

## Loss of heterozygosity: An independent prognostic factor of colorectal cancer

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### Abstract

**AIM:** Colorectal cancers result from the accumulation of several distinct genetic alterations. This study was to investigate the frequency and prognostic value of loss of heterozygosity (LOH) and microsatellite instability (MSI) at 14 genetic loci located near or within regions containing important genes implicated in colorectal tumorigenesis.

**METHODS:** We studied colorectal cancers with corresponding normal mucosae in 207 patients (139 males and 68 females, mean age at the time of tumor resection 66.2±12.4 years, range 22-88 years). There were 37 right-sided colonic tumors, 85 left-sided colonic tumors and 85 rectal tumors. The distribution of tumor staging was stage I in 25, stage II in 73, stage III in 68, and stage IV in 41. We analyzed the LOH and MSI of *HPC1*, *hMSH2*, *hMLH1*, *APC*, *MET*, *P53*, *NH23-H1*, *DCC*, *BAT25*, *BAT26*, *D17S250*, *MYCL1* and *D8S254* with fluorescent polymerase chain reaction and denatured gel electrophoresis. High-frequency LOH was determined to be greater than three, or more than 50% of the informative marker with LOH. High-frequency MSI (MSI-H) was determined as more than four markers with instability (>30%). Correlations of LOH and MSI with clinical outcomes and pathological features were analyzed and compared.

**RESULTS:** The occurrence of MSI-H was 7.25%, located predominantly in the right colons (7/15) and had a higher frequency of poor differentiation (6/15) and mucin production (7/15). LOH in at least one genetic locus occurred in 78.7% of the tumors and was significantly associated with disease progression. Of the 166 potentially cured patients, 45 developed tumor recurrence within 36 mo of follow-up. Clinicopathological factors affecting 3-year

disease-free survival (DFS) were TNM staging, grade of differentiation, preoperative CEA level, and high LOH status. Patients with high LOH tumors had a significantly lower DFS (50%) compared with patients with low LOH tumors (84%). Of the patients developing subsequent tumor recurrence, the number and percentage of LOH were 2.97 and 46.8% respectively, similar to the stage IV disease patients. TNM staging had the most significant impact on DFS, followed by high LOH status.

**CONCLUSION:** Clinical manifestations of LOH and MSI are different in colorectal cancer patients. High-frequency LOH is associated with high metastatic potential of colorectal cancers.

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**Key words:** Colorectal Cancer; Loss of Heterozygosity; Prognosis

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### INTRODUCTION

Cumulated genetic alterations could affect proto-oncogenes such as *Ki-ras* and tumor suppressor genes, including *APC*, *DCC* and *TP53*<sup>[1,2]</sup>. The common event that inactivates tumor suppressor genes is the loss of one allele frequently associated with an inactivating point mutation in the remaining wild-type allele. This process is referred to as loss of heterozygosity (LOH)<sup>[3]</sup>.

In another mutational pathway, colorectal cancers display increased rates of intragenic mutation characterized by generalized instability of short, tandemly repeated DNA sequences known as microsatellites<sup>[4]</sup>. High frequency of microsatellite instability (MSI-H) has been found in more than 90% of tumors in patients with hereditary nonpolyposis colorectal cancer<sup>[5,6]</sup>. The presence of MSI could be the result of defective DNA mismatch repair genes that were misaligned in repetitive sequences (caused by slippage of polymerases) and left unrepaired. In sporadic cases of colorectal cancer, MSI-H occurred in approximately 15% of tumors<sup>[4,5,7]</sup>.

Previous studies have demonstrated that tumors with more alleles lost have a considerably worse prognosis<sup>[8,9]</sup>. In contrast, the presence of MSI-H carries significant prognostic implications for colorectal cancer patients<sup>[10,11]</sup>. In this study,

we evaluated 207 colorectal cancer patients. LOH and MSI at 14 markers were detected by microsatellite analysis to evaluate the clinical manifestations of LOH and MSI in sporadic colorectal cancers.

