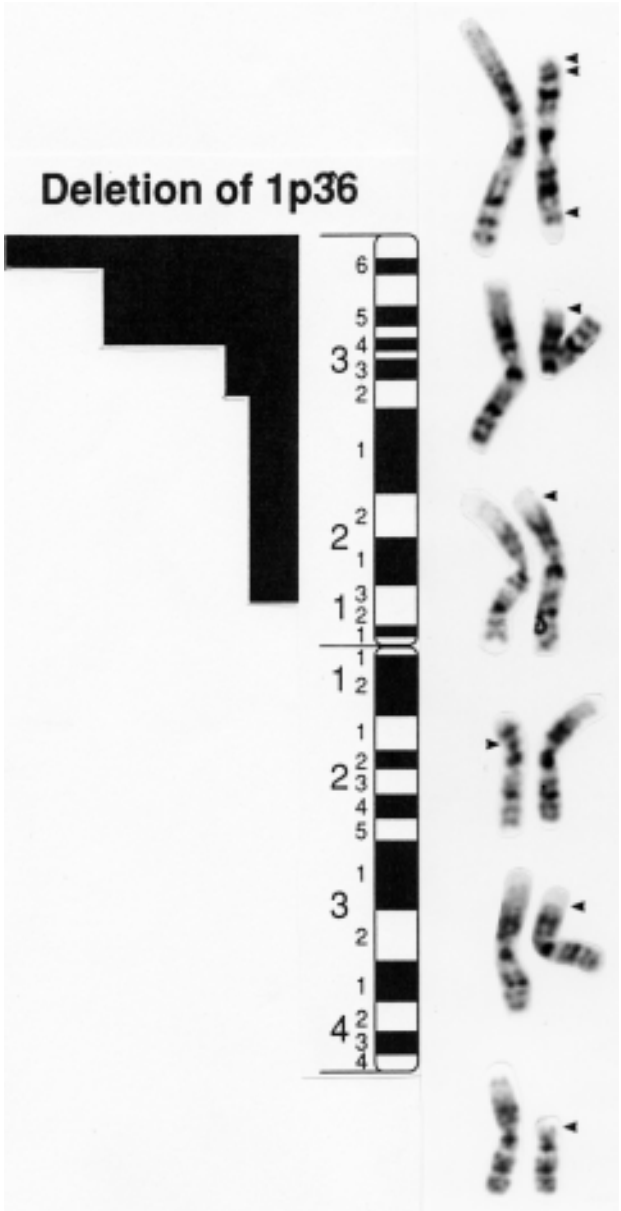

Figure 5. Partial karyotypes from 6 benign colon lesions showing 1p deletions of various size (right part). Ideogram of chromosome 1 illustrating the size of the deleted 1p segments. 1p36 is the minimum deleted segment is all cases.

Among the benign colorectal tumours with loss of 1p36 are not only adenomas but also hyperplastic polyps, i.e., lesions without any cellular atypia, and indeed more than one third of all investigated hyperplastic large bowel polyps have been shown to harbor clonal chromosomal abnormalities. Whether these polyps, too, have a tendency to progress toward carcinomas is not yet clear, but the cytogenetic data unequivocally indicate that they are genuine neoplasms with karyotypic features similar to those of small tubular adenomas. Rashid et al.[28], in a genotype-phenotype retrospective analysis of 129 hyperplastic polyps, identified allelic loss of 1p (1p32-1p36) as the most frequent genetic alteration. It is of interest that in that series, patients with 1p allelic loss had hyperplastic polyposis (>more than 20 hyperplastic polyps) and some of them had, in addition, family members with colorectal cancer. The authors therefore suggested, based on these findings, that 1p could be the "starting point' of a hyperplastic polyp- adenocarcinoma sequence.

