Clinical and molecular aspects of MUTYH- and APC-associated polyposis

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High frequency of copy-neutral LOH in *MUTYH*-associated polyposis carcinomas

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ABSTRACT

Genetic instability is known to drive colorectal carcinogenesis. Generally, a distinction is made between two types of genetic instability: chromosomal instability (CIN) and microsatellite instability (MIN or MSI). Most CIN tumours are aneuploid, whereas MSI tumours are considered near-diploid. However, for *MUTYH*-associated polyposis (MAP) the genetic instability involved in the carcinogenesis remains unclear, as near-diploid adenomas, aneuploid adenomas and near-diploid carcinomas have been reported. Remarkably, our analysis of 26 MAP carcinomas, using SNP arrays and flow sorting, showed that these tumours are often near-diploid (52%) and mainly contain chromosomal regions of copyneutral loss of heterozygosity (LOH) (71%). This is in contrast to sporadic colon cancer, where physical loss is the main characteristic. The percentage of chromosomal gains (24%) is comparable to sporadic colorectal cancers with CIN. Furthermore, we verified our scoring of copy-neutral LOH versus physical loss in MAP carcinomas by two methods: fluorescence in situ hybridization, and LOH analysis using polymorphic markers on carcinoma fractions purified by flow sorting. The results presented in this study suggest that copy-neutral LOH is an important mechanism in the tumorigenesis of MAP.

INTRODUCTION

MUTYH-associated polyposis (MAP) is the first colorectal cancer syndrome shown to be inherited in an autosomal recessive fashion. Biallelic mutations in the base excision repair (BER) gene *MUTYH* have been shown to cause colorectal adenomatous polyposis, and correlate with a high risk of developing carcinomas [1]. BER is a DNA repair mechanism that guards oxidative DNA damage and other metabolic DNA damage. Upon oxidative DNA damage, MUTYH removes incorrectly incorporated adenines opposite to an 8-oxo-guanine. Consequently, MAP patients show somatic G : C→ T : A mutations in crucial genes such as *APC* and *KRAS*.In *APC*, these G:C → T : A transversions seem to occur primarily in GAA sequences.[1,2] In *KRAS*, a specific GGT → TGT mutation (c.34 G → T, p.Gly12Cys) is found in up to 64% of MAP carcinomas. [3] Interestingly few *p53* and *SMAD4* mutations are found in MAP carcinomas, whereas these genes are frequently affected in sporadic colorectal cancer.[3]Although MUTYH deficiency triggers carcinogenesis by G : C → T : A transversions, the exact role of MUTYH deficiency in the tumour progression in MAP patients is still unknown.

For colorectal cancers, different types of genetic instability are known to drive carcinogenesis. The two main types of genetic instability are microsatellite instability (MIN or MSI) and chromosomal instability (CIN). CIN is defined as an accelerated rate of chromosomal missegregation resulting in an aberrant chromosomal content, and is found in the vast majority of sporadic colorectal cancers.[4] On the other hand, ~15% of the sporadic colorectal cancers show MSI, due to *MLH1* promoter hyper-methylation. [5] Moreover, MSI is typically seen in the carcinomas of Lynch syndrome patients. Colon carcinomas that display neither CIN nor MSI have also been described.[6] More recently, abnormal epigenetic modification has been described in colorectal cancer, exhibiting the CpG island methylator phenotype (CIMP).[7,8]

The genomic profile of MAP tumours has been described in three studies to date. Using flow cytometry, Lipton *et al* found MAP carcinomas to be predominantly near-diploid. Comparative genomic hybridization (aCGH) of two near-diploid MAP carcinomas showed no detectable chromosomal gains or losses. Furthermore, they analysed chromosomes 1p, 2p, 5q, 10p, 15q, 18q and 20q for LOH, using microsatellite markers, and reported a high frequency of LOH for chromosome 18q but low levels of LOH for the other regions[3] Recently, the same research group identified only a small number of copy number changes in MAP adenomas.[9] These changes were mainly restricted to chromosomes 1p, 13, 17p, 19 and 22. Additionally, in a single MAP adenoma, copy-neutral LOH (cnLOH) of whole chromosome 7 and 12 was reported. On the other hand, Cardoso *et al* identified chromosomal copy number aberrations in MAP adenomas using

aCGH analysis. The most prevalent aberrations identified were gains at chromosomes 7 and 13, as well as physical losses on chromosomes 17p, 19p and 22q [10]. However, the ploidy status of these adenomas was not determined.