## MATERIALS AND METHODS

### *Patients and clinicopathological data*

A total of 207 patients with colorectal adenocarcinoma who underwent resection in Taipei Veterans General Hospital from January 1999 to December 1999 were enrolled in this study. Patients were excluded if they received preoperative chemotherapy and/or radiotherapy, and had evidence of hereditary non-polyposis colorectal cancer according to the criteria of Amsterdam or familial adenomatous polyposis, a malignant tumor outside the colon within the previous five years, or died from surgical complications. Clinical data were recorded prospectively and stored in computerized files. The database included (1) patients' names, gender, age, family history, and major medical problems, (2) location, size, gross appearance, tumor-node-metastasis (TNM) stage, differentiation, and relevant pathologic prognostic features, and (3) types of operations, complications, recurrence of disease, and follow-up conditions. After operation, patients were followed up every 3 mo in the first 2 years, every 6 mo between 2 and 5 years, and annually thereafter. All patients were followed up for at least 3 years after operation or until death.

### *Tumortissues*

The Institutional Review Board of the Taipei Veterans General Hospital approved the study program. Consents to tissue collection were obtained from all patients. Following removal, specimens were cleaned thoroughly. The tumors were dissected meticulously and samples were collected from 4 different quadrants of the tumor for consideration of intratumoral heterogeneity. The corresponding normal mucosae taken at least 10 cm away from the primary tumor were collected. The fragments of samples were immediately frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . Sections of cancer tissues and the corresponding normal mucosae were identified, reviewed, and analyzed by a senior gastrointestinal pathologist who did not know the clinical outcome of the patients. The stage of the disease was classified according to the TNM classification of the International Union Against Cancer<sup>[12]</sup>. There were 139 (67.1%) men and 68 (32.9%) women in the study group. The mean age at the time of tumor resection was  $66.2 \pm 12.4$  years (range 22–88 years). There were 37 (17.8%) right side colonic tumors (cecum to splenic flexure), 85 (41.1%) left side colonic tumors (splenic flexure to sigmoid colon) and 85 (41.1%) rectal tumors. The distribution of tumor staging was stage I in 25 (12.1%), stage II in 73 (35.3%), stage III in 68 (32.9%) and stage IV in 41 (19.8%).

### *LOH and MSI analysis*

High-molecular-weight genomic DNA was prepared from each tumor and the corresponding normal tissue using the QIAamp tissue kit (QIAGEN GmbH, Hilden, Germany). Normal and tumor genomic DNA sample pairs were amplified using the microsatellite instability MSI/LOH assay

starter kit (Applied Biosystems, Foster City, CA). According to the international criteria for the determination of MSI<sup>[13]</sup>, five reference markers including *D2S123*, *D5S345*, *Bat25*, *Bat26* and *D17S250* were enrolled in our microsatellite panel. The chromosomal location of the microsatellite markers and genes surrounding the markers was described in a previous report<sup>[14]</sup>. In brief, 25 ng template DNA was amplified with fluorescent-labeled primer. PCR was carried out in a GeneAmp PCR System 9, 600 thermal cycler (Applied Biosystems) as follows: a 10-min pre-PCR incubation step at  $95^{\circ}\text{C}$ , 30 cycles of amplification, each at  $96^{\circ}\text{C}$  for 10 s, at  $55^{\circ}\text{C}$  for 30 s, at  $70^{\circ}\text{C}$  for 3 min, and a final extension at  $70^{\circ}\text{C}$  for 30 min.