Deletions of the short arm of chromosome 1 are also seen in large bowel carcinomas although, relatively speaking, not as frequently and often with a larger segment lost, as in adenomas. Usually the del(1p) in carcinomas is accompanied by several other anomalies, but cases also exist in which it was the only karyotypic change. Rests of adenomatous tissue were then often found in the tumours, and it has been suggested that the cells showing del(1p) as the only anomaly actually grew from these remnants.[12]

Based on the finding, in an allelic imbalance study, that colorectal carcinomas lacking 1p deletion in the primary tumour acquired such changes in their metastatic lesions, Thorstensen et al[29] suggested that loss of 1p35-36 is of importance both early and late in colorectal tumourigenesis. We share the view recently expressed by Couturier-Turpin et al[30] that one needs to distinguish between loss of 1p occurring in pseudodiploid cells and mostly as the sole cytogenetic change, which appears to be of importance in the initiation of carcinogenesis, and loss of 1p found in massively aneuploid cells, in which case the deletion may have been acquired as a result of complex chromosomal rearrangements occurring during tumour progression.

CLONAL RELATIONSHIP AMONG SYNCHRONOUSLY GROWING COLORECTAL TUMOURS

Synchronous adenomas

The phenotypic progression of colorectal tumours is driven by their step-by-step acquisition of genomic alterations which not only signify important changes in carcinogenesis, but also constitute highly informative markers of tumour clonality. In a series of 24 adenomas from 11 patients, we used chromosome banding analysis to examine the clonal relationship among synchronously growing, macroscopically distinct colorectal adenomas [16,17]. The main question we wanted to answer was whether these polyps had karyotypic similarities or whether the clonal

findings were unrelated, indicating that they arose independently. In 6 patients, we found similar clones in separate polyps within the same patient, polyps that were always located in the same part of the large bowel. In the remaining 2 patients, both with one rectal adenoma and one adenoma in the colon, no karyotypic similarity between the lesions was found. The findings indicate that when macroscopically distinct, synchronous adenomas are growing in the same part of the large bowel, they are karyotypically similar, in contrast to when they grow in different parts (colon, rectum, sigmoid). In the latter situation, they are macroscopic manifestations of parallel but pathogenetically independent neoplastic processes. In the former situation, on the other hand, two explanations are possible. Either the same etiologic agent has elicited identical pathogenetic responses from several cells within the same anatomical area (i.e., they have acquired the same chromosomal aberration, probably because that particular etiologic stimulus tends to induce one and the same genomic response), or the morphologically distinct but close adenomas are, in fact, seemingly separate lesions belonging to the same clone of neoplastically transformed cells.

Synchronous polyps and carcinomas

Many colorectal carcinomas arise from visible benign precursor lesions, adenomas, in what has been termed the adenoma-carcinoma sequence[31]. Most adenomas do not transform malignantly, however, in spite of the dysplastic changes that invariably characterize their epithelial component. Other carcinomas arise de novo, i.e., without a visible precursor lesion. In addition to adenomas, hyperplastic polyps are tumourous yet benign, and in most instances presumably nonneoplastic, lesions frequently seen in the colon and rectum. The identification of genetic similarities and differences between adenomas and carcinomas, and also between polyps that tend to and those that do not tend to transform to malignancy, is likely to shed light on the mechanisms driving tumourigenesis in the large intestine. The developmental relationship among various tumourous lesions present at the same time in the same patient is another interesting issue; are they clonally related or not?