Although these studies seem to be contradictory, Lipton *et al* studied carcinomas, whereas the other studies analysed adenomas. In addition, different technical platforms were used, i.e. flow cytometry vs. aCGH after amplification of laser capture microdissected DNA. In order to gain further insight into the genetic instability involved in MAP carcinogenesis, we analysed formalin-fixed paraffin-embedded tumour tissue from 26 carcinomas for patterns of chromosomal losses and gains and copy-neutral LOH using SNP arrays.[11,12]

Table 1. Characteristics of the MAP carcinomas

Tumour	Patient	*MUTYH* mutation	Site CRC*	Age at diagnosis	Tumour stage	DNA index
t1	1	Y165C/Y165C	Distal	52	I	0.9 + 1.7†
t2	2	Y165C/Y165C	Distal	49	II	1.1 + 1.4†
t3	3	Y165C/Y165C	Proximal	39	II	1.0
t4	4	Y165C/Y165C	Proximal	49	III	1.0 + 1.5†
t5	5	Y165C/Y165C	Distal	56	I	1.6
t6	6	Y165C/Y165C	Proximal	53	II	1.0
t7.1	7	Y165C/Y165C	Proximal	43	II	1.0 + 1.5†
t7.2	7	Y165C/Y165C	Distal	43	II	1.0 + 1.5†
t8.1	8	Y165C/Y165C	Proximal	41	III	na
t8.2	8	Y165C/Y165C	Proximal	41	III	na
t8.3	8	Y165C/Y165C	Proximal	41	III	1.0
t8.4	8	Y165C/Y165C	Distal	41	III	1.0
t9	9	Y165C/Y165C	Ileum	77	II	1.0
t10	10	Y165C/Y165C	Metastases‡	45	IV	1.5 + 2.7§
t11	11	Y165C/Y165C	Metastasis‡	64	IV	1.5
t12	12	Y165C/G382D	Proximal	67	III	1.0
t13.1	13	Y165C/G382D	Proximal	43	II	1.0 + 1.1†
t13.2	13	Y165C/G382D	Proximal	46	II	1.0
t14	14	Y165C/G382D	Proximal	59	II	1.0
t15.1	15	P391L/P391L	Proximal	37	III	1.1 + 1.4†
t15.2	15	P391L/P391L	Proximal	37	III	Na
t16	16	P391L/P391L	Distal	58	II	1.0
t17.1	17	1105delC/G382D	Distal	42	I	1.1
t17.2	17	1105delC/G382D	Distal	42	I	1.0
t18	18	R233X/P391L	Proximal	48	II	1.4
t19	19	G382D/P391L	Proximal	51	III	1.1

The tumours were located before (proximal) or after (distal) to the splenic flexura of the colon. T11 is a metastasis of an earlier colon carcinoma. T10 consists of two metastases of a colorectal carcinoma from patient 10.The DNA index was measured by multiparameter DNA flow cytometry. When two populations were identified in the keratin-positive fraction, the DNA index of both tumour fractions is shown in the table. Tumour staging was performed according to the TNM classification (http://tnm.uicc.org).

na, could not be analysed for technical reasons.

* All tumours were colorectal with the exception of t9 (ileum).

† Multiple clones.

‡ Exact location of the primary tumour in the colon not known.

§ Two metastases of a primary colorectal carcinoma with DNA index 1.5 and 2.7, respectively.

MATERIALS AND METHODS

Samples

From 19 MAP patients, 26 formalin-fixed paraffin-embedded (FFPE) carcinomas and corresponding normal tissue were selected (Table 1). This series of carcinomas included metastases of primary colon carcinomas (t10 and t11). Corresponding normal tissue was either histological normal colon tissue or tissue from unaffected lymph nodes. The carcinomas originated from 11 biallelic Y165C mutation carriers, two biallelic P391L mutation carriers, three Y165C/G382D compound heterozygotes, one 1105delC/G382D, one P391L/G382D and one P391L/R233X compound heterozygote. Clinical details of patients 2, 3, 8, 9, 10, 12, 13, 14, 15, 16 and 17 were previously described by Nielsen *et al*[13] (as the respective numbers 13, 4, 11, 12, 14, 20, 18, 16, 35, 34 and 30). Twenty-two previously published sporadic CRCs[14] were included as reference controls. The study was approved by the local Medical Ethical Committee (protocol P01.019); samples were handled according to the medical ethical guidelines described in the Code Proper Secondary Use of Human Tissue established by the Dutch Federation of Medical Sciences (www.federa.org). Tumour samples were enriched for tumour tissue by taking 0.6 mm tissue punches, using a tissue microarrayer (Beecher Instruments, Sun Prairie, WI, USA) guided by a haematoxylin and eosin (H&E)-stained slide. DNA was isolated by the previously described method, and subsequently cleaned using the Genomic Wizard kit (Promega, Leiden, The Netherlands) [15.] DNA concentrations were measured with the picogreen method (Invitrogen–Molecular Probes, Breda, The Netherlands).