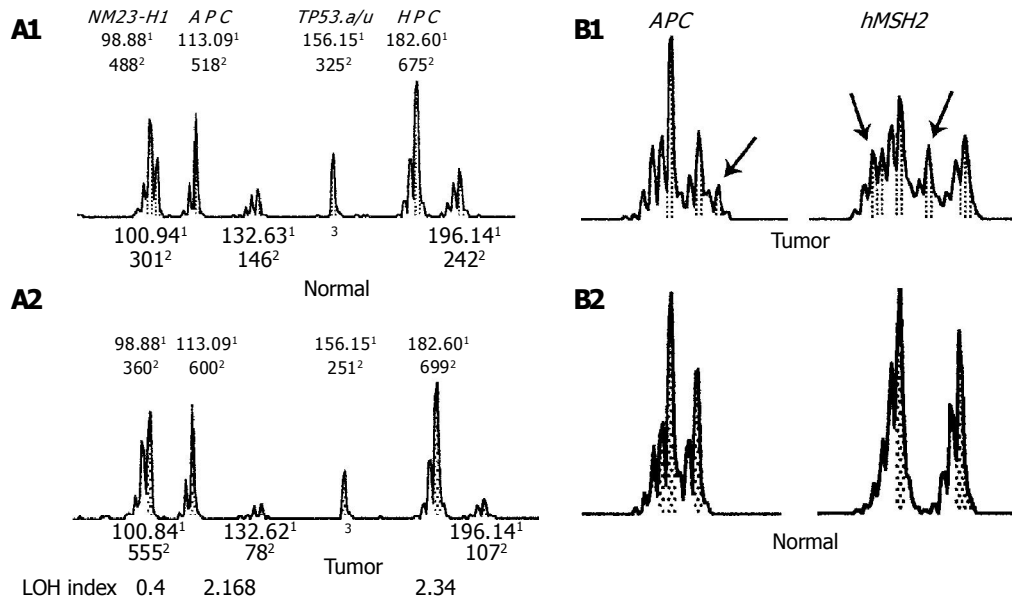
The amplification reactions were pooled and loaded onto a denaturing polyacrylamide gel, and the data were collected with an ABI 377 automated sequencer (Applied Biosystems). At the end of the run, each fluorescent peak was quantitated in terms of size (in base pairs), peak height, and area. Normal and tumor DNA pairs were compared for changes in peak height of each microsatellite marker *via* GeneScan analysis software (Applied Biosystems).

The LOH index was calculated using the modified method described by Cawkwell<sup>[15]</sup> for each paired normal and tumor samples. The peak height of two alleles in each tumor was divided by the peak height in normal samples:  $T1:T2/N1:N2$  where T1 and N1 are the peak heights of the tumor and normal samples, respectively for the corresponding allele one, and T2 and N2 for the corresponding allele two. Allele loss, according to the manufacturer's instructions, occurred with an LOH index of  $\leq 0.67$  or  $\geq 1.5$ . This allele loss also translated to a 33% decrease in peak height of one of the tumor alleles as compared with the normal alleles. The result of LOH analysis of a representative sample is shown in Figure 1. The tumors that exhibited LOH at more than 3 markers, or showed more than 50% of informative markers, were classified as the high-frequency LOH group (LOH-high). Others were classified as the low-frequency LOH group (LOH-low).

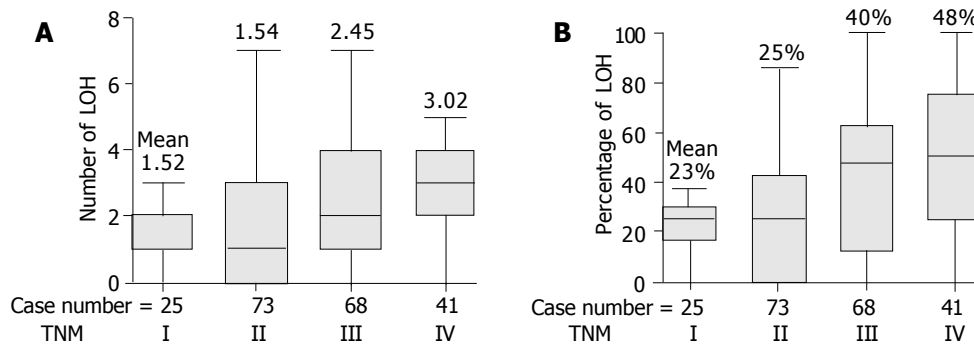
Tumor samples that exhibited novel allele peaks compared with the corresponding normal samples were classified as MSI at that marker. Such markers were considered uninformative for the LOH study. The pattern of MSI of a representative sample is shown in Figure 2. The analysis was performed twice if the data were controversial. According to the international criteria for the determination of MSI<sup>[13]</sup>, MSI in at least 4 or more loci was defined as high microsatellite instability (MSI-H); otherwise it was considered microsatellite stability (MSS).

