Chromosomal Instability in Microsatellite-Unstable and Stable Colon Cancer

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Abstract

Purpose: The genomic instability in colon cancer can be divided into at least two major types, microsatellite instability (MSI) or chromosomal instability (CIN). Although initially felt to be mutually exclusive, recent evidence suggests that there may be overlap between the two. The aim of this study was to identify chromosomal alterations at high resolution in sporadic colon cancers with high-level microsatellite instability (MSI-H) and to compare them to those present in a set of matched microsatellite stable (MSS) tumors.

Experimental Design: Array-based comparative genomic hybridization was used to analyze a set of 23 sporadic MSI-H and 23 MSS colon cancers matched for location, gender, stage, and age. The arrays consisted of 2,464 bacterial artificial chromosome clones.

Results: MSI and MSS colon cancers differed significantly with respect to frequency and type of chromosomal alterations. The median fraction of genome altered was lower among MSI-H tumors than MSS tumors (2.8% versus 30.7%, $P = 0.00006$). However, the MSI-H tumors displayed a range of genomic alterations, from the absence of detectable alterations to extensive alterations. Frequent alterations in MSI-H tumors included gains of chromosomes 8, 12, and 13, and loss of 15q14. In contrast, the most frequent alterations in MSS tumors were gains of 7, 13, 8q, and 20, and losses of 8p, 17p, and 18. A small, previously uncharacterized, genomic deletion on 16p13.2, found in 35% of MSI-H and 21% of MSS tumors, was confirmed by fluorescence *in situ* hybridization.

Conclusion: MSI and CIN are not mutually exclusive forms of genomic instability in sporadic colon cancer, with MSI tumors also showing varying degrees of CIN.

Genomic instability plays an essential role in the development and progression of colon cancer. Based on different forms of genomic instability, colon cancer can broadly be divided into two groups. In the first, tumors are characterized by the presence of defective DNA mismatch repair (MMR). These tumors show the presence of high-level microsatellite instability (MSI-H) and the absence of protein expression for one of the several proteins involved in the MMR pathway (1). The most commonly affected gene in sporadic colon cancer with defective MMR is hMLH1, with the primary mechanism of gene inactivation being hypermethylation of the promoter (2). These tumors account for ~15% of sporadic colon cancers. The majority of sporadic colon cancers (85%), however, are proficient in DNA MMR but show another form of genomic instability at the gross chromosomal level, which has been called chromosomal instability (CIN). Such CIN represents the end result of a number of processes, including mutations in mitotic checkpoint genes, microtubule spindle defects, and telomere dysfunction (3). Tumors with CIN are most often aneuploid, have an abnormal karyotype, and are microsatellite-stable (MSS), whereas the majority of MSI-H tumors are believed to be near-diploid and with few, if any, karyotypic abnormalities. Recent studies, however, suggest that some MSI-H tumors may also show evidence of CIN, although the extent and nature of this overlap remains uncertain (4–8).

The type and degree of genomic instability in colon cancers correlates with their clinical and phenotypic characteristics. Supporting this idea are the ample data that MSI-H and MSS colon tumors differ in their pathologic features, their prognosis, and response to therapy (9, 10). A more refined classification of colon cancer that recognizes the possible overlap between CIN and MSI-H tumors may prove to be of clinical relevance.

Array-based comparative genomic hybridization (CGH) allows the detection of DNA copy number alterations in

tumors at high resolution, providing an overview of the extent of genomic damage in a tumor at the chromosomal and subchromosomal level, and aids in the localization and identification of genes involved in molecular pathways critical to carcinogenesis (11, 12). Array-CGH was previously applied to a set of 130 primary colorectal tumors. Among that tumor set were seven MSI-H tumors, the majority of which, surprisingly, had concomitant chromosomal alterations to a varying degree (5). The current study sought to confirm and expand on this finding, by using array-CGH to assess DNA copy number alterations present in a new and larger set of sporadic MSI-H colon cancers, and to compare these alterations with those present in a set of MSS tumors matched for important clinical variables. The high resolution afforded by array-CGH allowed a more precise characterization of the different patterns of CIN in sporadic MSI-H colon cancers than has previously been undertaken, and led to the identification of recurrent alterations in small genomic regions, which may harbor target genes relevant to the carcinogenesis of sporadic MSI-H colon cancer.

Materials and Methods

Patient material. After institutional review board approval, a total of 23 MSI-H and 23 MSS sporadic colon cancers were identified from a group of tumors that was prospectively collected at the Mayo Clinic, Rochester, MN from 1995 to 1998. The MSI-H and MSS tumors were matched for gender, location, stage, and age. Tumor blocks were trimmed on a cryostat to remove normal and necrotic tissue. After sectioning, DNA was extracted using standard phenol/chloroform methods. H&E sections were reviewed to confirm that tumor cells comprised >70% of the specimen.

DNA MMR status. The MMR status for each tumor was determined by a combination of immunohistochemistry and MSI testing (13). For the immunohistochemical analysis, tumors were tested for the presence or absence of protein expression for hMLH1, hMSH2, and hMSH6. For the MSI analysis, normal and tumor pairs were tested for the presence of MSI using a panel of seven markers, one mononucleotide (BAT26) and six dinucleotide markers (D5S346, TP53, D18S34, D18S49, D18S61, and ACTC). Tumors demonstrating instability at >30% of these markers were classified as MSI-H.

