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RAPID COMMUNICATION

Frequent loss of heterozygosity in two distinct regions, 8p23.1 and 8p22, in hepatocellular carcinoma

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Abstract

AIM: To identify the precise location of putative tumor suppressor genes (TSGs) on the short arm of chromosome 8 in patients with hepatocellular carcinoma (HCC).

METHODS: We used 16 microsatellite markers informative in Japanese patients, which were selected from 61 published markers, on 8p, to analyze the frequency of loss of heterozygosity (LOH) in each region in 33 cases (56 lesions) of HCC.

RESULTS: The frequency of LOH at 8p23.2-21 with at least one marker was 63% (20/32) in the informative cases. More specifically, the frequency of LOH at 8p23.2, 8p23.1, 8p22, and 8p21 was 6%, 52%, 47%, and 13% in HCC cases. The LOH was significantly more frequent at 8p23.1 and 8p22 than the average (52% *vs* 22%, $P = 0.0008$; and 47% *vs* 22%, $P = 0.004$, respectively) or others sites, such as 8p23.2 (52% *vs* 6%, $P = 0.003$; 47% *vs* 22%, $P = 0.004$) and 8p21 (52% *vs* 13%, $P = 0.001$; 47% *vs* 13%, $P = 0.005$) in liver cancer on the basis of cases. Notably, LOH frequency was significantly higher at *D8S277*, *D8S503*, *D8S1130*, *D8S552*, *D8S254* and *D8S258* than at the other sites. However, no allelic loss was detected at any marker on 8p in the lesions of nontumor liver tissues.

CONCLUSION: Deletion of 8p, especially the loss of 8p23.1-22, is an important event in the initiation or promotion of HCC. Our results should be useful in identifying critical genes that might lie at 8p23.1-22.

Key words: Loss of heterozygosity; Chromosome; Hepatocarcinogenesis; Hepatocellular carcinoma; 8p

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INTRODUCTION

Primary liver cancer is one of the most frequent neoplasms worldwide, with both an incidence and a mortality rate that are increasing markedly. According to a recent report, the global number of new cases annually rose from 437 400 to 564 000 between 1990 and 2000, and is expected to continue to rise in the future[1-4].

Hepatocellular carcinoma (HCC), the predominant histological subtype of primary liver cancer, mostly arises against a background of chronic liver disease, usually in association with cirrhosis. Several risk factors for HCC have been reported, such as chronic infection with hepatitis B virus (HBV), and C virus (HCV) or both, alcohol-induced liver injury, and dietary exposure to aflatoxin B1 and others. Prolonged exposure to these risk factors is thought to cause an accumulation of chromosomal aberrations and altered gene expression, and eventually results in hepatocarcinogenesis[4-6]. In Japan, more than 70% of HCCs develop in patients with chronic infections with HCV[7]. Carcinogenesis is mainly researched based on virology and the viral gene itself. However, the mechanisms by which inflammation and cirrhosis contribute to tumor development and/or progression remain unclear. After the human genome was sequenced, the mechanism of generation and subsequent progression was researched at a molecular level for HCC. Histological findings suggest that the initiation and subsequent development of HCC are multistep processes involving qualitative and quantitative changes in sequentially expressed genes, especially the inactivation of tumor suppressor genes (TSGs) related to the deletion of chromosomal regions critical for hepatocarcinogenesis[8,9]. A typical alteration in many TSGs, the mutation of one allele, can be detected as a loss of heterozygosity (LOH) with informative markers in TSG regions. Therefore, LOH assays have been widely used as an indirect approach in the search for a new TSG[10]. In the last few years, genetic approaches to the detection of genome-wide LOH using microsatellite markers and chromosomal aberrations detected by comparative genomic hybridization (CGH) have indicated that frequent allelic loss in many different chromosomal regions, including 1p[11,12], 3p[13],

4q[14], 6q[15], 8p[16-19], 9p[20], 10p[21], 13q[22], 22q[23], 16q, 17p and Xq[24,25] , is closely associated with the tumorigenesis of HCC.