To approach these questions, we cytogenetically analyzed 30 tumourous lesions of the large bowel, including carcinomas, adenomas, and non-adenomatous polyps, from 7 patients with colorectal cancer[13]. We found clonal chromosomal abnormalities in all adenomas and carcinomas, but only in 37.5% of the non-adenomatous polyps. Whereas the majority of hyperplastic polyps displayed a normal chromosome complement, some showed clonal aberrations that in general seemed to be simpler than those of dysplastic polyps. It is possible that the subset of hyperplastic polyps with cytogenetic aberrations may have had small dysplastic areas that went undetected by conventional histological examination, or the chromosome aberrations they carry, which are indistinguishable from those of small tubular adenomas, are not dysplasia-specific but rather related to the hyperproliferation taking place in the intestinal mucosa. Finally, the very occurrence of clonal chromosome aberrations in a proportion of hyperplastic polyps, evidence that these lesions are neoplastic, could be viewed as the genetic corollary of a hyperplastic polyp-carcinoma sequence even in the absence of corresponding histopathological or clinical indications to this effect. Leggett et al.[32] recently confirmed our earlier finding that some hyperplastic polyps do have genetic changes by reporting microsatellite instability in 3 and k-ras mutations in 8 of 47 hyperplastic polyps examined.

Although some chromosome aberrations were found in carcinomas but not in adenomas, for example der(8;17)(q10;q10), indicating that they may be specifically associated with malignant transformation of large bowel mucosa, adenomas and carcinomas occurring simultaneously in the same patient by and large shared most of their chromosomal features (Figure 6). This karyotypic similarity between the malignant and benign tumours in the same patient, and also sometimes among non-malignant polyps in the same individual, can be interpreted to indicate that the macroscopically distinct lesions arose as part of a single clonal expansion, in spite of the fact that the distance between them was more than 3cm. The only alternative explanation would be that the same oncogenetic environmental factor induced identical chromosomal rearrangements in more than one cell.

Ried et al[33] using CGH confirmed that the frequency and degree of genetic aberrations increases with progression from low-grade adenoma through high-grade adenoma to carcinoma. Only three of the 14 low-grade and five high-grade adenomas showed chromosome abnormalities by CGH, compared with 14 of 16 carcinomas in their study. The most frequently gained chromosome regions found by them were 20q, 13q, 8q, 7p, and 7q, whereas frequent losses were observed from 8p, 18q, 4q, and 17p. Furthermore, the frequency of specific alterations increased with advancing stages of tumour progression. Also Meijer et al.[34] found that the average number of chromosomal aberrations increased from adenoma to carcinoma; frequent gains involved 13q, 7p, 7q, 8q,

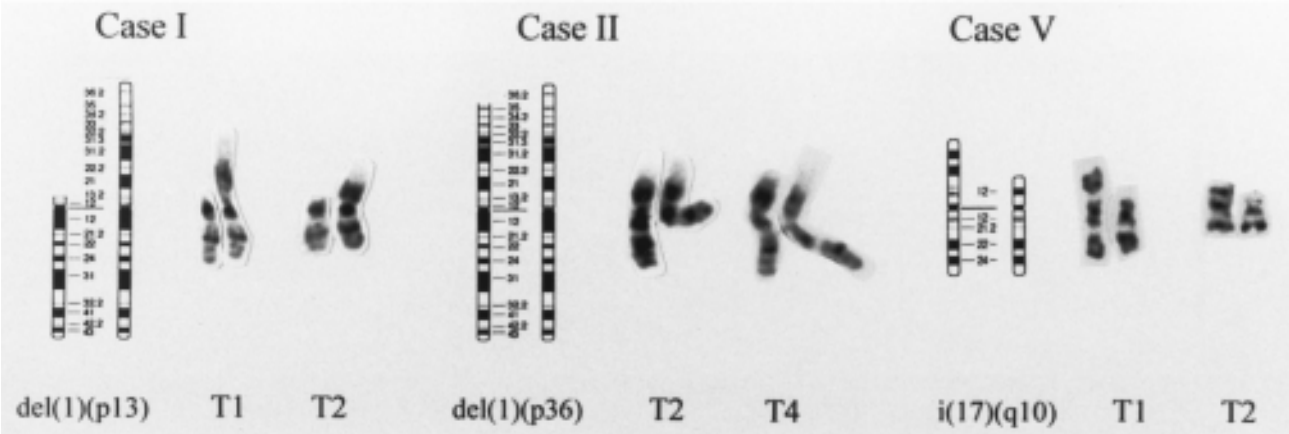

Figure 6. Ideograms and partial karyotypes illustrating three structural chromosome aberrations, each detected in two macroscopically distinct tumors from the same patient: del(1)(p13) was found in the carcinoma (T1) and the hyperplastic polyp (T2) of case I; del(1)(p36) was found in two of the tubulovillous adenomas (T2 and T4) of case II; and I(17q10) was found in the carcinoma (T1) and the tubular adenoma (T2) of case V

