Distinct Chromosomal Imbalances in Nonpolypoid and Polypoid Colorectal Adenomas Indicate Different Genetic Pathways in the Development of Colorectal Neoplasms

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Cytogenetic changes are widely unknown for nonpolypoid (synonymously termed as “flat” or “depressed”) colorectal adenomas. A comparison with polypoid adenomas will contribute to the discussion whether different genetic pathways for colorectal tumorigenesis depending on its origin from nonpolypoid or polypoid adenomas exist. Tissue samples of nonpolypoid ($n = 22$), polypoid ($n = 28$) adenomas, carcinomas ex-nonpolypoid adenomas ($n = 9$), carcinomas ex-polypoid adenomas ($n = 14$), and normal colonic mucosa ($n = 9$) were investigated by comparative genomic hybridization of whole genomic DNA. Chromosomal imbalances were detected from average comparative genomic hybridization profiles for each entity. Nonpolypoid adenomas show recurrent chromosomal losses on chromosomes 16, 17p, 18, 20, and 22 and gains on chromosomes 2q, 4q, 5, 6, 8q, 12q, and 13q. In polypoid adenomas losses of whole chromosomes 16, 18, and 22 and gains of chromosomes 7q and 13 were detected. The frequency of copy number changes was higher in nonpolypoid compared to polypoid adenomas and early onset of chromosomal changes became apparent in low-grade dysplasias of nonpolypoid adenomas. Gains on chromosomes 2q, 5, 6, 8q, and 12q and losses on chromosomes 17p and 20 occurred exclusively in nonpolypoid adenomas, whereas 16p dele-

tions are significantly more frequent in nonpolypoid than in polypoid adenomas. Carcinomas ex-nonpolypoid adenomas are characterized by more complex aberration patterns compared to nonpolypoid adenomas exhibiting frequent losses on chromosomes 8p, 12q, 14, 15q, 16, 17p, 18, and 22 and gains on 3q, 5, 6, 7, 8q, 12q, and 13, respectively. Normal colonic mucosa showed no chromosomal imbalances. Distinct differences of chromosomal imbalances between nonpolypoid and polypoid colorectal adenomas have been characterized that support the hypothesis that different genetic pathways may exist in the development of colorectal adenomas exhibiting nonpolypoid and polypoid phenotype. (*Am J Pathol* 2003; 163:287–294)

Besides polypoid adenomas, another type of colorectal neoplasia has endoscopically been identified as a nonpolypoid adenoma.¹ The terms superficial, flat, and depressed adenoma are all used synonymously to describe this entity. Because flat or depressed adenomas display little or no mucosal elevation, endoscopical diagnosis can be very difficult. This type of nonpolypoid adenoma is associated with a higher potential of malignancy.^{2–4} Nonpolypoid colorectal neoplasias exhibit histological features that differ from those of polypoid adenomas, being mainly tubular structures that may show high-grade dysplasia at an early stage.^{5–7} The grade of dysplasia in nonpolypoid colorectal neoplasias may not necessarily be related to size or to a villous component.

During the past few years, colorectal tumorigenesis has been associated with the progressive acquisition of a variety of genomic alterations by neoplastic cells, some of these have been linked to early stages of evolution. Two groups have been distinguished according to their genetic alterations.⁸ The major group, called LOH+ (loss of heterozygosity) or microsatellite stability (MSS), is

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characterized by frequent allelic losses associated with mutations that inactivate tumor suppressor genes (APC, p53) and account for at least two-thirds of tumors; the second group of tumors exhibit a high frequency of replication error at microsatellite loci, thus termed RER+ (replication error) or microsatellite instability (MSI), and were shown to be impaired in the DNA mismatch repair pathway. Furthermore, genes of the ras family have been found activated by missense mutations in 45% of colorectal neoplasias.

It has been reported that there may be a different clinical behavior and histopathological character between nonpolypoid and polypoid adenomas,²⁻⁷ suggesting an alternative pathway in the genesis of colorectal cancer.^{9,10} However, the clinical and molecular genetic dignity of nonpolypoid neoplastic lesions still remain rather unclear.