### *Statistical analysis*

The statistical end point in our analysis was the occurrence of metastasis or death. The group distribution of each clinicopathological characteristic according to the presence or absence of LOH or MSI was compared using the  $\chi^2$  test. The numerical value was compared with the Student's *t* test. Statistical significance was defined as  $P < 0.05$  (SPSS for Windows version 10.0). The variables that resulted in *P* values  $< 0.1$  were entered into the Cox proportional hazards model for the determination of the independent prognostic factor for colorectal cancer.



**Figure 1** LOH at three genetic loci in a 52-yr-old male with stage IV disease (A) and MSI at two genetic loci in a 47-yr-old male with stage II disease (B) shown as representative results of GeneScan. A: LOH at three genetic loci in a 52-yr-old male with stage IV disease; B: MSI at two genetic loci in a 47-yr-old male with stage II disease. <sup>1</sup>Size of the PCR product (in bp). <sup>2</sup>Fluorescence intensity of peak. <sup>3</sup>Homozygosity. The arrowhead indicated novel alleles.



**Figure 2** The number and percentage of LOH. Panel A: The number of LOH; Panel B: The percentage of LOH.

## RESULTS

Markers with detectable heterozygous alleles were defined as informative. One hundred and sixty-three tumors (78.8%) exhibited LOH in at least one microsatellite marker. LOH in more than 3 markers (LOH-high) was noted in 93 tumors (44.9%). The highest frequency of LOH in the tumor suppressor gene was *TP53.alu* (65%), followed by *DCC* (64.3%), *D8S254* (51.7%) and *APC* (47.8%) (Table 1, Figure 1A). There was no difference in the distribution of gender, age, preoperative CEA level and location of tumors between the LOH-high and LOH-low groups. Considering the pathological variables, there was a linear trend between TNM staging and the LOH-high tumors ( $P < 0.001$ ). The mean number and percentage of LOH in stage I tumors were 1.52% and 22.8%, respectively, with an increase to 3.02% and 48%, respectively, in stage IV tumors (Figure 2). The other pathological variables such as lymph-vascular invasion, mucin component, invasive pattern of tumors, and grade of differentiation showed no significant association with the LOH-high tumors (Table 2).

**Table 1** Frequency of LOH and MSI of tumor suppressor genes in sporadic colorectal cancers

Marker	Informative / Total (%)	LOH/Informative (%)	MSI No./Total (%)
<i>NM23-H1</i>	125/207 (60.4)	43/125 (31.6)	4/164 (2.4)
<i>APC</i>	121/207 (58.5)	56/121 (47.8)	10/151 (6.6) <sup>1</sup>
<i>Tp53.alu</i>	103/207 (49.8)	67/103 (65.0)	1/140 (0.7)
<i>HPC1</i>	127/207 (61.4)	15/127 (11.8)	6/192 (3.1)
<i>D8S254</i>	89/207 (43.0)	46/89 (51.7)	5/161 (3.1)
<i>DCC</i>	98/207 (47.3)	63/98 (64.3)	3/144 (2.1)
<i>hMSH2</i>	153/207 (73.9)	24/153 (15.7)	19/183 (10.4) <sup>1</sup>
<i>hMLH1</i>	99/207 (47.8)	26/99 (26.3)	11/181 (6.1)
<i>MET</i>	91/207 (44.0)	27/91 (29.7)	7/180 (3.9)
<i>Tp53.pcr15</i>	80/207 (38.6)	31/80 (38.8)	5/174 (2.9)
<i>MYCL1</i>	110/207 (53.1)	38/110 (34.5)	5/169 (3.0)
<i>Bat-25</i>	30/207 (14.5)	3/30 (10)	22/204 (9.8) <sup>1</sup>
<i>Bat-26</i>	41/207 (19.8)	4/41 (4.4)	15/203 (7.4) <sup>1</sup>
<i>Mfd15</i>	33/207 (15.9)	2/33 (7.2)	9/205 (4.4) <sup>1</sup>

<sup>1</sup>Reference markers according to the international criteria for the determination of MSI<sup>[13]</sup>.