For the tumors found to have defective MMR (MSI-H and absence of protein expression), DNA from peripheral blood leukocytes was subjected to DNA sequence analysis to test for the presence of a germ line alteration in one of the MMR genes. Additionally, for those cases with the involvement of hMLH1 (as detected by immunohistochemistry) the methylation status of the promoter was evaluated (2). All of the MSI-H tumors selected for this study were caused by the inactivation of hMLH1 and all but two were due to hypermethylation of the hMLH1 promoter. No germ line mutations in hMLH1 were detected in this group.

Array-CGH. Hum 3.1 arrays were obtained from the University of California San Francisco Cancer Center Array Core facility. The arrays consisted of 2,464 bacterial artificial chromosome (BAC) or P1 clones printed in triplicate, covering the human genome with a resolution of ~1 Mb. Pooled lymphocytic DNA from normal male individuals was used as a reference for the hybridizations (Promega, Madison, WI). Preparation of the arrays and hybridization of the DNA were done as described previously (5). Briefly, 100 ng of tumor and reference DNA were first amplified and then fluorescently labeled with Cy3- and Cy5-dCTP (Amersham Pharmacia, Piscataway, NJ) by random priming (Invitrogen, Carlsbad, CA). Hybridization was done for 48 hours in a formamide-based buffer in the presence of Cot-1 DNA (Invitrogen).

Three, 16-bit fluorescence, single-color intensity images were collected from each array using a custom-built CCD camera system. A tiling BAC array was used for higher resolution analysis of the chromosome 16 deletion.

Data analysis. Images were analyzed using University of California San Francisco Spot and Sproc software (14). Sproc automatically excludes data points based on low 4',6-diamidino-2-phenylindole intensity, poor correlation between Cy3 and Cy5, and a low reference/4',6-diamidino-2-phenylindole signal. For each clone, the average ratio of test to reference intensity was calculated from the three replicate clones on the array. The data were further filtered to exclude polymorphic and unmapped clones, clones whose ratio was derived from only one of the triplicates or with a triplicate $\log_2$ SD >0.2, and for clones that were present in <75% of all samples. A set of common outlier clones was also excluded from further analysis, reducing the average clone number to 1,818 clones per case. The July 2003 freeze of the human DNA sequence draft at http://genome.ucsc.edu/ was used to map clones. The $\log_2$ ratios for each case were median centered to zero. Primary and filtered array-CGH data for all samples are available as supplementary data files (http://cc.ucsf.edu/people/waldman/Colon/Trautmann.html).

The array-CGH data were analyzed using circular binary segmentation to translate noisy intensity measurements into regions of equal copy number (15). Each probe was assigned a segment value referred to as its smoothed value. The scaled median absolute deviation of the difference between the observed and smoothed values was used to estimate the tumor-specific experimental variation. The gain and loss status for each probe was defined using the merged level procedure (16).

The amplification status for a clone was determined by considering the width of the segment to which that clone belonged and a minimum difference between the smoothed value of the clone and the segment means of the neighboring segments. The clone was declared amplified if it belonged to the segment spanning <26 Mb and the minimum difference was greater than $\exp(-x3)$ where x is the final smoothed value for the clone. Clones with $\log_2$ ratios less than -0.75 were considered low-level deletions.

Statistical analysis. Clones with significantly different copy numbers between MSI-H and MSS tumors were identified using a *t*-statistic with pooled variance. Adjustments for multiple comparisons were made using false discovery rates. The Kruskal-Wallis nonparametric test was used to assess the significance of the different measures of genomic instability between MSI-H and MSS tumors. The following measures of genomic instability were considered: the fraction of genome altered (FGA), number of whole chromosome changes, number of chromosomal break points, number of chromosomes containing break points, and the number of chromosomes with amplifications. The fraction of the genome gained or lost for each case was calculated as the sum of genomic distances represented by each clone on Hum 3.1 (17). Unsupervised hierarchical clustering was done for tumors using the complete linkage as a measure of similarity with Spearman rank correlation as a metric.

Fluorescence in situ hybridization. Dual-color fluorescence *in situ* hybridization was done to confirm the deletion of clone RP11-160D13 at 16p13.2. The following probes were used: centromere 16 (PHUR 195), RP11-160D13, RP11-167B4, and RP11-18H23. Clone RP11-167B4 was chosen as an additional test clone spanning an overlapping region to RP11-160D13. To distinguish between failure of hybridization and deletion of the test probes, we used a control clone (RP11-18H23), which was simultaneously hybridized on a different area of the same tumor section. Fluorescence *in situ* hybridization was done as described previously (18). PHUR 195 was labeled with FITC and BAC DNA with Cy3 using standard nick translation. Slides were denatured in 1 mol/L of sodium sulfocyanate at 80°C for 30 minutes, and digested in pepsin (0.5 mg/mL) at 37°C for 15 minutes prior to hybridization at 37°C. Visualization and scoring of hybridization signals was done as described previously (18).