Flow cytometry and cell sorting

For 23 carcinomas, the DNA index was determined by flow cytometry, as described previously with minor modifications [16]. In short, cell suspensions were prepared from FFPE samples and stained for keratin (*APC*), vimentin (RPE) and DNA (DAPI). Samples were analysed on a LSRII flow cytometer (BD Biosciences, San Jose, CA, USA). From five MAP carcinomas (t2, t4, t10, t12 and t18) and one sporadic carcinoma (sp1), those cell fractions that were vimentin-positive, keratin-negative (V^+K^-) and vimentin-negative, keratin-positive (V^-K^+)were flow-sorted using a FACSAria cell sorter (BD Biosciences).

Single nucleotide polymorphism arrays

Illumina BeadArrays were used in combination with the linkage mapping panel IV B4b (Illumina, San Diego, CA, USA) [11], which consists of four panels. Panel I covers chromosomes 1, 2, 3 and 22; panel II covers chromosomes 5, 6, 7, 8 and 9; panel III covers chromosomes 10, 11, 12, 13, 14, 15 and 21; and panel IV covers chromosomes

4, 16, 17, 18, 19, 20, X and Y. The GoldenGate assay was performed according to the manufacturer's protocol, with minor adjustments: 1 µg input DNA was used for multi-use activation and resuspended in 60 µl RS1.[17] Genotypes were extracted using GenCall (version 6.0.7) and GTS Reports (version 4.0.10.0; Illumina). Tumours t3, t9, t10 and t14 could only be analysed for copy number abnormalities, since corresponding normal tissue was unavailable to determine cnLOH. For t1, t17.2 and t18 only three panels could be analysed, due to limited availability of the FFPE tumour DNA. We corrected for this missing information in our calculations.

Analysis of copy numbers and loss of heterozygosity

Copy number and cnLOH profiles were generated by analysing the carcinomas and corresponding normal tissue in 'Beadarray SNP'.[12] Criteria for the scoring of copy number aberrations were based on previous experiments.[12] LOH was determined as follows. The ratio between the GenCall Score (GCS) and the GenTrain Score (GTS) was computed as a relative measure for the quality of the clustering of the SNP. All high-quality heterozygous SNPs (GCS/GTS >0.8) in the normal sample were included in the analysis. For homozygous SNPs and those with a GCS/GTS <0.8 in the tumour, LOH was assigned. LOH at one or two SNPs was ignored. In practice, regions of LOH always presented as stretches of markers showing LOH. When both a copy number change and LOH were detected at a specific region, the detected LOH was considered to be a consequence of the copy number alteration. If no copy number change was detected, LOH was interpreted as cnLOH.

For verification, conventional LOH analysis was performed for chromosomes 17p and 18q, using microsatellite markers (D17S938, D17S921, D18S877, D18S65, D18S460 and D18S1137) in pure tumour DNA of five MAP carcinomas obtained after flow sorting. Normal DNA was used as a reference. As a positive control, one sporadic carcinoma with known physical loss of chromosomes 17p and 18q was included. A standard PCR protocol was used for amplification. Mixtures of 9.5 µl HiDi formamide, 0.5 µl ROX 500 size standard and 2.0 µl PCR product were run on an ABI 3130 Genetic Analyser (Applied Biosystems) and analysed using GeneMapper version 4.0 (Applied Biosystems).

Interphase fluorescence in situ hybridization (FISH)

FISH was performed on flow-sorted nuclei that were spotted onto glass slides, as described previously [18]. The nuclei of five carcinomas were hybridized with a BAC on 17p13.1 (RP11-199F11, spanning the *p53* locus), a BAC on 18q21.1 (RP11-748M14, spanning the SMAD2 locus) and centromere probes for chromosome 17 and 18. For all carcinomas 50 nuclei were scored. For heterogeneous tumours, each cell population

that represented at least one-third of the scored nuclei was considered as a separate fraction.