In a total of 2,451 MSI analyses, excluding LOH markers, 122 markers had MSI (4.97%, Figure 1B). Fifty-five tumors

**Table 2** Comparison between clinico-pathological features of loss of heterozygosity-high and loss of heterozygosity-low in colorectal cancer, *n* (%)

Features	Total	LOH-high	LOH-low	<i>P</i>
Number of cases	207	93 (44.9)	114 (55.1)	
Age (yr)	66.2±12.4	67.2±12.4	65.4±12.3	0.292 <sup>1</sup>
Gender (male/female)	139/68	61/32	78/36	0.778 <sup>2</sup>
Location of tumor				0.544 <sup>3</sup>
Right colon	37 (17.9)	16 (17.2)	21 (18.4)	
Left colon	85 (41.1)	42 (45.2)	43 (37.7)	
Rectum	85 (41.1)	35 (37.6)	50 (43.9)	
TNM stage				<0.001 <sup>4</sup>
I	25 (12.1)	7 (7.5)	18 (15.8)	
II	73 (35.3)	22 (23.7)	51 (44.7)	
III	68 (32.9)	37 (39.8)	31 (27.2)	
IV	41 (19.8)	27 (29.0)	14 (12.3)	
Invasive pattern				0.286 <sup>2</sup>
Expanding	52 (25.1)	20 (21.5)	32 (28.1)	
Infiltrative	155 (74.9)	73 (78.5)	82 (71.9)	
Grade				0.263 <sup>3</sup>
Well-differentiated	5 (2.4)	2 (2.2)	3 (2.6)	
Moderate differentiated	182 (87.9)	81 (87.0)	101 (88.6)	
Poorly differentiated	20 (9.7)	10 (10.8)	10 (8.8)	
Lymphovascular invasion				0.102 <sup>2</sup>
Yes	48 (23.2)	27 (56.3)	21 (43.7)	
No	159 (76.8)	66 (41.5)	93 (58.5)	
Mucin production				0.155 <sup>2</sup>
Yes	29 (14.0)	9 (9.7)	20 (17.5)	
No	178 (86.0)	84 (90.3)	94 (82.5)	

<sup>1</sup>Student *t* test, <sup>2</sup>Fisher exact test, <sup>3</sup>Pearson  $\chi^2$  test, <sup>4</sup> $\chi^2$  test with linear-by-linear association.

**Table 3** Comparison between clinico-pathological features of MSI-H and MSS in colorectal cancer, *n* (%)

Features	Total	MSI-H	MSS	<i>P</i>
Number of cases (%)	207	15 (7.25)	192 (92.7)	
Age (yr)	66.2±12.4	69.7±14.8	65.9±12.2	0.248 <sup>1</sup>
Gender(male/female)	139/68	8/7	131/61	0.369 <sup>2</sup>
Location of tumor				0.005 <sup>3</sup>
Right colon	37 (17.9)	7 (46.7)	30 (15.6)	
Left colon	85 (41.1)	2 (13.3)	83 (43.2)	
Rectum	85 (41.1)	6 (40.0)	79 (41.2)	
TNM stage				0.780 <sup>4</sup>
I	25 (14.2)	1 (6.7)	24 (12.5)	
II	73 (36.8)	6 (40.0)	67 (34.9)	
III	68 (31.6)	6 (40.0)	62 (32.3)	
IV	41 (17.4)	2 (13.3)	39 (20.3)	
Invasive pattern				1.0 <sup>2</sup>
Expanding	52 (25.1)	4 (26.7)	48 (25.0)	
Infiltrative	155 (74.9)	11 (73.3)	144 (75.0)	
Grade of differentiation				<0.001 <sup>3</sup>
Well	5 (2.4)	(0)	5 (2.6)	
Moderate	182 (87.9)	9 (60.0)	173 (90.1)	
Poor	20 (9.7)	6 (40.0)	14 (7.3)	
Lymphovascular invasion				0.209 <sup>2</sup>
Yes	48 (23.2)	1 (6.7)	47 (24.5)	
No	159 (76.8)	14 (93.3)	145 (75.5)	
Mucin component				0.001 <sup>2</sup>
Yes	29 (14.0)	7 (46.7)	22 (11.5)	
No	178 (86.0)	8 (53.3)	170 (88.5)	

<sup>1</sup>Student *t* test, <sup>2</sup>Fisher exact test, <sup>3</sup>Pearson  $\chi^2$  test, <sup>4</sup> $\chi^2$  test with linear-by-linear association.