We have performed a genome-wide search for LOH with human genetic markers in several types of human cancer and confirmed that loss of 8p is the most frequent chromosomal alteration in prostate cancer, especially allelic loss at 8p22, which not only is an important event in the initiation of tumor, but also is closely associated with the progression of primary cancer to metastatic cancer[26].

In our comprehensive allelotyping, less than 30% of microsatellite markers located at 8p21-23, were recognized as informative for Japanese patients. We therefore undertook an allelotype based study of 33 HCCs using the selected informative markers to obtain a comprehensive view of the LOH on the most frequent altered chromosome, and to identify the location of the putative TSGs in HCC.

MATERIALS AND METHODS

Tissue collection, histopathology, and DNA extraction

Thirty-three patients with hepatocellular carcinoma who underwent liver resection were included in this study. Of these, fifty six tumor lesions and 33 adjacent morphological non-tumor lesions were obtained from surgically resected specimens. All specimens were formalin-fixed, and paraffin wax-embedded tissues were processed with routine histological methods. Use of the tissues was approved by the Ethics Committee of the Jikei University School of Medicine before the study. The study group included 26 men and 7 women, ranging in age from 31 to 76 years. Of the 33 patients, 24 (73%) had a chronic infection with HCV, HBV or both and 15 (45%) had cirrhosis in the background liver tissues. Histological diagnoses were made according to the WHO Histological Classification of Tumors of the Liver and Intrahepatic Bile Ducts (2000). According to histological grade, HCC was classified into well differentiated (WD), moderately differentiated (MD), and poorly differentiated (PD) types. In this study, clinicopathological characteristics were also classified, such as solitary or multiple tumor, growth pattern of tumor (expansive or infiltrative), infiltration of capsule or not, histological grading of tumor (well, moderately or poor differentiation), and with or without vascular and bile duct infiltration. Simultaneously, we also compared LOH frequency and etiological factors, such as chronic hepatitis with HCV or HBV infection, and cirrhosis in the background liver tissues. Fibrosis degree was classified as F1, F2, F3, and F4 according to the histological grading and staging of chronic hepatitis. In this system, liver cirrhosis was classed as F4, which is the end-stage form of liver fibrosis. Of the 33 patients who underwent liver resection, 18 had a solitary tumor nodule and 15 had multiple tumor nodules. All lesions from each case were selected and reviewed by two pathologists in order to confirm the original diagnosis. The tumor (T) and corresponding non-tumor hepatocytes (H), and remaining nonhepatocytes that were portal vein lesions (*P*) were micro-dissected from 15-μm tissue specimens after deparaffinization and nuclear staining. Normal tissues were obtained from the gallbladder or lymph nodes collected from the same patients (Figure 1). DNA was extracted using the standard phenol/chloroform method as described previously[26].

LOH analysis

Matched tumors, corresponding non-tumor liver tissues, and normal tissue DNAs were analyzed for LOH by amplification of polymorphic microsatellite markers using the polymerase chain reaction (PCR). Sixty-one published microsatellite markers, located at 8p23.3, 8p23.2, 8p23.1, 8p22, and 8p21, were selected from the Genome Database (available at http: //www. gdb. org). A total of 16 microsatellite markers were identified as informative in Japanese patients and used (Table 1).

DNA amplification was performed in 10-μL volumes containing 100 ng of genomic DNA as a template. Each PCR mixture contained 1.5 mmol/L $MgCl_2$, 100 μmol/L forward and reverse primers, 200 μmol/L each of dATP, dGTP, dTTP and dCTP, 10 μCi of [α-^{32}P] dCTP (6000 Ci/mmol, Amersham, Biosciences Corp., Piscataway, NJ), 1 U of Taq DNA polymerase (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 1 × PCR buffer. After the initial denaturation at 94℃, 35 PCR cycles, each consisting of denaturation at 94℃ for 30 s, annealing at 65℃ -50℃ for 30 s, elongation at 72℃ for 1 min, and a final extension at 72℃ for 5 min, were performed in a 96-well Hybaid thermocycler (Gene Amp PCR System 9600, Takara, Tokyo, Japan). Ten microliters of PCR products were denatured with 30-60 μL of dye solution (95% formamide, 10 mmol/LEDTA (pH 8.0), 0.2% xylene cyanol FF, and 0.02% bromophenol blue) at 95℃ for 3 min and then cooled on ice immediately. Three microliters of denatured products were separated on a 6% urea-formamide-polyacrylamide gel and electrophoresed at 40 W for 2-3 h at room temperature. The dried gel was exposed to Hyperfilm MP (Amersham Biosciences Corp.) for 3-7 d and reexposed to another film for 2-3 wk.