Statistics

The amount of chromosomal aberrations identified in the MAP carcinomas and the sporadic carcinomas was compared using a Mann–Whitney U-test for independent samples. The analyses were performed using SPSS 12.0.1.

RESULTS

We studied a series of 26 Dutch carcinomas from 19 biallelic *MUTYH* mutation carriers. All patients were diagnosed with >10 colon polyps (median age at diagnosis 49 years, range 37–77 years), ranging from 10–50 polyps to polyposis with >50–100 polyps. The carcinomas were predominantly located proximal to the splenic flexura (15/24 reported) (Table 1). Most carcinomas were stage II (11/26 or 42%) or stage III (9/26 or 35%); 68% of the MAP carcinomas contained a somatic mutation in KRAS (16/17 mutations: c.34 G > T, p.Gly12Cys). A low level of mutations (12%) in the mutation cluster region of *APC* was identified and all carcinomas were microsatellite-stable (data not shown).

Using SNP arrays suitable for analysis of FFPE tissue, we were able to study the 26 carcinomas for genome-wide copy number abnormalities and genome-wide copy-neutral loss of heterozygosity (cnLOH) (see Supplementary Table 1, available at: http://www.interscience.wiley.com/jpages/0022-3417/ suppmat/path.2375.html).Remarkably, this analysis revealed that 71% of all changes in the MAP carcinomas concerned cnLOH, whereas only 29% comprised copy number abnormalities (mainly chromosomal gains). On average, 5.1 (range 1–14) cnLOH events were identified per carcinoma. The cnLOH involved chromosome arms or complete chromosomes, but cnLOH of smaller chromosomal regions was also frequently identified. The regions most commonly affected by cnLOH in these tumours were chromosome 17p (57%), 18q (52%) and 15q (52%). Copy-neutral LOH was also frequently present at chromosome 6p (36% of the carcinomas). Lower frequencies of cnLOH were found for chromosomes 4p (24%), 4q (29%), 6q (23%), 8p (23%), 10q (24%), 18p (24%), 21q (24%) and 22q (29%) (Figure 1, Supplementary Table 1).

The MAP carcinomas we studied displayed only a few copy number abnormalities (on average 2.5, range 0–9). This is in contrast to sporadic colorectal cancer, where many chromosomal gains and losses are generally seen [4]. In all patients, the tumours showed five or fewer changes, except for patients 10 and 11, who showed eight, nine and six

aberrations, respectively. Gain of chromosome 13q was the most prevalent aberration, seen in 9/26 (35%) carcinomas. Chromosome 11q was amplified in 6/26 (23%) carcinomas. Very limited physical chromosomal loss occurred in the MAP carcinomas (Figure 1, Supplementary Table 1).

The absence of gross chromosomal copy number alterations in our series of MAP carcinomas may reflect a near-diploid genome. For 23 carcinomas, we were able to measure ploidy status using flow cytometry. This analysis concluded that 12/23 (52%) MAP carcinomas were, indeed, near-diploid (DNA index, 1.0 ± 0.1). We found three cases with a neartriploid DNA index (1.5 ± 0.1). In addition, flow cytometry revealed that seven carcinomas contained two fractions, each with a different DNA index. In all seven of these carcinomas, one of the fractions was near-diploid, while the other fraction had a DNA index of 1.5 in five of the seven cases. Tumour 10 consisted of two metastases from the same primary tumour, each with a different DNA ploidy (Table 1).

We further compared the 19 MAP carcinomas to the CIN profile of sporadic carcinomas (Figure 1). For accurate comparison, we used a series of 22 microsatellite-stable sporadic carcinomas with CIN that were analysed previously using the same SNP methodology[14] and displayed the typical CIN profile of sporadic carcinomas [4]. Our comparison showed that the amount of cnLOH in MAP carcinomas is significantly increased compared to sporadic carcinomas ($p < 0.001$). Moreover, the amount of physical chromosomal losses is significantly ($p < 0.001$) decreased compared to sporadic carcinomas (Figure 1). No differences were seen in the number of chromosomal gains between MAP carcinomas and the sporadic carcinomas. The majority of chromosomal events that are targeted by cnLOH in MAP comprise physical loss instead of cnLOH in sporadic CRC.