and 20q and losses most often occurred at 18q, 4q, and 8p. The results of CGH analysis in these studies are in almost complete accordance with the pattern of imbalances previously detected in colorectal adenomas and carcinomas by G-banding analysis. Only losses of 4q, which were maybe hidden in unidentified chromosome markers, appeared to be underestimated in G-banding studies compared to the CGH data in colorectal cancer. In spite of the similar findings, however, one should keep in mind, while interpreting the combined G-banding and CGH results, that CGH can only detect DNA copy number changes that are present in at least 50% of the examined tumour cell population. Karyotypic analysis is in this sense superior because it can also detect balanced changes and very small clones consisting of as little as two abnormal mitoses.

Primary and metastatic colorectal carcinomas

The major cause of death in colorectal cancer patients is metastasis rather than localized disease. It is therefore clear that a better understanding of the metastatic process and the finding of ways to prevent it stand out as prime goals in colorectal cancer research. Given their importance, it is somewhat surprising that so little is known about the genetic profile of metastatic deposits. As a consequence, markers of prognosis or response to therapy are often assessed against the backdrop of data on the primary tumour, with the often implicit assumption that they also reflect the situation in the secondary disease loci.

To provide information on the karyotypic characteristics of colorectal cancer metastases, we examined cytogenetically 18 tumours from 11 patients with metastatic disease[14]. In all cases with matched samples from the primary tumour and lymph node metastases, cytogenetic similarities were found between the primary and the secondary lesions, indicating that many of the chromosomal aberrations were acquired before disease spreading took place. Compared with the primaries, the metastases appeared to exhibit decreased clonal heterogeneity (probably reflecting clonal selection within the primary tumour from which the metastasis was derived) but, concurrently, an increase in the karyotypic complexity of individual clones. In addition to the aberrations del(1)(p34), i(17)(q10), -18, -21, +7, and +20, which were found recurrently in both primary and metastatic lesions, the del(10)(q22) found in metastases (Figure 7) has not so far been associated with primary colorectal carcinomas. The finding of loss of 10q24-ter in two more cases in another series of colorectal metastases to the liver[20] provides further support for an association between loss of 10q and tumour progression. In a recent LOH study, Fawole et al.[35] used a panel of 9 highly polymorphic microsatellite markers spanning the long arm of chromosome 10 to examine 114 sporadic colorectal adenocarcinomas. They suggested that loss within this region, with the highest LOH frequency observed at 10q21.1, is a late event in colorectal tumourigenesis.

Using CGH, Paredes-Zalgul et al.[36] compared the genetic composition of colorectal tumours that had set up distant metastases and those that had not and found a distinct predominance of genetic losses in the meta-