The molecular genetic characteristics of nonpolypoid colorectal adenomas has been described in relatively few publications. K-RAS mutations were significantly associated with the polypoid phenotype of the adenomas. Further, loss of heterozygosity at chromosomes 3p, 2p, 5q, 17p, and 18q have been reported¹¹ and the frequency of somatic mutations of the APC and p53 genes in nonpolypoid colorectal adenomas¹² has been described. Other studies have focused on the replication error status and transforming growth factor- β RII mutations paying attention also to mutations of APC and K-RAS.¹³ A recent publication reports on the aberrant expression of G₁ phase cell-cycle regulators in nonpolypoid and polypoid colorectal adenomas.¹⁴ To date these sparse molecular genetic investigations, with the exception of low K-RAS mutation rate in nonpolypoid adenomas, indicate that nonpolypoid adenomas seem to follow the tumorigenesis pathways very similar to those identified in polypoid adenomas and carcinomas.

All of the molecular genetic results mentioned above are based mainly on the analysis of individual genes that are known to be involved in the colorectal tumorigenesis pathway and they do not satisfactorily explain the differences that seem to exist between colorectal adenomas with polypoid and nonpolypoid phenotype.

The aim of this study is to investigate the entire genome of nonpolypoid adenomas for chromosomal imbalances by means of comparative genomic hybridization (CGH) profiling and to examine whether there is a specific genotype for nonpolypoid adenomas, which could explain the different clinical behavior and histopathological character between both phenotypes of colorectal adenomas. In this study we investigated 22 nonpolypoid adenomas, 9 carcinomas ex-nonpolypoid adenomas, and 28 polypoid adenomas by CGH and compared resulting chromosomal gains and losses to obtain any clues for differences in the tumorigenic pathway.

Materials and Methods

Patients and Tissue Samples

The tissue samples selected for CGH analysis in this study have been either removed at colonoscopy (polypoid and nonpolypoid adenomas, carcinomas ex-non-

polypoid adenomas) or by surgical resection (carcinomas ex-polypoid carcinomas). A total of 22 nonpolypoid adenomas and 9 carcinomas ex-nonpolypoid adenomas from 27 patients (5 females, 22 males) were investigated in the present study. Additionally, 28 polypoid adenomas from 27 patients (13 female, 14 male) as well as 14 polypoid carcinomas from 14 patients were studied for comparison. Nine normal mucosa samples were examined for control. Histopathological data of the patients are summarized in Tables 1 and 2. For nonpolypoid neoplasias tumor size ranges from 3 to 35 mm (mean, 11.3 mm), tumors were located for 14 cases in the right colon and for 17 cases in the left colon and patients had an age range of 30 to 91 years (mean, 69 years). For polypoid neoplasias the tumor size was determined between 5 and 40 mm (mean, 10.6 mm), 5 tumors showed a location in the right and 23 tumors in the left colon and patients (14 males, 13 females) had an age range of 56 to 84 years (mean, 69 years).

Tissue Samples

Formalin-fixed and paraffin-embedded tissue specimens have been cut and stained with hematoxylin and eosin (H&E) according to standard procedures for routine histopathological examination. For CGH analyses representative areas of the neoplasms were microdissected from tissue sections.

The histopathological classification of each sample was re-evaluated independently by two pathologists. Predominantly tubular adenomas were present ($n = 19$ for nonpolypoid and $n = 17$ for polypoid adenomas). Only a minority of cases showed serrated ($n = 2$ for nonpolypoid and polypoid adenomas each), tubulovillous ($n = 9$ for polypoid adenomas), or villous ($n = 1$ for nonpolypoid adenomas) subtypes.

Endoscopic Criteria Used in this Study

Nonpolypoid (ie, nonprotruding) adenomas were defined as lesions lacking an exophytic, polypoid configuration at colonoscopy, usually consisting of slightly elevated dysplastic mucosal plaques never more than two times the thickness of the adjacent nondysplastic segments at histopathological examination. Polypoid (ie, exophytic) adenomas were defined as protruding lesions either with (pedunculated) or without (sessile) stalks.

Morphological Criteria Used in this Study

Histopathological classification was performed according to World Health Organization.¹⁵ In particular, criteria for defining nonpolypoid (flat) adenomas include the presence of slightly elevated lesions with flat or depressed surface lacking an exophytic polypoid configuration. Those lesions that were classified as carcinomas ex-nonpolypoid (flat) adenomas demonstrated in addition to the above described criteria the presence of invasion, containing invasive carcinoma. Lesions that were classified as carcinomas ex-polypoid adenomas are exophytic cancers with presence of residual (polypoid) adenoma.