The observed pattern of cnLOH versus physical loss was confirmed for five representative MAP carcinomas (t2, t4, t10, t12 and t18) after flow sorting, by FISH for chromosome 17p and 18q on tumour nuclei, in combination with LOH analysis using microsatellite markers. One sporadic carcinoma was included as a control (Table 2). The SNP arrays revealed that four of these five MAP carcinomas exhibited cnLOH on chromosome 17p (t2, t4, t12 and t18) and three exhibited cnLOH on chromosome 18q (t2, t12 and t18). Two MAP cases and the sporadic CRC displayed physical loss of chromosomes 17p and/or 18q. All FISH results that could be obtained were in agreement with our estimation based on the DNA index in combination with the SNP array results. For example, in the tumours with a near-diploid genome content, two copies of chromosome 17p and 18q were identified by FISH in case of cnLOH and in tumours with a neartriploid genome three copies were identified in case of cnLOH (Figure 2). However, within MAP carcinoma t18 (DI = 1.4) only half of the tumour nuclei showed three chromosomal arms of 18q, indicating intratumour heterogeneity. The sporadic carcinoma also harboured two cell

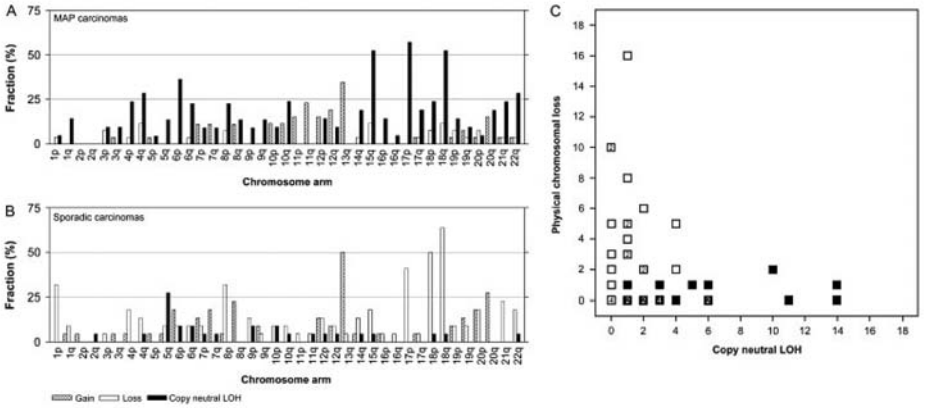

Figure 1. Chromosomal aberrations in MAP carcinomas versus sporadic CRCs. (A, B) The bars indicate the percentage of the 26 MAP carcinomas and 22 sporadic carcinomas, respectively, that exhibit an event of gain, loss or cnLOH of a chromosome. This percentage has been calculated for the respective chromosome arms. White bars, chromosomal gains; checked bars, physical losses of chromosomes; black bars, cnLOH. (C) In this graph the number of cnLOH events versus the number of physical losses is depicted for the 19 MAP carcinomas for which all genomic information was collected (see Materials and methods) versus 22 sporadic carcinomas. White squares, sporadic carcinoma; black squares, MAP carcinoma. The numbered squares represent multiple carcinomas that share the same amount of copy-neutral LOH and physical chromosomal loss

populations, with different copy numbers on chromosomal arms 17p and 18q. LOH was unambiguously identified for all informative microsatellite markers in all these cases, also in the cases with cnLOH in the context of a triploid genome content (implying the presence of three copies of a single allele), except for D17S921 in the diploid fraction of MAP carcinoma t4, which showed retention. These results are concordant with the results obtained with the SNP array analysis.

DISCUSSION

Three studies have reported on the genetic profiles of MAP tumours.[3,9,10] Unfortunately, the results of these studies are seemingly contradictory. Copy number changes in adenomas have been reported, as well as near-diploidy in adenomas and carcinomas. In order to gain more insight into the genetic instability in MAP tumours we studied a series of 26 MAP carcinomas using SNP array analysis in FFPE tissue. In contrast to sporadic colorectal cancer, copy-neutral LOH (cnLOH) appears to be a prevalent characteristic of MAP carcinomas, while only a few copy number abnormalities were identified (4). However, the percentage of chromosomal gains (24%) is comparable to sporadic colorectal cancers with CIN. Such a genomic tumour profile of colon cancer has, to our knowledge, not been described before. With the recent availability of SNP

arrays, more detailed information can be obtained on genome-wide cnLOH and several studies now report on cnLOH in cancers.[19,20] However, no study has described cnLOH to the extent seen in our series of MAP carcinomas.