Results

Genomic profiles of MSI-H and MSS colon cancers. Array-based CGH was done on 23 MSI-H (Fig. 1A) and 23 MSS (Fig. 1B) colon cancers. The clinicopathologic characteristics for these cases are summarized in Table 1.

For the entire set of MSI-H tumors, the median fraction genome altered was 2.8% (mean, 6.8 ± 10%). The median genome gained was 2.3% (mean, 5.1 ± 8.3%) and the median genome lost was 0% (mean, 1.7 ± 4%). The most frequent imbalances in the MSI-H cancers were gains of chromosome 8, mainly involving 8q22-24 (35% of the cases), chromosome 13, especially 13q13-14 (26%), and chromosome 12 (13%). Losses occurred predominantly at 15q14 (17%).

Table 1. Clinical characteristics

	MSI-H tumors	MSS tumors
Stage		
1	5	2
2	8	10
3	9	11
4	1	0
Location		
Proximal	22	22
Distal	1	1
Sex		
Female	13	13
Male	10	10
Age		
51-60	2	5
61-70	4	6
71-80	14	8
81-90	3	3
91-100	0	1

MSS colon cancers displayed a much higher degree of CIN than MSI-H tumors, with an overall median FGA of 30.7% (mean, 37.3 ± 21.2%). The median fraction of genome gained was 17.2% (mean, 17.3 ± 9.3%) and the median lost was 19.9% (mean, 20 ± 16.1%). The most commonly altered chromosomal regions in MSS tumors were gains of 20q (95% cases), 13 (91%), 7 (68%), and 8q (59%), as well as losses of chromosome 18 (77%), 8p (68%), and 17p (73%).

High-level amplifications were identified in 8 of the 23 MSS cancers (five tumors with one amplification and three tumors with two), whereas only one MSI-H tumor (no. 11) had a single amplicon detected (at 15p11, see Fig. 2A). The most frequently seen loss of a single clone was the deletion of RP11-160D13 on 16p13.2 (6.5 Mb) A list of all clones reporting amplifications and low-level deletions in MSI-H and MSS cancers with their chromosomal location is shown in Supplementary Table S1.

Patterns of chromosomal alterations in sporadic MSI-H tumors. MSI-H tumors displayed several patterns of CIN following array-CGH. Five tumors (22%) had extensive chromosomal changes (median FGA, 21.2%; mean, 22.8 ± 13.1%; Fig. 2A) including gains and losses of multiple chromosomes. However, losses of whole chromosomes were very rare in MSI-H tumors, affecting only two tumors (nos. 11 and 95), both of which displayed extensive chromosomal changes. The sole common whole chromosome loss was loss of chromosome 4, which occurred in both tumors. Seven MSI-H tumors (30%) had alterations of a single chromosome or large chromosomal segment (median FGA, 5%; mean, 4.5 ± 1.6%; Fig. 2B). The identified chromosomal changes occurred predominantly in the form of chromosomal gains, although two tumors showed losses in the region 15q14. Eleven out of 23 MSI-H tumors (48%) revealed almost no chromosomal changes by array-CGH (median FGA, 0%; mean, 0.8 ± 0.1%; Fig. 2C), with four of those tumors showing alterations of very small chromosomal regions encompassing only a few BACs, most commonly in the form of small telomeric deletions.