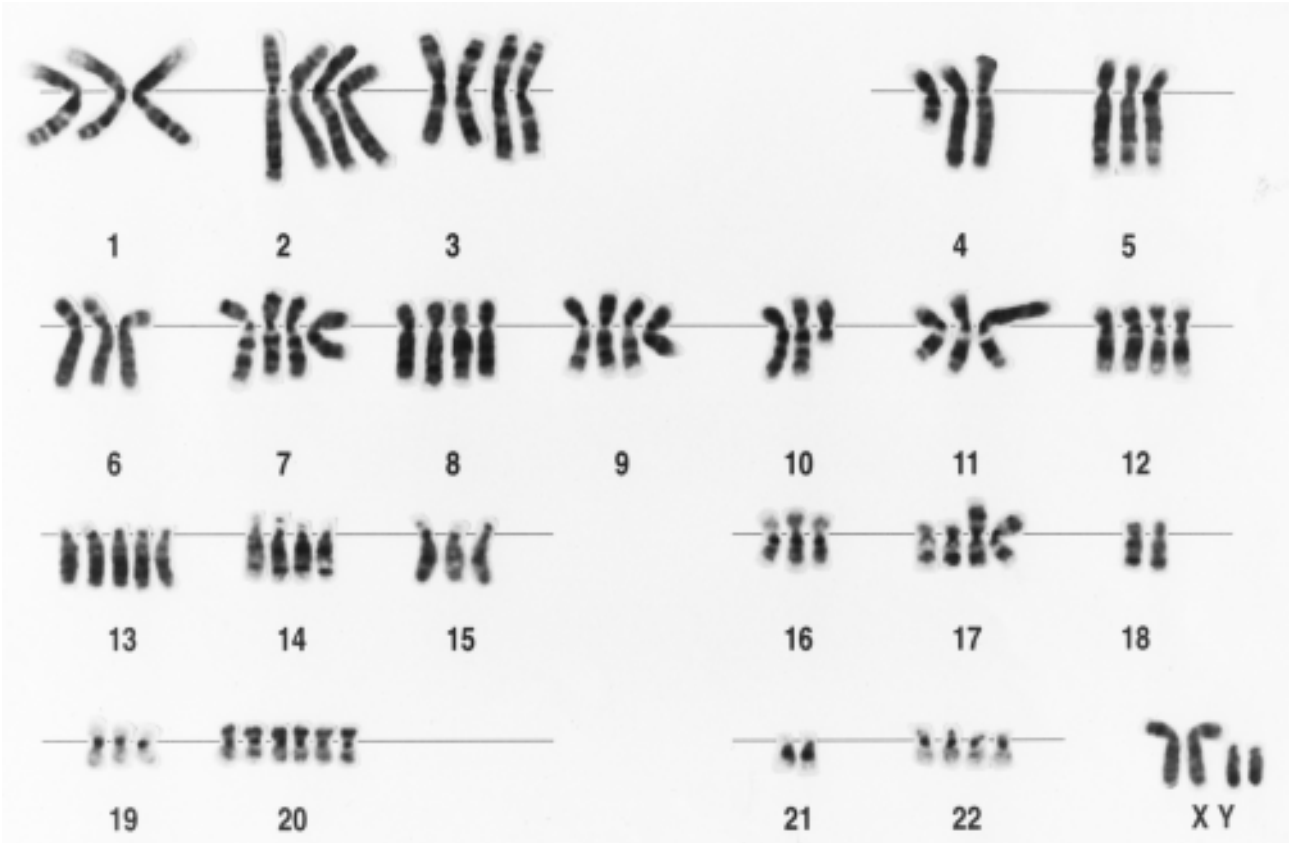

Figure 7. Representative karyogram from a metastatic colon adenocarcinoma displaying 10q deletion among other numerical and structural chromosomal anomalies.

static lesions. Although not all aberrations were the same as those found by us, they too identified two patterns of alterations; (a) changes (+8q, +13q, -4p, -8p, -15q, -17p, -18q, -21q, and –22q) that were more often found in liver metastases than in primary tumours, and (b) changes (-9q, -11q, and –17q) that were unique to the metastatic lesions.

In our series, a comparison between metastatic lesions in the same patient but at different sites[14] showed common aberrations but not in a single case was the karyotype of one metastatic lesion exactly identical to that of any other metastasis, even when both metastatic samples were to lymph nodes. Similar genomic differences between primary and secondary tumours and among metastatic lesions to distinct sites were also reported by Al-Mulla et al.[37], who used CGH analysis to examine paired primary colorectal carcinomas and metastases to either lymph nodes or liver.