Criteria for LOH

A pair of regular and longer-exposed autoradiographs was reviewed independently by two of the authors (T. L. and CX. M.). Informative pairs were judged by visual inspection to show LOH, no loss or to be noninformative.

LOH was defined as a loss of intensity of 60% or greater in 1 or more alleles in the tumor (T) or corresponding hepatocytes (H) compared with the identical allele in the normal tissue (N) (Figure 1).

Statistical analysis

The differences in LOH frequency between tumor, nontumor and normal tissues for individual markers and background values were determined with Fisher's exact test.

RESULTS

The distribution of the frequency of LOH at 8p23.2, 8p23.1, 8p22 and 8p21 for hepatocellular carcinoma is shown in Table 2. Allelic loss at 8p23.2-21 was detected with at least 1 marker in 18 of 32 (56%) cases of liver cancer. More specifically, the frequency of LOH at 8p23.2, 8p23.1, 8p22, and 8p21 with at least 1 marker was 6% (1

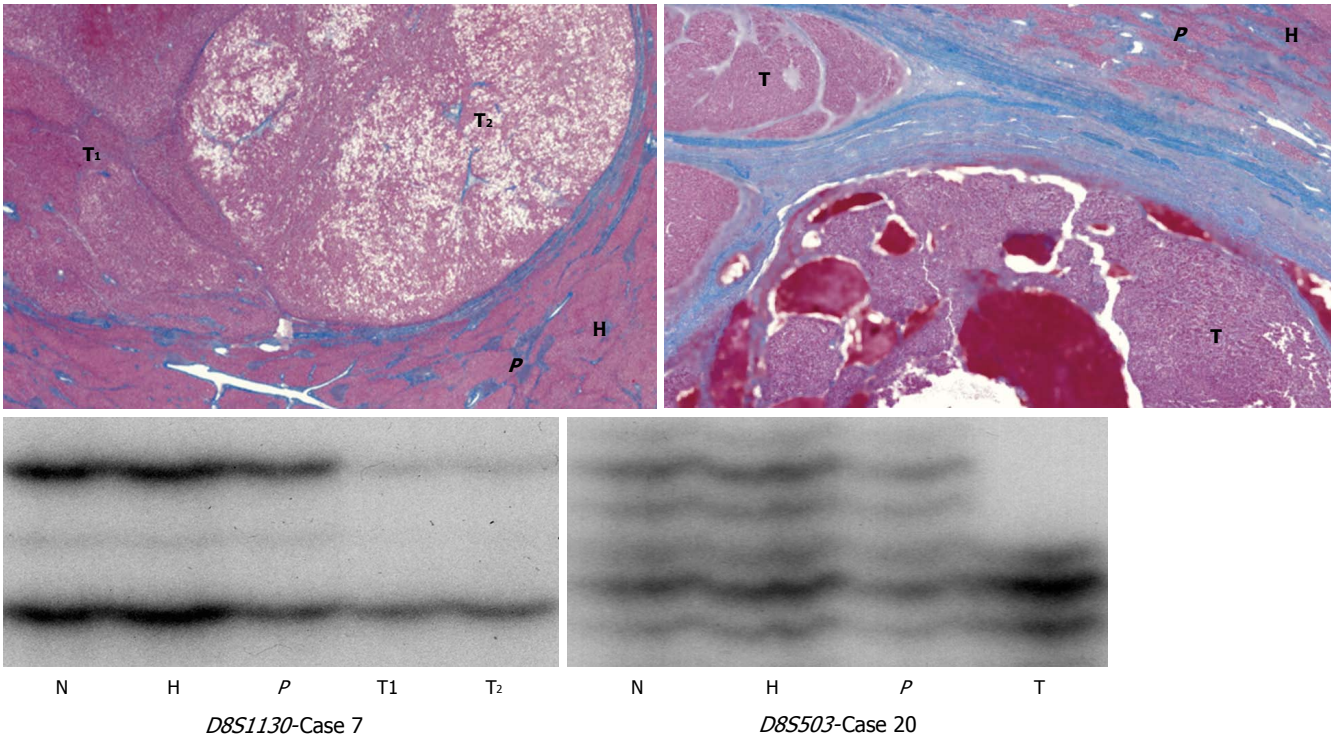

Figure 1 Representative examples of LOH in two cases of hepatocellular carcinoma (N: normal; H: hepatocytes; P: portal vein; T: tumor; number beside T indicates different lesion): case 7 showing partial loss of upper alleles in lesions of tumor 1 and tumor 2 but not in lesions of hepatocytes and portal vein at *D8S1130*; case 20 showing complete loss of upper alleles in lesion of tumor but not in surrounding non-tumor tissues at *D8S503*.

of 16), 52% (16 of 31), 47% (15 of 32), and 13% (4 of 32) for liver cancer cases respectively (Table 2). A similar result was obtained in the lesion-to-lesion comparison (data not shown). In contrast, no allelic loss at any markers on 8p was detected in the background liver tissue. The average frequency of LOH at 8p23.2-21 was 22% (58 of 264) in informative cases. We found that LOH at the 8p23.1 and 8p22 loci was significantly higher than the average in HCC cases ($P = 0.0008$, and $P = 