Comparison between MSI-H and MSS colon cancers. Unsupervised hierarchical cluster analysis of the smoothed array-CGH data grouped the tumors into two main clusters, with one cluster containing all but two of the MSI-H and the second
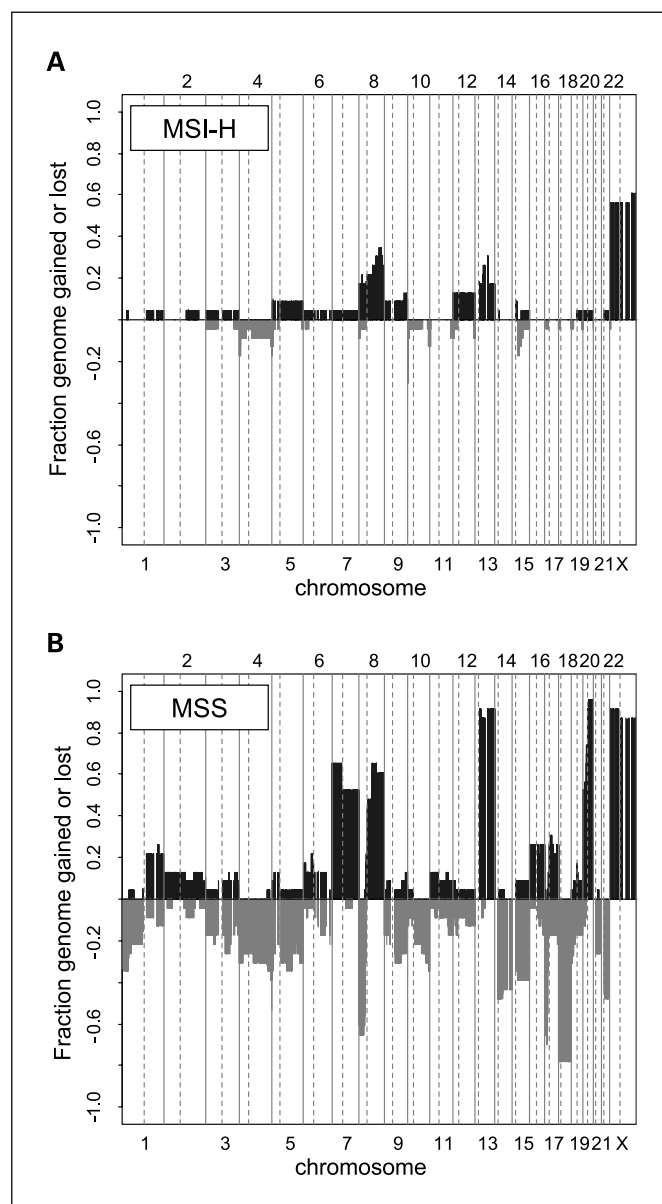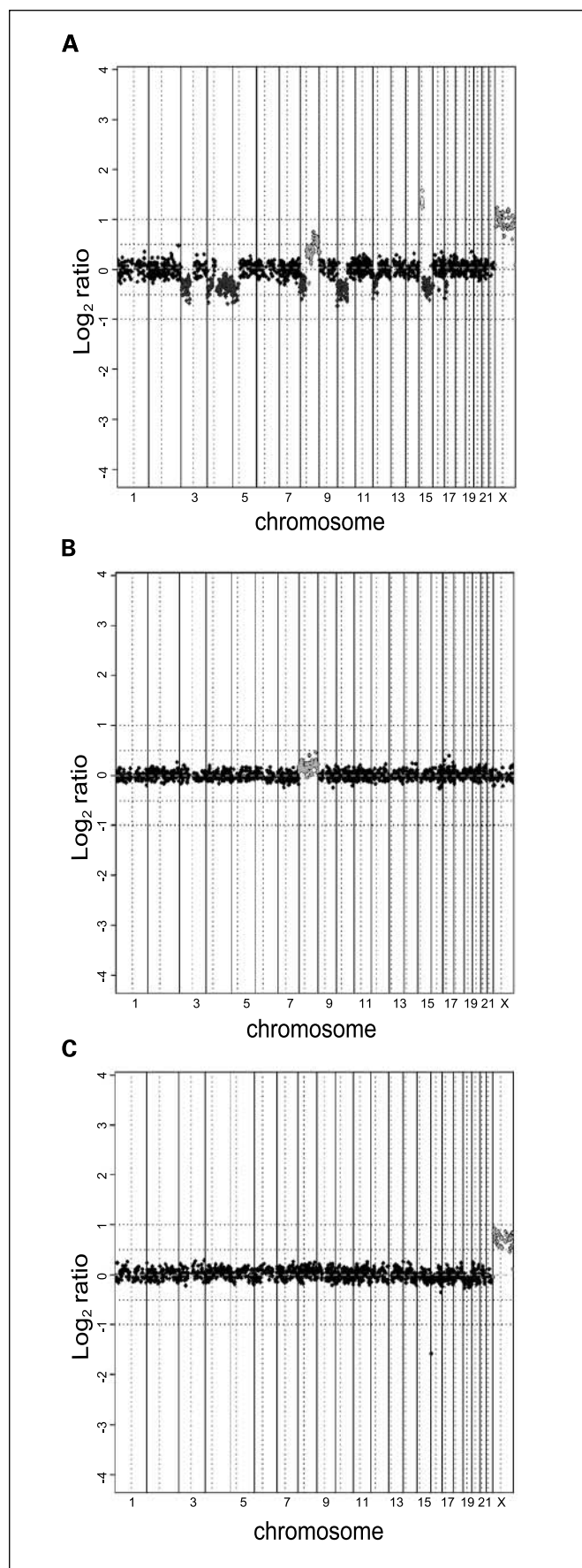

Fig. 1. Array-CGH alterations in 23 MSI-H (*A*) and 23 MSS (*B*) colon cancers. Clones are ordered according to their chromosomal position. Gains are shown above and losses below the 0 horizontal line; *dashed lines,* the centromere location.

cluster containing all MSS tumors (Fig. 3A). The two MSI-H cases that clustered with the MSS cancers were both characterized by multiple chromosomal alterations.

Sporadic MSI and MSS tumors differed significantly with respect to the frequency and type of chromosomal alterations (Table 2). The median fraction of the genome altered was lower among the MSI tumors than the MSS tumors (P = 0.00006), affecting both gains and losses. Other measures of genomic instability, representing more specific mechanisms of chromosome alterations, were also significantly different between MSI-H and MSS colon cancers. These included the number of whole chromosome changes, the number of chromosomal break points, the number of chromosomes containing break points, and the number of chromosomes with amplifications.

A direct comparison of MSI-H and MSS tumors was made to identify individual clones significantly altered between the two groups. Gains of clones located on chromosomes 20q, 7, and 13q, and losses of clones located on chromosome 18, 17p, and 8p were significantly more common in the MSS cancers. A comprehensive list of these clones and a graphical representation of their chromosomal distribution can be found in Supplementary Table S2 and Supplementary Fig. S1.