Table 1. Summary of Clinical and Histopathological Characteristics of Nonpolypoid and Polypoid Colorectal Adenomas

Case no.	Size (mm)	Localization	Age*/gender	Histology [†]
Nonpolypoid adenomas				
1	20	Coecum	82/F	LGD/TA
2 [‡]	6	Coecum	71/M	LGD/TA
3 [‡]	12	Coecum	71/M	LGD/TA
4 [‡]	8	Coecum	71/M	LGD/TA
5	7	C. descendens	79/M	LGD/TA
6	6	C. transversum	73/M	LGD/TA
7	5	C. descendens	63/M	LGD/TA
8	7	C. sigmoideum	60/M	LGD/TA
9	10	C. descendens	73/M	LGD/TA
10	10	C. transversum	30/M	LGD/TA
11	5	C. ascendens	81/M	LGD/TA
12	20	C. ascendens	70/F	LGD/TA
13	10	C. sigmoideum	63/M	LGD/TA
14	5	C. sigmoideum	68/F	LGD/TA
15	3	C. transversum	57/M	LGD/TA
16	10	C. ascendens	73/M	LGD/TA
17	4	C. sigmoideum	56/M	LGD/TA
18	5	C. sigmoideum	56/M	LGD/SA
19	6	C. transversum	39/M	LGD/SA
20	5	C. sigmoideum	91/F	HGD/TA
21	10	C. descendens	45/M	HGD/TA
22	4	C. descendens	44/M	HGD/VA
Polypoid adenomas				
32	5	C. sigmoideum	63/M	LGD/TA
33	10	C. sigmoideum	76/F	LGD/TA
34	10	C. transversum	75/F	LGD/TA
35	5	C. sigmoideum	64/F	LGD/TA
36 [‡]	10	C. sigmoideum	54/F	LGD/TA
37 [‡]	10	C. sigmoideum	54/F	LGD/TA
38	7	Rectum	62/M	LGD/TA
39	7	Rectum	66/M	LGD/TA
40	10	C. descendens	67/F	LGD/TA
41	40	C. transversum	71/F	LGD/TVA
42	10	Rectum	56/M	LGD/TVA
43	5	C. sigmoideum	56/M	LGD/TVA
44	8	C. sigmoideum	80/M	LGD/TVA
45	10	C. descendens	84/M	LGD/TVA
46	7	C. transversum	54/M	LGD/TVA
47	15	Rectum	83/F	LGD/TVA
48	7	C. sigmoideum	61/F	LGD/SA
49	5	Rectum	56/F	LGD/SA
50	15	Coecum	56/M	HGD/TA
51	7	C. sigmoideum	64/F	HGD/TA
52	10	Rectum	61/M	HGD/TA
53	5	C. sigmoideum	55/M	HGD/TA
54	15	Rectum	62/M	HGD/TA
55	15	Coecum	56/M	HGD/TA
56	10	C. sigmoideum	68/F	HGD/TA
57	10	C. sigmoideum	79/M	HGD/TA
58	20	C. descendens	78/F	HGD/TVA
59	10	C. sigmoideum	67/F	HGD/TVA

* Age at diagnosis in years; M, male; F, female.

[†] TA, Tubular adenoma; TVA, tubulovillous adenoma; SA, serrated adenoma; VA, villous adenoma; Intramuc. C, intramucosal carcinoma; LGD, low-grade dysplasia; HGD, high-grade dysplasia.

[‡] Samples of different loci from one patient.

DNA Extraction and Hybridization

The analyses were performed on archival material from formalin-fixed tissues embedded in paraffin. Serial sections were cut for tissue microdissection (5 μ m). The first and the last section were stained with H&E. Microdissection was performed on hematoxylin-stained sections under an inverted microscope yielding at least 80 to 90% pure neoplastic cells for DNA extraction. At least four to six serial sections were sampled, depending on the size

of area. The cells were transferred into a sterile microcentrifuge tube and the DNA was extracted with NucleoSpin Tissue Kit (Clontech Laboratories, Inc., Palo Alto, CA) as specified by the manufacturer.