0.004$, respectively). But allelic loss at 8p23.2 and 8p21, the loci on either side of the 8p23.1-22 region, tended to be lower than the average. On the other hand, no allelic loss (0 of 52 lesions) was detected at any informative markers on 8p23.2-21 in the surrounding liver tissues. Moreover, allelic loss at *D8S277, D8S503, D8S1130, D8S552, D8S1109, D8S254*, and *D8S258* was 25%, 42%, 39%, 43%, 24%, 43% and 50%, respectively, significantly higher than that elsewhere and the average frequency at 8p.

Correlations between LOH frequency and clinicopathological variables are summarized in Table 3. To determine whether allelic loss at 8p was associated with clinicopathological characteristics and reveal its biological role in the initiation and/or progression of tumors, we compared the frequency of LOH based on almost all of the clinicopathological findings. Corresponding to the result described above, the LOH frequency tended to be higher at 8p23.1 and 8p22 loci than at 8p23.2 and 8p21 loci for all clinicopathological findings, but no significant difference in LOH frequency was found between the liver cancer positive or negative for malignant factors. In other words, no association was detected between the deletion of 8p23.1-22 and subsequent progression of the tumors.

The distribution of LOH frequency based on the fibrosis (F) of background liver tissues, which is usually thought to be associated with hepatocarcinogenesis, was also analyzed. The frequency of LOH at 8p23.1 or 8p22 in F1, F2, F3, and F4 was 75% (3 of 4), 78% (7 of 9), 20% (1 of 5), and 38% (5 of 13), or 100% (2 of 2), 56% (5 of 9), 40% (2 of 5), and 46% (6 of 13), respectively. No statistically significant difference in LOH frequency was found on the basis of the fibrosis staging at 8p. Allelic loss at 8p even tended to be slightly more frequent in cases of tumor with earlier-stage fibrosis than in cases with advanced stage fibrosis of the background liver tissues.