Deletion of clone RP11-160D13. The most frequently seen loss of a single clone was deletion of RP11-160D13 on 16p13.2 (6.5 Mb), which was lost in 8 out of 23 MSI-H and 8 out of 23 MSS tumors. For this clone, the low tumor to reference $\log_2$ ratio met the criteria for a low-level deletion, which is highly indicative of a homozygous loss in three of the MSI-H and in one of the MSS tumors. Similarly, this clone (RP11-160D13) was homozygously lost in the MLH1-defective colon cancer cell line, HCT116. Array-CGH analysis of HCT116 using a tiling BAC array confirmed the low-level deletion on 16p13.2. On that array, the deletion spanned a total of eight BACs encompassing the region between 6091158 and 7019966 kb on 16p as shown in Fig. 4. Fluorescence *in situ* hybridization was done on paraffin sections from a total of eight MSI-H tumor cases, four of which showed the deletion by array-CGH. RP11-160D13 was found homozygously deleted (Fig. 3A and B) in all of these tumors, but in none of the control samples. One of the cases demonstrating a deletion displayed a significant amount of intra-tumoral heterogeneity with some areas being homozygously deleted and others showing no deletion.

Discussion

MSI and CIN have been considered to be mutually exclusive forms of genomic instability. The distinction seems to be of clinical importance because recent data strongly suggests that biological behavior, including response to therapy, differs between MSI-H and MSS tumors (9). However, the genomic classification of colon cancers based on MSI and CIN status

Fig. 2. Representative array-CGH profiles from MSI-H colon cancers demonstrating (*A*) extensive CIN, (*B*) few whole chromosome changes, and (*C*) no changes. Profiles show copy number alterations relative to normal DNA ordered by chromosome with each spot representing one clone. Chromosomal gains and losses are defined as described. Gains are shown above and losses below. Note the amplification on chromosome 15p11 (*A*) and the single BAC deletion on 16p13.2 (*C*).

may be overly simplistic, and tumors may have evidence of both types of instability or neither. For example, recent data indicate that the CpG island methylator phenotype can be found in up to 25% of sporadic colon tumors and that those

Fig. 3. *A,* unsupervised hierarchical clustering of all MSI-H and MSS tumors. Dendrogram shows two main clusters largely separating MSI-H (*yellow*) and MSS (*blue*) tumors. *Red,* increased copy numbers; *green,* decreased copy numbers. Note that two MSI-H cases with extensive chromosomal changes cluster with the MSS tumors. *B,* dual-color fluorescence *in situ* hybridization analysis (magnification, ×100) of a single MSI-H tumor using a centromeric probe for chromosome 16 (*green*) in combination with BAC RP11-18H23 (*red*), a control clone localizing to 16p11.2, showing two green and two red signals. *C,* analysis with chromosome 16 (*green*) in combination with BAC RP11-160D13 (*red*), showing two green and zero red signals, confirming the homozygous deletion of RP11-160D13 on 16p13.2.

tumors may not progress independently through either the MSI or the CIN pathways (4, 19, 20). An even more precise genomic and epigenetic classification of tumors, beyond the simple MSI versus CIN dichotomy, may prove to further delineate important subgroups of colon cancers with respect to clinical behavior.

In this study, array-CGH was employed to detect DNA copy number gains and losses at high resolution in a set of sporadic MSI-H tumors, and to compare the extent and type of alterations found with a set of MSS tumors matched for sex, location, stage, and age. Matching was intended to reduce the effect of factors other than MSI status on the differences in DNA copy number alterations observed. Our goal was to more precisely determine the extent and patterns of chromosomal alterations present in sporadic MSI-H tumors. The major finding of this study was that MSI and CIN were not found to be mutually exclusive forms of genomic instability. Although the overall frequency of DNA copy number alterations was much lower in the MSI than the MSS tumors, two of the MSI-H tumors had such extensive chromosomal alterations that they clustered with the MSS tumors in an unsupervised hierarchical cluster analysis of the smoothed array-CGH data. In addition, the majority of MSI-H tumors displayed some level of chromosomal alterations detectable by array-CGH.

Although evidence for some degree of CIN could be found in the majority of MSI-H tumors, the specific alterations identified differed significantly between the two tumor groups. The most common alterations in the MSI-H tumors were gains of chromosomes 8, 12, 13, and losses of 15q14, while the profile of gains and losses in the MSS tumors with high degrees of CIN was very similar to that previously reported (5, 6), with gains of 7, 8q, 13, and 20q, and losses of 8p, 17p, and 18 being most common. A clone-by-clone comparison between the two groups confirmed that these chromosomal loci harbored most of the clones whose pattern of gain or loss significantly differed between the MSI-H and MSS cancers.