Amplification and Labeling of DNA

For amplification, *Mse*I restriction endonuclease digest followed by ligation-mediated polymerase chain reaction

Table 2. DNA Copy Number Changes (≥ 2 Alterations) in Nonpolypoid Neoplasias ($N = 31$) and Polypoid Neoplasias ($n = 42$), Total $n = 73$

Pathological diagnosis	Nonpolypoid neoplasias	Polypoid neoplasias
Low-grade adenoma	10/19	3/18
High-grade adenoma	1/3	4/10
Carcinoma	9/9	14/14
Total	20/31	21/42

(PCR) were performed on DNA extracts according to Klein and colleagues.¹⁶ Briefly, the *MseI*-digested DNA was ligated by annealing of the primers *MseLig* 21 and *MseLig* 12. At 15°C the T4-DNA-ligase (Roche Diagnostics, Mannheim, Germany) was added and primers and DNA fragments were ligated overnight. After primary PCR amplification the size of DNA fragments was checked by agarose gel electrophoresis and should range between 500 and 1500 bp.

The amplified DNA was indirectly labeled by PCR using biotin-16-dUTP (Roche Diagnostics) for the tumor DNA and digoxigenin-11-dUTP (Roche Diagnostics) for the female or male reference DNA. The amplification products were then *MseI* digested for 3 hours at 37°C. After purification the DNAs were used for subsequent CGH analysis.

CGH and Image Analysis

For each CGH hybridization 1 μ g of tumor DNA and 800 ng of normal female or male DNA, plus 25 μ g of *CotI* DNA (Invitrogen, Karlsruhe, Germany) and 20 μ g of herring sperm DNA (Sigma-Aldrich, Taufleivchen, Germany) were co-hybridized to denatured metaphases for 72 hours at 37°C. After hybridization, biotin-labeled tumor DNA was detected with avidin-FITC DCS (Vector Laboratories, Wertheim-Bettingen, Germany), the digoxigenin-labeled control DNA with anti-digoxigenin-rhodamine (Roche Diagnostics). Slides were counterstained with 4,6-diamidino-2-phenylindole in anti-fade solution and mounted in Vectashield (Vector Laboratories, Burlingame, CA). For CGH analysis, at least 10 metaphases were captured and karyotyped after visualization with a Zeiss Axioplan 2 fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with filter sets (single-band excitation filters for 4'-6-diamidino-2-phenylindole, Cy2, and Texas Red). Averaged profiles were generated by CGH analysis software (ISIS 3, V2.84; MetaSystems, Altlußheim, Germany) from at least 10 to 15 homologous chromosomes and interpreted according to published criteria^{17,18} using statistical confidence limits based on *t*-statistics. A chromosomal gain was classified as high-level amplification when the CGH ratio exceeded a value of 1.5, or if the Cy2 fluorescence showed a strong, distinct signal by visual inspection and the corresponding ratio profile was diagnostic of overrepresentation. Telomeric regions, heterochromatic regions, and the Y chromosome were all excluded from analyses, because of interindividual variations within these regions.

Ligation-mediated PCR-amplified DNA obtained from morphologically normal mucosa DNA was co-hybridized

with male or female reference DNA to metaphase preparations. In these experiments no chromosomal changes were detected except for chromosome region 1p34-36 and chromosome 19. These regions are known to show artifactual results by CGH.¹⁹⁻²¹

Statistical Analysis

The study cohort consists of patients from a hospital-based case control study. For control cases (polypoid adenomas, normal mucosa) patients have been selected randomly from the patient cohort. For comparison of average aberration frequencies in each entity, Fisher's exact test, binomial test, and pairwise comparison analysis were applied.

Results

CGH was used to identify DNA copy number changes that occur in nonpolypoid adenomas in the course of colorectal tumorigenesis. The histopathological data for the individual adenoma cases are summarized in Table 1. The summary of the CGH results including DNA gains and losses for each of the 82 samples investigated are presented in Tables 2 and 3. Chromosomal imbalances are additionally compared between nonpolypoid and polypoid adenomas in Figure 1 and examples are shown in Figure 2.