CORRELATION BETWEEN KARYOTYPIC AND PHENOTYPIC FEATURES

Cytogenetic pattern and tumour site

Colorectal cancers that arise proximal (right) or distal (left) to the splenic flexure exhibit differences in incidence depending on their site and the patient's age and gender. Together with observations that tumours in the hereditary cancer syndromes HNPCC and FAP (*reference*) occur predominantly in the right and left colon, respectively, the existence of 2 categories of colorectal cancer based on the site of origin in the large bowel was proposed more than a decade ago. Differences between normal right-sided and left-sided colonic segments that could favour progression through different tumourigenic pathways have been recognized[38]. Right- and left-sided tumours exhibit different sensitivities to chemotherapy, probably related to the genetic characteristics of the tumours, with microsatellite instability phenotypes being associated with right-sided tumours and chromosomal instability with left-sided tumours[38]. To date well-defined

classification systems for colorectal cancer based on their pattern of acquired chromosomal aberrations have not been set out clearly, in spite of the rather extensive information on such changes outlined above and which has been available for some years now.

In our series of colorectal carcinomas, we found that carcinomas located in the proximal colon and rectum, tumours that are often near-diploid with simple numerical changes and displaying cytogenetically unrelated clones, probably arise through different mechanisms than do tumours located in the distal colon, which more often have near-triploid to near-tetraploid karyotypes with massive chromosomal aberrations (Figure 8).

Cytogenetic pattern and Histology

A statistically significant association was found between the karyotype of colorectal carcinomas and their degree of differentiation when cytogenetically abnormal tumours were divided into those with only numerical changes and those also having structural aberrations[10]. Carcinomas carrying structural chromosomal rearrangements were more often poorly differentiated, whereas well- and moderately differentiated tumours more often had only numerical aberrations or normal karyotypes.

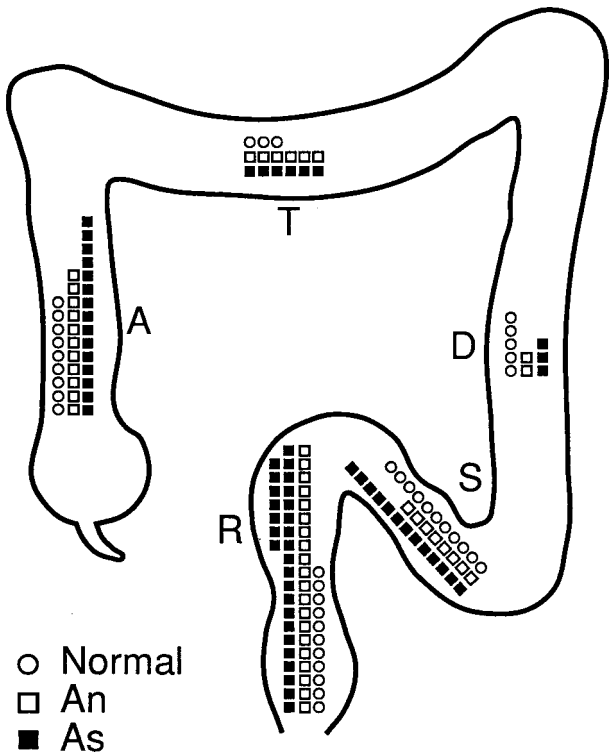

Figure 8. Site distribution of colorectal adenocarcinomas examined cytogenetically. m karyotypically normal tumors, o tumors with numerical/simple chromosome aberrations, n tumors with structural/complex chromosome changes. A ascending colon, T tranverse colon, D descending colon, S sigmoid and R rectum.