Several other studies have found that MSI and CIN can occur in the same tumor (4–8, 21). Using microsatellite markers to assess loss of heterozygosity, Goel et al. (4) identified at least one locus of loss of heterozygosity in 7 out of 30 (23%) MSI-H tumors, and employing chromosomal CGH, Li and colleagues (7) found chromosomal alterations in 31% of 39 MSI-H colorectal cancers. In accordance with our findings, more recent studies employing chromosomal or array-CGH in small sets of MSI-H tumors have concluded that the majority of MSI-H cancers have some degree of CIN (6, 8). All of these studies differed to some extent in terms of the particular loci most commonly altered among the MSI-H tumors. Some of these discrepancies regarding the specific loci altered may be methodologic, due to differences between array- and metaphase-based CGH or loss of heterozygosity approaches. Another explanation for the diversity of chromosomal alterations seen may be the inclusion in past studies of tumors from patients with hereditary nonpolyposis colorectal cancer, whereas in the current study, only sporadic MSI-H tumors with lack of expression of MLH1 were analyzed. There is ample evidence that the molecular changes in sporadic versus hereditary colon cancer with defective MMR differ (22).

This study expands on previous reports in several important respects. First, it seems that not all MSI-H tumors show roughly equal and low amounts of chromosomal aberrations; rather,
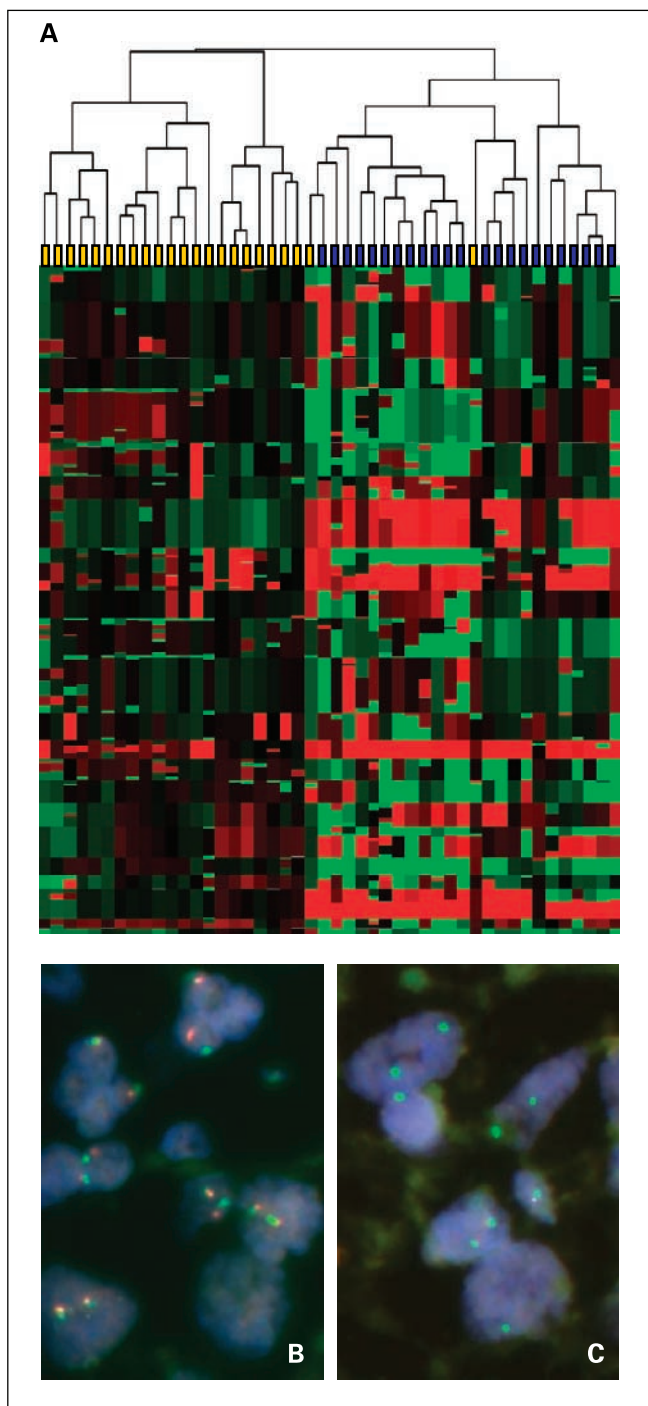
Table 2. Median frequency of chromosomal alterations in MSI-H versus MSS tumors

	MSI-H	MSS	Kruskal-Wallis
Fraction of genome altered	0.028	0.307	0.00006
Fraction of genome gained	0.019	0.172	0.002
Fraction of genome lost	0	0.199	0.00005
Number of whole chromosome changes	0	4	0.0002
Number of chromosomal break points	2	8	0.003
Number of chromosomes containing break points	1	6	0.002
Number of chromosomes with amplifications	0	0	0.009*

*Although the median number of chromosomes with amplifications was 0 for both groups, their mean number differed (MSI, 0.043; MSS, 0.478).

the extent of CIN in MSI-H tumors may follow one of three general patterns. One group of MSI-H cancers (48%) had no detectable signs of CIN following array-CGH, another group (30%) had a small number of alterations, and a third group (22%) displayed multiple chromosomal alterations, with the extent of alterations approaching those seen in MSS tumors. Second, the types of chromosomal alterations seen in MSI-H tumors differed from that in MSS tumors, with the almost complete absence of whole chromosome or chromosome arm losses in MSI-H tumors, whereas these losses are common in MSS tumors. Third, the use of array-CGH, with its extremely high resolution, allows a precise delineation of the differences in target loci between MSI-H and MSS tumors, and the identification of a number of very small chromosomal alterations in MSI-H cancers, some of which have not been previously described.