Normal Mucosa

No chromosomal aberrations were detected in this series of normal mucosa. The DNAs from normal specimens were amplified by PCR and labeled as test DNAs. The ratios for the sex chromosomes were consistent with the gender of the patients in all cases.

Nonpolypoid Adenomas

Recurrent chromosomal aberrations (at least two alterations) were observed as DNA copy number losses on chromosomes 16, 17p, 18, 20, and 22 as well as gains of chromosomal regions on chromosomes 2q, 4q, 5q, 6, 8q, 12q, and 13q (Figure 1). These chromosomal imbalances were detected in 11 low-grade adenomas (11 of 19) as well as in 3 high-grade adenomas (3 of 3).

Polypoid Adenomas

Eighteen low-grade adenomas and 10 high-grade adenomas were investigated. Recurrent DNA losses were detected on chromosomes 16, 18, and 22 and gains were identified on chromosomes 7q and 13 (Figure 1). Chromosomal changes were preferentially detected in high-grade adenomas, whereas low-grade adenomas showed in single cases only copy number changes for whole chromosomes indicating aneuploidy.

Table 3. Frequent Chromosomal Imbalances from CGH Analysis of Nonpolypoid and Polypoid Neoplasias

Chromosomal changes*†	Nonpolypoid colorectal neoplasias			Polypoid colorectal neoplasias		
	LGD	HGD	Ca	LGD	HGD	Ca
Gains						
2q[2q32–33]	3/19	–	2/9	–	–	–
3q[3q26.1–26.3]	–	–	3/9	–	–	–
4q[4q21–26]	2/19	–	2/9	–	–	–
5	2/19	–	5/9	–	–	–
6[6q22–26]	2/19	–	3/9	–	–	–
7[7q31]	–	–	7/9	–	2/10	–
8q[8q13–24]	2/19	–	3/9	–	–	–
12q[12q14–21]	2/19	–	3/9	–	–	–
13[13q21–22]	3/19	–	8/9	–	2/10	–
20[20q12–13]	–	–	2/9	–	–	3/14
Losses						
2p[2p22–24]	–	–	–	–	–	2/14
3p[3p21]	–	–	–	–	–	2/14
4p	–	–	2/9	–	–	2/14
5[5q31qter]	–	–	2/9	–	–	2/14
7[7q32-qter]	–	–	2/9	–	–	–
8p[8p21–22]	–	–	3/9	–	–	2/14
8q[8q22]	–	–	–	–	–	2/14
10q[10q25]	–	–	–	–	–	4/14
12q[12q23-qter]	–	–	4/9	–	–	3/14
14[14q31-qter]	–	–	3/9	–	–	4/14
15q[15q24-qter]	–	–	3/9	–	–	4/14
16[16p13.1–13.3]	6/19	2/3	3/9	2/18	2/10	4/14
16q[16q22–23]	–	–	–	–	–	2/14
17p[17p12–13]	5/19	–	6/9	–	–	2/14
17q[17q21–24]	–	–	–	–	–	3/14
18[18q]	3/19	–	6/9	–	2/10	8/14
20[20q]	3/19	–	–	–	–	–
22[22q]	8/19	–	5/9	–	3/10	4/14

* Only chromosomal imbalances occurring in more than two cases are shown.

† Minimal common regions are given in brackets.

–, No aberrations were detected.

Carcinomas Ex-Nonpolypoid Adenomas (n = 9)

DNA losses were frequently detected on chromosomes 8p, 12q, 14, 15q, 16, 17p, 18, and 22 as well as DNA gains on chromosomes 3q, 5, 6, 7, 8q, 12q, and 13. Very complex aberrations were detected in some cases. A summary of aberration frequencies is shown in Table 2.

Carcinomas Ex-Polypoid Adenomas (n = 14)

Frequent DNA losses were detected on chromosomes 10q, 12q, 14q, 15q, 16p, 17q, 18, and 22 as well as DNA gain on chromosome 20. Also typical deletions on chromosomes 5q and 8p became apparent in two cases each. Some cases showed very complex aberrations similar than in carcinomas ex-nonpolypoid adenomas. Aberration frequencies are also demonstrated in Table 2.