DISCUSSION

Previous studies of LOH have reported that allelic loss of 8p is the most frequent chromosomal alteration in a variety of human cancers and have suggested that one or several tumor suppressor genes (TSGs) may lie within the short arm of chromosome 8[16-19]. To further identify the precise location of the putative TSGs that might potentially be involved in the tumorigenesis of HCC, we performed a high-density LOH study of HCC at 8p using recently developed microsatellite markers. Only 16 of 61 (less than 30%) were identified as informative for Japanese patients. Furthermore, among the informative markers, the informative cases for all specimens were usually lower (from 20% to 70%) for Japanese than for Westerners. The same-general tendency has been found in various other types of cancer, possibly because Japan is not multiracial like Western countries. This has led us to suggest that using this characteristic might be more efficient for identifying

Table 1 Informative microsatellite markers were selected and used in this study

No.	Locus	Markers	Genetic map (cM)	Forward	Reverse	PCR product size (bp)	result[1]
1	8p23.3	D8S7	Not listed	ACCCTGACAGCAGAGGTTTC	ACCCTGACGTTCTCCCAGTA	250-252	ni
2	8p23.2	D8S1164	Not listed	CACAAATCAGATTTTTGAAGTTGC	GGGTTAGACGGACAACCTCA	225	ni
3	8p23.2	D8S264	0.7	ACATCTGCGTCGTCTTCATA	CCAACACCTGAGTCAGCATA	121-145	in
4	8p23.2	D8S262	4.3	AGCTCAAAAGCGAAGGTGAT	GGCAACAAAGTGAGATCCTG	114-128	in
5	8p23.1	sts-X53793	Not listed	TCGACTACCCAGTGGTCTTG	GTTCAAAATGCTTGCTCGC	127	ni
6	8p23.1	D8S1742	Not listed	CCCCCACCAAGACACA	CTCAAGGGATATGAAGGGCA	130	in
7	8p23.1	D8S277	8.2	GATTTGTCCTCATGCAGTGT	ACATGTTATGTTTGAGAGGTCTG	121	in
8	8p23.1	D8S1918	Not listed	GAATGTCATGCTGGGAACG	GTAGCTCTCAAAGCAAATTATGAGC	108	ni
9	8p23.1	D8S1819	10	TCACTGAGGGACTTGGC	CGTGCTGAGAATGAGACC	207	in
10	8p23.1	D8S1140	Not listed	GACAACATCCGATAATGCTG	GAGGACATCTAGATAATTGGAAGA	378	ni
11	8p23.1	D8S503	16.2	GGTTACGAGTTTTGTCCTTTG	GAAACAAACCAATGTAGGAGTG	136	in
12	8p23.1	D8S1672	Not listed	AACTGAGATCACGCCACTCC	CCCATTGGTTTTAGAGTGGC	149	ni
13	8p23.1	AFM234ve1	Not listed	TACCGCAAAACACACCA	GCAGCCTTAGTTGACAACA	245	ni
14	8p23.1	D8S2045	Not listed	CCGATTGCTTCATCGGGAC	CGCCTCCTCCTCTGAAATCCT	120	ni
15	8p23.1	D8S1130	22.4	GAAGATTTGGCTCTGTTGGA	TGTCTTACTGCTATAGCTTTCATAA	145	in
16	8p23.1	D8S1946	Not listed	GCACAAGATCAGAGAGGTTGTG	GAGGAGAGATGGTGTTGGGA	102	ni