The biological, mechanistic, and clinical importance of the different patterns of chromosomal alterations among the MSI-H tumors observed in this study needs to be established in a larger tumor set with known outcomes to determine if MSI-H tumors with different degrees of CIN have different clinico-pathologic characteristics and clinical behavior, including response to therapy. The failure to see almost any loss of large chromosomal regions in the MSI-H tumors, for example, may be related to the fact that MSI-H tumors are likely diploid or near diploid, and therefore, major chromosomal loss may lead to potentially lethal alterations in gene dosage. On the other hand, MSS tumors are frequently aneuploid, so loss of large

chromosomal regions may be tolerated and may provide a growth advantage given relative changes in gene dosage. Array-CGH was the only method employed to assess chromosomal alterations in this study, and ideally, future studies might also include other methodologies. Array-CGH is limited by its inability to detect chromosomal alterations, such as balanced translocations, which do not alter the overall DNA copy number in the tumor compared with the reference. It is possible that MSI-H tumors have a preponderance of these types of alterations as a cause of their low FGA, but this would be very unlikely.

One clear advantage of the array-CGH is its extremely high-resolution for the alterations detected, thereby allowing a precise identification of differences in the loci altered between MSI-H and MSS tumors. The differences in altered loci between the two tumor groups likely reflects differences in target genes and supports the growing body of evidence that gene expression profiles differ markedly between MSI-H and MSS cancers (23–25). The high-resolution analysis afforded by array-CGH also aids in the identification of a number of very small chromosomal alterations in MSI-H cancers, some of which have not previously been described. The loss of a single clone on 16p13.2 (RP11-160D13) was identified in eight MSI and five MSS colon cancers. The presence of a homozygous deletion of RP11-160D13 in four of the MSI-H cases was confirmed by fluorescence *in situ* hybridization. Previous array-CGH studies have found a deletion of that region in 15% to 20% of all colon tumors and in a number of colon cancer cell lines, especially in the MLH-1-defective colon cancer cell line, HCT116 (5, 6, 26). RP11-160D13, which comprises a 130 kb region, lies within the Ataxin-2-binding-protein-1 (*A2BP1*) gene. A2BP1 encodes a protein that is known to interact with the spinocerebellar ataxia-type-2 (SCA2) protein (27). No involvement of A2BP1 in cancer has yet been reported. However, its binding partner, SCA2, was recently reported to act as a tumor suppressor by sensitizing neuroblastoma cells for apoptotic stimuli (28). Additional studies will need to address the specific role of A2BP1 in sporadic colon cancer.

Another common region of chromosomal loss in MSI-H tumors occurred at 15q14, with a single overlapping clone (RP11-70A17) spanning a 170 kb region that contains connexin-36, a member of the connexin gene family. Connexins code for gap junction proteins and are known to act as tumor suppressors in a variety of cancers (29). A rare amplicon on 15p11 (peak clone CTD-1018N9) was also found among the MSI-H cases, occurring in a single case that showed
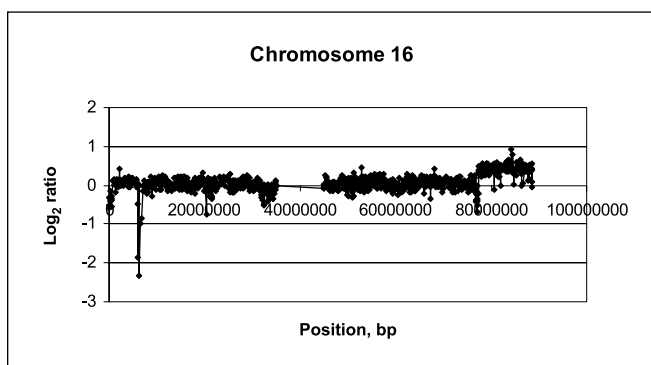

Fig. 4. Copy number profile of chromosome 16 in cell line HCT116 using a tiling array with 32,145 BACs. The homozygous deletion on 16p13.2 spans a total of 8 BACs.

multiple chromosomal alterations. It contains the gene ubiquitin-protein ligase E3A (UBE3A). This gene is known to interact with the E6 protein of human papillomavirus types 16 and 18, resulting in ubiquitination and proteolysis of tumor protein p53.

Array-CGH also was able to detect small telomeric deletions encompassing only a few megabases in several of the MSI-H cancers that showed no other signs of CIN. This unusual array-CGH pattern may represent an early stage of telomere-associated crisis eventually leading to more obvious chromosomal alterations. Data from animal models of cancer have shown that telomere dysfunction and resultant fusion-bridge-breakage cycles represent one mechanism that drives copy number alterations across cancer genomes (3). Recent studies indicate that telomere instability occurs in MSI-H tumors and may even be more common than in MSS cancers (30, 31).

In summary, MSI-H tumors show CIN to a variable degree, with the extent of CIN seeming to fall into one of three broad, overlapping patterns. However, the extent of abnormalities, and the specific chromosomal abnormalities seen, clearly differ from those in MSS tumors. Whether or not MSI tumors with differing degrees of CIN may have distinctive clinical behavior is uncertain. Future studies on prognostic assessment and response to therapy in colon cancer should therefore take into account this previously underestimated heterogeneity of sporadic MSI-H cancers with regard to CIN.