In large bowel adenomas, an association between cytogenetic pattern and the tumours' degree of dysplasia, histologic type, and size was found[15-17]. All villous and tubulovillous adenomas, i.e., the adenomas most likely to progress to carcinomas, had structural chromosome aberrations. Adenomas with numerical changes only were mildly dysplastic, whereas all but one of the adenomas with structural rearrangements showed either moderate or severe dysplasia. Furthermore, polyps with a normal karyotype had either mild or moderate, but never severe, dysplasia. Polyps with structural chromosomal aberrations were, on average, larger than polyps with only numerical changes or those with a normal karyotype. The data strongly indicate that the accumulation of chromosomal changes in adenomas correlates with pathologic features: The more malignancy-like the phenotype, the more complex the karyotype. Presumably, this correlation reflects a causal relationship, with the acquired genetic changes enabling the cells to assume an increasingly malignant growth pattern.

Cytogenetic pattern and prognosis

In a relatively small study performed by us in 1993, a statistically significant correlation between tumour karyotype and patient survival was demonstrated[8]. Patients with complex tumour karyotypes had shorter survival times than those whose tumours had no or only few and simple chromosome anomalies. It is currently unknown whether the karyotype represents an independent indicator of survival or, if it does not, which of the more conventional prognostic factors it covaries with. Recently, Risques et al.[39] examined a series of 131 colorectal cancer patients and found a statistically significant correlation between aneuploidy and Dukes' stage as well as metastases. A high aneuploidy index predicted a poorer outcome in univariate and multivariate analyses.

Little is known about the prognostic impact of particular aberrations or aberration patterns. Laurent-Puig et al.[40] used multivariate analysis to show that loss of heterozygosity on the short arm of chromosome 17 was independently associated with shorter survival. They concluded that loss of 17p alleles was a marker of tumour aggressiveness. Recently, imbalances of 8p, 18q, and 20q have been associated with tumour progression with changes of 8p and 18q being able to predict poor prognosis in patients with early-stage tumours[41,42].

Cytogenetic pattern and clinical management of colorectal cancer

The majority of colorectal cancers are not diagnosed in the earlier stages of disease because the tumours often do not give rise to any symptoms or signs at all until they are quite large, sometimes until they have already metastasized. Therefore, the development of diagnostic systems based on the early genetic characteristics of colorectal lesions, either as part of high risk population screening programs or at regular check up examination, stands out as a prime goal. Already available commercial stool tests, based on the use of genetic markers for presymptomatic detection are examples of how genetic profiling may be used in a clinical setting[43].

The appropriate management of individuals with precursor polyps is of utmost importance; since some of these individuals will develop adenomas again, even after endoscopic removal, and a small percentage will go on to have colorectal cancer. An appropriately selected panel of genetic markers associated with adenoma recurrence or progression to carcinoma holds the promise of paving the way for individualized management of colorectal cancer patients based on the pathogenetic elements in tumour development. The recent introduction of molecular cytogenetic techniques, especially the application of FISH probes in interphase cells of histological sections, may serve as a genetic diagnostic system to assess the potential of excised colorectal lesions, by detecting chromosome or gene alterations, e.g. loss of 17p, that are linked to the carcinoma transition independently of the initiating chromosomal events.

A system for genetic staging and prognosis evaluation would help clinicians to properly manage colorectal cancer patients in the gray zone of stage II. The identification of chromosome alterations that are associated with poor prognosis, e.g., loss of chromosome arms 8p and 18q, as well as the karyotypic complexity and the total DNA copy number changes found in each individual tumour, may serve as prognostic indicators that could be introduced as adjunctive tools to assess the aggressiveness of each tumour. In addition, inter-individual genetic differences are now being considered in the development of new drugs, which may soon lead to individualized therapies of patients with colorectal cancer. The ultimate goal is to arrive at therapies that are at the same time, both rational and individualized: rational because they rely on medications designed to counteract the pathogenetic events that lie at the heart of colorectal carcinogenesis, and individualized because they pay proper attention to the genetic peculiarities of both "individuals" locked in combat in cancer diseases, the genetic make up of the tumour cells and that of the host, the patient.

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