The relative absence of chromosomal loss in our series of MAP carcinomas indeed reflects a near-diploid genome. Ploidy analysis using flow cytometry concluded that 12/23 (52%) MAP carcinomas analysed were near-diploid (DNA index, 1.0 ± 0.1). Lipton *et al*[3] found a near-diploid genome in 12/13 MAP carcinomas tested, with one carcinoma showing a polyploid status. We found three cases with a near-triploid DNA index (1.5 ± 0.1). In addition, flow cytometry revealed that seven carcinomas contained two fractions, each with a different DNA index. In all seven of these carcinomas, one of the fractions was near-diploid, while the other fraction had a DNA index of 1.5 in five of the seven cases. Interestingly, the distribution of the DNA ploidy of the MAP carcinomas is very different from sporadic colorectal cancers, which are primarily highly aneuploid. A DNA index of ~1.5 is uncommon in sporadic CRC, although near-triploidy has been described for sporadic CRC.[21,22] We confirmed the scoring of our SNP results in a purified set of tumours by a combination of FISH and LOH analysis, using polymorphic chromosomal microsatellite markers on chromosomes 17p and 18q. In the tumours with a near-diploid genome content, two copies of chromosome 17p and 18q were identified by FISH in case of cnLOH, and in tumours with a near-triploid genome three copies of a single allele were identified in case of cnLOH. Possible limited sensitivity in detecting copy number aberrations, especially in heterogeneous tumours, is unlikely in view of the FACS sorting in combination with FISH and conventional LOH analysis. Moreover, the results we obtained on the sporadic CRC are reassuring in this respect, since these are analysed and scored in exactly the same way as the MAP tumours.

Recently, we studied by SNP analysis of FFPE tissue a series of microsatellite-unstable sporadic and Lynch syndrome colon carcinomas, often with a near-diploid DNA content. All MSI-H carcinomas showed few chromosomal aberrations. CnLOH was infrequent in these tumours and usually confined to the locus harbouring a pathogenic mutation in MLH1, MSH2 or PMS2.[23]

These results further underline the uniqueness of the phenotype of the MAP carcinomas. Interestingly, the cnLOH events identified in the MAP carcinomas frequently involve the same chromosomes affected by physical loss in sporadic colorectal cancer, indicating that the same tumorigenic pathway may be involved in tumour initiation and progression. For example, chromosomes 17p and 18q are commonly affected by physical loss in sporadic colorectal cancer, whereas cnLOH is identified primarily on these chromosome arms in MAP carcinomas.

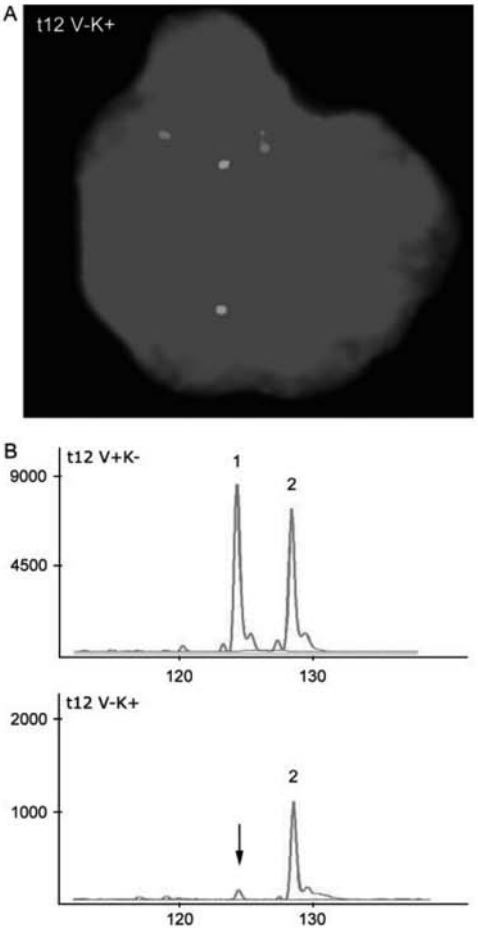

Figure 2. Microsatellite LOH analysis and fluorescent *in situ* hybridization on chromosome 18q21.1 after flow sorting of MAP carcinoma t12 (see also Table 2). (A) FISH showed two centromeric chromosome 18 signals (red) and two signals on 18q21.1 (green) for MAP carcinoma t12 (DNA index = 1.0). (B) Microsatellite LOH analysis (D18S877) on the flow-sorted MAP carcinoma t12 is shown: (upper panel) vimentin-positive, keratin-negative (normal) fraction; (lower panel) the vimentin-negative, keratin-positive (tumour) fraction. Unambiguous LOH is seen of allele 1 in the tumour. In combination with the FISH result shown in (A), copy-neutral LOH for chromosome 18q can be concluded