(26.6%) exhibited MSI in at least one genetic locus. MSI in at least 4 or more loci (MSI-H) was noted in 15 tumors (7.25%). The distribution of MSI at different markers is shown in Table 1. The highest frequency of MSI was *hMSH2* (10.4%), followed by *Bat25* (9.8%), and *Bat26* (7.4%). The information on the clinicopathological features of MSI-H and MSS colorectal cancers is shown in Table 3. Seven (46.7%) of the 15 MSI-H cancers were located in the right colon, a significantly greater number than that of the MSS

cancers (15.6%). Poor tumor differentiation was found in 40% of MSI-H cancers (6/15), significantly higher than that found in MSS cancers (7.3%, 14/192). High mucin component of tumors was found in 46.7% of MSI-H cancers (7/15), significantly higher than that found in MSS cancers (22/192). There was no difference in the distribution of gender, TNM stage, invasive pattern of tumors, and lymph-vascular invasion between the MSI-H and MSS cancers.

Of the 166 potentially cured patients, 45 developed tumor recurrence within a median of 36 mo of follow-up. The sites of tumor recurrence were: liver (20), lung (18), bone (5), peritoneum (5), remote nodes (4), and other sites (3). As shown in Table 4 and Figure 3, the clinicopathological factors affecting the 3-year DFS were TNM staging, grade of differentiation, preoperative CEA level, and high LOH status. Using multivariate analysis, we identified that TNM staging showed the most significant impact on DFS [hazard ratio (HR) = 3.31; 95% confidence interval (CI) = 1.82-6.01], followed by high LOH status (HR = 3.18; 95% CI = 1.68-5.98) and CEA level (HR = 2.30; 95% CI = 1.21-4.37). Of the respective markers, LOH of *Tp53.alu*, *D8S254*, *DCC*, and *Tp53.pcr15* had a significant impact on DFS, as shown in Table 5.

**Table 4** Correlation of the clinico-pathological factors with three-year disease-free survival in potentially cured colorectal cancer patients (stage I, II, III patients)

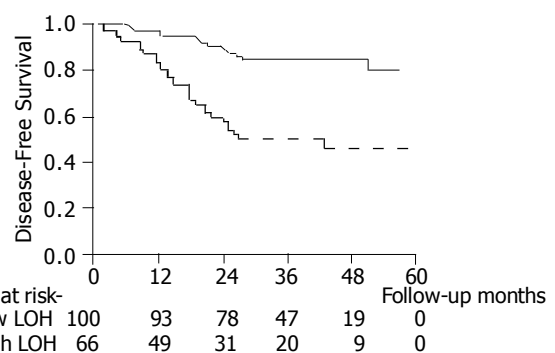
Variables	Number of cases	3-yr disease-free survival	P <sup>1</sup>
Gender			
Male	116	70	0.614
Female	50	75	
Preoperative CEA level			
<5 ng/mL	80	82	0.003
>5 ng/mL	86	63	
TNM stage			
I	25	95	<0.001
II	73	84	
III	68	47	
Lymphovascular permeation			
No	129	73	0.091
Yes	37	63	
Invasive pattern of tumor			
Expansive	45	73	0.517
Infiltrative	121	68	
Grade of differentiation			
Well	3	100	0.004
Moderate	146	75	
Poor	17	48	
Mucinous component			
<50%	143	72	0.642
>50%	23	68	
Microsatellite instability			
MSI-H	13	69	0.910
MSS	153	71	
LOH status			
Low	100	84	<0.001
High	66	50	
Multivariate analysis <sup>2</sup>			
Factors	Hazard ratio	95% CI	P
LOH (High vs Low)	3.18	1.68-5.98	<0.001
TNM	3.31	1.82-6.01	<0.001
CEA level			
>5 ng/mL vs <5 ng/mL	2.30	1.21-4.37	0.011
Grade of differentiation	1.12	0.45-2.77	0.791
Lymphovascular invasion	1.0	0.49-2.01	0.981

<sup>1</sup>Log-rank test; <sup>2</sup>The P value results from the hypothesis that the relative risk as determined by a multivariate binary logistic regression analysis equaled 1.0.