References

1. Baudhuin LM, Burgart LJ, Leontovich O, Thibodeau SN. Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for Lynch syndrome. Fam Cancer 2005;4: 255–65.
2. Cunningham JM, Christensen ER, Tester DJ, et al. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res 1998;58:3455–60.
3. Grady WM. Genomic instability and colon cancer. Cancer Metastasis Rev 2004;23:11–27.
4. Goel A, Arnold CN, Niedzwiecki D, et al. Characterization of sporadic colon cancer by patterns of genomic instability. Cancer Res 2003;63:1608–14.
5. Nakao K, Mehta KR, Fridlyand J, et al. High-resolution analysis of DNA copy number alterations in colorectal cancer by array-based comparative genomic hybridization. Carcinogenesis 2004;25:1345–57.
6. Douglas EJ, Fiegler H, Rowan A, et al. Array comparative genomic hybridization analysis of colorectal cancer cell lines and primary carcinomas. Cancer Res 2004;64:4817–25.
7. Li LS, Kim NG, Kim S, et al. Chromosomal imbalances in the colorectal carcinomas with microsatellite instability. Am J Pathol 2003;163:1429–36.
8. Camps J, Armengol G, Del Rey J, et al. Genome-wide differences between microsatellite stable and unstable colorectal tumors. Carcinogenesis 2006;27:419–28.
9. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247–57.
10. Raut CP, Pawlik TM, Rodriguez-Bigas MA. Clinicopathologic features in colorectal cancer patients with microsatellite instability. Mutat Res 2004;568: 275–82.
11. Pinkel D, Segraves R, Sudar D, et al. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 1998;20:207–11.

12. Snijders AM, Nowak N, Segraves R, et al. Assembly of microarrays for genome-wide measurement of DNA copy number. Nat Genet 2001;29:263–4.
13. Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet 2001;69:780–90.
14. Jain AN, Tokuyasu T, Snijders AM, Segraves R, Albertson DG, Pinkel D. Fully automatic quantification of microarray image data. Genome Res 2002;12: 325–32.
15. Olshen AB, Venkatraman ES, Lucito R, Wigler M. Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics 2004;5: 557–72.
16. Willenbrock H, Fridlyand JA. Comparison study: applying segmentation to array CGH data for downstream analyses. Bioinformatics 2005;21:4084–91.
17. Mehta KR, Nakao K, Zurae M, et al. Fractional genomic alteration detected by array-based comparative genomic hybridization independently predicts survival after hepatic resection for metastatic colorectal cancer. Clin Cancer Res 2005;11:1791–7.
18. Balazs M, Carroll P, Kerschmann R, Sauter G, Waldman FM. Frequent homozygous deletion of cyclin-dependent kinase inhibitor 2 (MTS1, p16) in superficial bladder cancer detected by fluorescence in situ hybridization. Genes Chromosomes Cancer 1997;19:84–9.
19. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A 1999; 96:8681–6.
20. Samowitz WS, Albertsen H, Herrick J, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology 2005;129:837–45.
21. Westra JL, Boven LG, van der Vlies P, et al. A substantial proportion of microsatellite-unstable colon tumors carry TP53 mutations while not showing chro-

mosomal instability. Genes Chromosomes Cancer 2005;43:194–201.
22. Young J, Simms LA, Biden KG, et al. Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. Am J Pathol 2001;159: 2107–16.
23. Giacomini CP, Leung SY, Chen X, et al. A gene expression signature of genetic instability in colon cancer. Cancer Res 2005;65:9200–5.
24. Kim H, Nam SW, Rhee H, et al. Different gene expression profiles between microsatellite instability-high and microsatellite stable colorectal carcinomas. Oncogene 2004;23:6218–25.
25. di Pietro M, Bellver JS, Menigatti M, et al. Defective DNA mismatch repair determines a characteristic transcriptional profile in proximal colon cancers. Gastroenterology 2005;129:1047–59.
26. Snijders AM, Fridlyand J, Mans DA, et al. Shaping of tumor and drug-resistant genomes by instability and selection. Oncogene 2003;22:4370–9.
27. Shibata H, Huynh DP, Pulst SM. A novel protein with RNA-binding motifs interacts with ataxin-2. Hum Mol Genet 2000;9:1303–13.
28. Wiedemeyer R, Westermann F, Wittke I, Nowock J, Schwab M. Ataxin-2 promotes apoptosis of human neuroblastoma cells. Oncogene 2003;22: 401–11.
29. King TJ, Bertram JS. Connexins as targets for cancer chemoprevention and chemotherapy. Biochim Biophys Acta 2005;1719:146–60.
30. Stewenius Y, Gorunova L, Jonson T, et al. Structural and numerical chromosome changes in colon cancer develop through telomere-mediated anaphase bridges, not through mitotic multipolarity. Proc Natl Acad Sci U S A 2005;102:5541–6.
31. Pickett HA, Baird DM, Hoff-Olsen P, et al. Telomere instability detected in sporadic colon cancers, some showing mutations in a mismatch repair gene. Oncogene 2004;23:3434–43.