Comparison of Chromosomal Changes between Nonpolypoid Adenomas and Polypoid Adenomas

A comparison of frequent chromosomal alterations (≥ 2 alterations) between nonpolypoid and polypoid adenomas and carcinomas is summarized in Table 3. Statistical analysis revealed that cases with more than two aberrations are significantly more frequent for nonpolypoid ad-

enomas (Fisher's exact test, $P = 0.035$). In contrast to polypoid adenomas, nonpolypoid adenomas exhibit frequent chromosomal aberrations already in low-grade lesions. A pairwise comparison test of single chromosomal aberrations revealed a significant higher frequency of 16p deletions in nonpolypoid adenomas compared to polypoid adenomas (Fisher's exact test, $P = 0.0026$). This aberration type is significantly more frequent than any other chromosomal aberration in nonpolypoid adenomas ($P < 0.001$, binominal test). There is an accumulation of chromosomal imbalances in the adenoma-carcinoma sequence for both entities. As demonstrated in Table 3 the aberration pattern for DNA losses is very similar between carcinomas ex-nonpolypoid adenomas and carcinomas ex-polypoid adenomas, however, differences exist for DNA gains.

Discussion

It is a generally accepted hypothesis that most of colorectal cancers develop from a pre-existing adenomatous polyp. Such benign lesions are mostly of polypoid origin, while a small proportion of adenomas are described as nonpolypoid lesions. The latter morphological category has therefore been proposed to exhibit specific genetic alterations. To confirm this assumption we have investigated 82 tissue specimens for chromosomal imbalances

Figure 1. Summary of DNA copy number change detected by CGH in nonpolyploid colorectal adenomas indicated in blue and in polyploid colorectal adenomas indicated in orange. Lines to the left and the right of the ideograms of chromosomes represent losses and gains of chromosomal regions, respectively.

consisting of 22 nonpolyploid and 28 polyploid adenomas. Nine carcinomas ex-nonpolyploid adenomas, 14 polyploid carcinomas, and 9 specimens of normal epithelium were analyzed for control.

This is the first comprehensive CGH study to detect chromosomal imbalances between nonpolyploid and polyploid adenomas of the colon. Our results show that recurrent chromosomal aberrations in nonpolyploid adenomas of the colon are found as DNA copy number losses on chromosomes 16, 17p, 18q, 20, and 22. Gains of chromosomal regions were observed on chromosomes 2q, 4q, 5q, 6, 8q, 12q, and 13q (Table 3). Carcinomas ex-nonpolyploid adenomas that represent intramucosal carcinomas are characterized by complex aberration patterns showing frequently chromosomal losses on 8p, 12q, 14, 15q, 16, 17p, 18, and 22, gains on 3q, 5, 6, 7, 8q, 12q, and 13 (Table 3). Although more amplifications were detected in carcinomas ex-nonpolyploid adenomas than in carcinomas ex-polyploid adenomas; many of the deletions detected occurred in both tumor types (eg, deletions on 17p and 18q).

So far, there are no cytogenetic data on nonpolyploid adenomas of the colon. For comparison, polyploid ade-

nomas were additionally investigated in this study. Our subset of polyploid adenomas exhibited frequent losses of whole chromosomes 16, 18, and 22 as well as gains of chromosomes 7q and 13, however, a lower frequency of chromosomal aberrations was detected compared to nonpolyploid adenomas. Moreover, loss of whole chromosomes is the most frequent finding indicating that aneuploidy is a very early event in polyploid adenomas, which is in agreement with an earlier study of Ried and colleagues²² reporting on crude aneuploidy in low- and high-grade polyploid adenomas.

The most frequent affected chromosomal regions in nonpolyploid and polyploid adenomas from this study are summarized in Table 3 and Figure 1. Nonpolyploid adenomas show chromosomal changes very early in low-grade dysplasias, which is different to the observation in polyploid adenomas, although an accumulation of chromosomal imbalances became apparent in the adenoma-carcinoma sequence comparable to the situation in polyploid adenomas. Moreover, cases with more than two aberrations are significantly more frequent in nonpolyploid than in polyploid adenomas ($P = 0.035$, Fisher's exact test). This represents another indication for a different carcinogenic pathway in both lesions. More striking evidence for this hypothesis comes from a detailed analysis of single aberrations. It is obvious from Figure 1 that gains on chromosomes 2q, 5q, 6, 8q, and 12q occurred exclusively in nonpolyploid adenomas. In addition loss on chromosome 17p became apparent in very early lesions (low-grade dysplasia) of nonpolyploid adenomas, which cannot be confirmed for polyploid adenomas. It is of special interest that 16p deletions are significantly more frequent than any other aberration in nonpolyploid adenomas (8 of 36 aberrations, $P < 0.001$, binominal test) and that pairwise comparison using Fisher's exact test reveals also a significant difference for this aberration between nonpolyploid and polyploid adenomas ($P = 0.0026$).