**Table 5** Informative markers that influenced three-year DFS in potentially cured colorectal cancer patients (stage I, II, III patients)

Markers	Number of cases	3-yr disease-free survival	P <sup>1</sup>
<i>Nm23-H1</i>			
Non-LOH	68	75	0.266
LOH	28	62	
<i>APC</i>			
Non-LOH	54	75	0.881
LOH	45	72	
<i>Tp53.alu</i>			
Non-LOH	29	92	<0.001
LOH	55	51	
<i>HPC1</i>			
Non-LOH	87	75	0.743
LOH	12	70	
<i>D8S254</i>			
Non-LOH	39	75	0.030
LOH	31	52	
<i>DCC</i>			
Non-LOH	27	79	0.042
LOH	45	55	
<i>MSH2</i>			
Non-LOH	107	73	0.972
LOH	15	75	
<i>MLH1</i>			
Non-LOH	62	66	0.287
LOH	19	57	
<i>Met ONC</i>			
Non-LOH	50	72	0.350
LOH	16	64	
<i>Tp53.pcr15</i>			
Non-LOH	42	76	<0.001
LOH	20	33	
<i>MYCL1</i>			
Non-LOH	59	71	0.421
LOH	29	65	

<sup>1</sup>Log-rank test; The markers that had frequency of informative tumors lower than 30% were excluded in the survival analysis.



**Figure 3** Comparison of the clinicopathological factors with 3-yr survival in colorectal cancer patients. — : Low LOH group; ----: High LOH group.

## DISCUSSION

Our approach consisted of examining 14 microsatellite markers located near or within regions containing important genes implicated in the complex process of colorectal tumorigenesis. The use of automated assessment of loss of heterozygosity and microsatellite instability with fluorescent-PCR was described by Cawkwell<sup>[15]</sup> and could be adapted to the analysis of both LOH and MSI, two important mechanisms of colorectal carcinogenesis. In our results, the frequency of MSI exhibited a trend to cluster

in *bMSH2*, *BAT25* and *BAT26* loci, but not in markers in regions displaying high levels of allelic loss (e.g., *p53* and *DCC*). The higher susceptibility of mononucleotide repeats to instability in MSI tumors has been reported by Ionov<sup>[4]</sup> and Dietmaier<sup>[16]</sup>. The microsatellite markers with a low frequency MSI might provide prognostic information about LOH. The present study demonstrated a significant inverse correlation between the two molecular events (LOH vs MSI). The tumors with MSI-H phenotypes had a lower frequency of LOH than those with MSS phenotype.

In the present series of patients with resected primary colorectal carcinomas, the frequency of MSI-H phenotypes was 7.25%, lower than in previous reports<sup>[7,17]</sup>. This may be attributed to several markers having a low-frequency MSI. If only considering five reference markers<sup>[13]</sup> as the current definition of MSI-H, the frequency of MSI-H could be as high as 11.1% of tumors. Previous reports indicated that MSI-H colorectal cancers were associated with distinct clinicopathological features, including poor differentiation, extracellular mucin accumulation, younger age at onset, and proximal tumor location<sup>[18-20]</sup>. Our MSI-H tumors displayed similar clinicopathological findings.