Table 2. Confirmation of copy-neutral LOH by FISH and microsatellite analysis

		p53 locus (17p)				SMAD4 and SMAD2 locus (18q)					
Tumour	DNA index	SNP array Chr. 17	D17S938	D17S921	FISH 17p	SNP array Chr. 18	D18S877	D18S65	D18S460	D18S1137	FISH 18q
t2 K^+ Dip	1.1	17p cnLOH*	LOH	LOH	na	18pq cnLOH*	LOH	na	na	LOH	na
t2 K^+ An	1.4	17p cnLOH*	LOH	LOH	3/3	18pq cnLOH*	LOH	na	LOH	na	na
t4 K^+ Dip	1	17pq cnLOH*‡	U	R	2/2	18pq phLoss*	na	LOH	LOH	U	2/2†
t4 K^+ An	1.5	17pq cnLOH*‡	U	LOH	3/3	18pq phLoss*	LOH	LOH	LOH	U	
t10 K^+	1.5	17p phLoss	U	U	na	18q phLoss	LOH	LOH	U	na	na
t12 K^+	1	17p cnLOH	LOH	LOH	2/2	18pq cnLOH	LOH	na	LOH	na	2/2
t18 K^+	1.4	17p cnLOH	U	LOH	3/3	18pq cnLOH	LOH	LOH	U	LOH	2/2, 3/3
sp1 K^+	1	17p phLoss	U	LOH	2/1, 1/1	18pq phLoss	LOH	na	U	na	2/2, 1/1

Microsatellite LOH analysis and FISH after flow sorting of five MAP carcinomas and one sporadic carcinoma was concordant with our estimation based on the DNA index and SNP array results. Chr., chromosome, K^+, keratin-positive, vimentin-negative (tumour) fraction after flow sorting; Dip, diploid fraction; An, aneuploid fraction; PhLoss, physical loss; cnLOH, copy-neutral LOH. For the LOH analysis: LOH, loss of heterozygosity; R, retention of both alleles; U, uninformative; na, could not be analysed for technical reasons. For the FISH results, the first number indicates the amount of centromeres and the second number indicates the amount of chromosomal arms 17p and 18q, respectively.

* Assay performed on unsorted tumour material.

† The FISH for t4 on chromosome 18q was, due to technical limitations, not performed on flow-sorted tumour nuclei, but on a tissue slide.

‡ cnLOH of complete chromosome 17.

How frequent the genes that are targeted in sporadic colorectal cancer on these respective chromosomes, e.g. *p53* and the SMAD genes, are targeted in MAP carcinomas remains elusive. Lipton *et al* found only three *p53* (located on chromosome 17) somatic mutations in 14 MAP carcinomas analysed, although immunohistochemistry for p53 over-expression (indicative for mutation) was positive in four tumours that were negative for mutation testing. SMAD4 mutations on 18q were not found in the MAP carcinomas analysed by Lipton *et al*,[3] although analysis of two chromosome 18q microsatellite markers showed a high frequency of 18q LOH in 7/14 cases analysed.

Our studies also indicate that chromosome 15q is often targeted by cnLOH in the MAP carcinomas. Physical loss of this chromosome has been associated with distant metastasis of sporadic colorectal cancer.[4] Copy-neutral LOH can arise via mitotic recombination, non-disjunction, or deletion and reduplication events. In our series, we identified cnLOH on whole chromosomes and on parts of chromosomes. The high prevalence of cnLOH in MAP carcinomas suggests a relationship between mitotic recombination and the *MUTYH* deficiency. However, it is difficult to explain why MAP cancers show few copy number aberrations. First, the occurrence of copy-neutral LOH might be directly linked to BER malfunctioning. Secondly, in parallel to a mismatch repair deficiency, the mutational burden might be relatively high due to the BER defect, favouring mitotic recombination but not physical loss. Therefore, further research into this possible relation is important.

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