The successive molecular changes proposed to occur at different stages in the adenoma-carcinoma sequence were primarily based on DNA studies of polyploid adenomas. An accumulation of genetic changes in tumor suppressor genes and oncogenes such as adenomatous polyposis coli (APC), K-RAS, and TP53 has been reported in this type of colorectal carcinogenesis.^{23,24} For nonpolyploid adenomas and carcinomas ex-nonpolyploid adenomas such genetic changes are widely unknown for the adenoma-carcinoma sequence. The low incidence of KRAS mutations reported for nonpolyploid colorectal adenomas^{10,11,13} suggested a different genetic basis for the transformation process in these lesions. Also RAS mutations, a frequent genetic change in early colorectal tumor development of polyploid origin^{8,25} have been reported to be not involved in the carcinogenesis of ex-nonpolyploid adenoma.²⁶ Similar conclusions about different avenues for colorectal cancer formation have been drawn from recent loss of heterozygosity studies reporting about differences in the KRAS locus and on microsatellite loci on chromosome 3p between polyploid and nonpolyploid lesions.²⁷ It became also apparent from a very recent study of Hermsen and colleagues²⁸ demonstrating the presence of varying combinations of few

Figure 2. CGH profiles of colorectal neoplasias showing exemplary gains on chromosomes 5q, 6p/q, 8q, 12, and 13q, and losses on chromosomes 16, 17p, 18q, and 22, which are typical changes in nonpolypoid neoplasms. **Red** and **green lines** represent thresholds for chromosome losses and gains, respectively. Chromosome numbers and numbers of chromosomes calculated are indicated below each profile.

chromosomal abnormalities during adenoma-to-carcinoma progression, which indicate also the existence of multiple chromosomal instability pathways. As indicated in the same study²⁸ gains on chromosomes 8q23 and 13q21-22 and losses on chromosomes 8p21, 17p12-13, and 18q12-21 are associated with tumor progression present in advanced lesions (adenomas containing carcinoma foci or developed carcinomas). Interestingly, most of these alterations could be detected in our tissue samples preferentially in nonpolypoid adenomas showing low-grade dysplasia and in nonpolypoid intramucosal carcinomas (Table 3). No single polypoid adenoma with low-grade dysplasia in our subset of cases has shown chromosomal alterations in the critical loci indicated by

Hermesen and colleagues.²⁸ In our cohort of low-grade dysplasias only nonpolypoid adenomas revealed chromosomal imbalances as mentioned above. Therefore, this phenomenon may reflect a higher malignant potential of nonpolypoid adenomas, which also show a higher extent of genetic instability than polypoid adenomas. Assuming a higher malignant potential for nonpolypoid adenomas one may also speculate that a genetic link between the specific growing pattern of nonpolypoid neoplasms and the malignant potential may exist.

A puzzling finding in colorectal neoplasms is the overexpression of pRB and amplification of the RB1 gene,¹⁴ which is paradoxical to the common inactivation of the RB gene in most types of human cancers.^{29,30} The finding of frequent

13q gain for nonpolypoid and polypoid adenomas in this study accounts for this puzzling situation and may contribute to support one of several hypotheses from elevated pRB being a secondary neutral change resulting from chromosome 13 gains, to a potential oncogenic role of pRB.^{14,31,32}

The present study summarizes chromosomal changes detectable in nonpolypoid adenomas of the colon. It has been demonstrated that distinct differences to polypoid adenomas exist. This observation supports the assumption from earlier genetic and microsatellite studies^{10,11,26} that different genetic pathways for tumor progression may exist for colorectal neoplasias of polypoid and nonpolypoid phenotype. Moreover, this study provides a basis for search of new gene alterations in nonpolypoid adenomas starting at frequent chromosomal imbalances.

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