Loss of heterozygosity, i.e., loss of one allele at a constitutional heterozygous locus indicates the probability of loss of a tumor suppressor gene, which might promote neoplastic progression<sup>[21,22]</sup>. The frequency of an informative tumor for each microsatellite marker varied in the present study (range 14.5-73.9%). Analysis of a single marker may be biased by non-informative tumors, especially in the marker in which the frequency of an informative tumor was less than 50%. Therefore, we defined the LOH-high group as LOH at more than 3 markers, or more than 50% of informative markers for considering both the number and percentage of LOH. In our results, high-frequency LOH could be an independent prognostic factor affecting tumor recurrence. Of the 166 potentially cured patients, those with a high LOH tumor showed a DFS of 50%, which was poorer than those with low LOH tumors (84%). Of the 45 patients who developed tumor recurrence, the mean number of LOH was 2.97 and the percentage of LOH in the primary tumor was 46.8%, which were similar to those tumors that already had distant metastasis at operation. These findings imply that high LOH tumors behave comparably to those stage IV diseases.

Metastasis is a complicated multistep process. The cells must detach and acquire motility, invade through the basement membrane and extracellular matrix, gain access to and survive in the vascular system, adhere to and immigrate into the target organ, and then proliferate in the new site<sup>[23]</sup>. It is reasonable to believe that multiple genes control the complicated process. Previous reports have hypothesized that accumulation of various genetic abnormalities is more important in tumorigenesis than the sequence of genetic alterations<sup>[2,24]</sup>.

Additionally, a previous report demonstrated that *p53* and *DCC* were the most common mutated tumor suppressor genes in colorectal cancer, and occurred mainly at the late phase of carcinogenesis<sup>[1]</sup>. In our study, the frequency of LOH was the highest at *p53* (65.0%) followed by *DCC* (64.3%), *D8S254* (51.7%) and *APC* (47.8%). There were

four markers associated with metastatic potential, including *p53*, *DCC*, and a tumor suppressor gene located in the 8p22. In our series, the incidence of LOH of *p53* was similar to a previous report<sup>[24]</sup>. The *p53* gene has been thought to modulate two discrete functions, both in response to stress (particularly DNA damage), cell cycle arrest, and apoptosis<sup>[25,26]</sup>. The loss of *p53* in the final stages of colon cancer not only allows the cells to divide at an unrestrained rate, but this rapid replication further promotes the development of even more genetic mutations because the cell cycle is not interrupted when errors occur. Several authors have found that mutations of *p53* or allelic loss of the *p53* was correlated with poor outcomes<sup>[27-29]</sup>. Our study yielded a similar finding. The incidence of allele loss of *DCC* in colorectal cancer has been reported to be from 33%<sup>[15]</sup> to 75%<sup>[29,31]</sup>. According to previous reports, LOH or decreased *DCC* expression was associated with liver and lymph node metastasis<sup>[29,31]</sup>. In our study, LOH of *DCC* was significantly associated with lymph node metastasis (data not shown). The *DCC* protein was a transmembrane glycoprotein similar to the neural cell adhesion molecules<sup>[32]</sup>. LOH of *DCC* could thus lead to impaired contacts between cells, thereby contributing to tumor growth and invasion. The prognostic value of LOH of *DCC* has been confirmed in several previous studies<sup>[31,33,34]</sup>. Our observations also showed that LOH of *DCC* was significantly associated with metastasis potential.

Another putative tumor suppressor gene located in the short arm of chromosome 8 had a high frequency of LOH in our study, as shown in a previous report<sup>[35]</sup>. Several studies showed that this novel tumor suppressor gene locus was related to the stage progression of colorectal cancer<sup>[36,37]</sup>, similar to our findings.

In the multivariate analysis, TNM stage was the most significantly independent prognostic factor for colorectal cancer, followed by high-frequency LOH and preoperative CEA level. The outcome of patients with colorectal cancer was determined by the presence or absence of metastasis at the time of operation. We further analyzed the prognostic impact of high LOH status on early stage tumors (stage I or II). Of the 29 stage I or II tumor patients with high-frequency LOH, 9 patients (32.1%), developed distant metastasis within three years after surgery, which was significantly higher than those with low-frequency LOH (8.7%,  $P = 0.037$ ). This finding implies that early stage tumors with LOH-high have a stronger metastatic potential.

In conclusion, genetic change in both MSI and LOH phenotypes may occur in sporadic colorectal tumors, as established by microsatellite analysis. Variation of genetic phenotypes is related to different clinical manifestations, an important finding that may be useful in identifying subgroups of patients with different prognostic risks.

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