17	8p23.1	D8S1640	Not listed	TGCAGTCTGCGGGAGTTC	AGCAGGGTGACTGTAAAGAAGG	175	ni
18	8p23.1	D8S2060	Not listed	CTCTCCGGGAATGTAATACTGC	GAGCTGGGAGTTACTGCCTG	256	ni
19	8p23.1	D8S552	26.4	CCTGTACCATACCCCTGTATC	AAGGTTTGAATCTCTCAGTGG	132	in
20	8p23.1	D8S1109	26.4	TTCTCAGAATTGCTCATAGTGC	TCAGCTCCTCTTCTGCTGAT	241	in
21	8p23.1	D8S2066	Not listed	TTTTCTCCATCCGGTGACTC	CCAACTACGGCATGGTTTCT	175	ni
22	8p23.1-22	D8S1106	26.5	TTGTTTACCCCTGCATCACT	TTCTCAGAATTGCTCATAGTGC	149	ni
23	8p22	D8S1451	Not listed	AACCTAAGGTTCTGTGCTACATCA	AACTTACCAAGGCCGTTTAGG	149	ni
24	8p22	EST465487	Not listed	TTTGTTTGGGTGGAGGACTC	TGGACATCTGCCTAGGTCCT	250	ni
25	8p22	D8S1647	Not listed	CCAGAATTTTGAAATAGATTCATCC	AAATTTTGTAAATATCAGTGTTCCG	174	ni
26	8p22	stSG29388	32	GCAGTGAGATTTTGCTTCTGG	ATGAACATTCAATGAATCAGCA	125	ni
27	8p22	D8S1713	Not listed	CAGGGGCTGATTGTCAGAAC	GTGGCTGTCACCAAGGTCTC	113	ni
28	8p22	SGC33312	Not listed	AGGGCCTTGGGAACACTC	TCAGTTTAAATGGATGGTTTTTACT	137	ni
29	8p22	D8S2080	Not listed	GACTCAAAGAGAACCTGCCG	TAGGTTGGTGAGCACACGTC	132	ni
30	8p22	D8S2081	Not listed	ACCCAGTTACAGCACTGTAATATCA	CTCTACCCCGAAATGATGGA	147	ni
31	8p22	SHGC-24261	Not listed	AAGCAGAGATAAGCCCGACA	TTTCTTTAGATGGAGTCCATTGC	123	ni
32	8p22	SHGC-52401	Not listed	ACAGGATAGTGTTAGGCTCACATG	CATTCTCTGTATCTTTTGGGGG	120	ni
33	8p22	D8S254	Not listed	TGCCGGACATACATTAGTGA	TTGTAAACACCACAAGCAGG	65-75	in
34	8p22	D8S2001	Not listed	GACATTGAATTCCAGTATTTGTGC	GGACAAATGCCACTGCAAC	138	ni
35	8p22	SHGC-5873	Not listed	GACACACACATACAGAAAACCA	CTTACCATGAATGGAGCTTG	225	ni
36	8p22	D8S261	35.8	TGCCACTGTCTTGAAAATCC	TATGGCCCAGCAATGTGTAT	128	in
37	8p22	AFM234vf4	Not listed	GGGCACAGGCATGTGT	GGCTGCATTCTGAAAGGTTA	260-272	ni
38	8p22	D8S1948	Not listed	TTACAAAACATACCCAGTGTTTGG	CTTTTTAGTGCTTGAGACTGTCTCC	110	ni
39	8p22	D8S2028	Not listed	TCAAAAGTTTTGTTTCTATTCAGGG	TTTTTTCTGTTCCCCTCCG	178	ni
40	8p22	D8S258	40.3	CTGCCAGGAATCAACTGAG	TTGACAGGGACCCACG	144-154	in
41	8p22	D8S1949	Not listed	TGTCTTACAGCTCTCCCTCTCC	CAGTAAGGATCACCAAGACAAGG	106	ni
42	8p22	D8S1983	Not listed	ATTGGAAGAGGCAAATGGTG	TATGTACTGGATGAAGCAGGACA	175	ni
43	8p22	D8S1786	Not listed	CGAAAGATTGAGACCCCAT	GTTTCCACACCGAAGCC	209	ni
44	8p22	D8S298	42.7	AGGCTTACACCCATGGACC	ACGCAGCACACAACATCAT	155-167	in
45	8p21.3	D8S2050	Not listed	TGCCAATATCAGTGGAAGAGG	TCCTTTTTCCCTTGTTGCC	162	ni
46	8p21.3	D8S1752	Not listed	TCCTGGATCAGGCAGAAA	TCAGAGTTGGGTGAGCGA	140	in
47	8p21.3	D8S1734	44.9	GCTATCCACTTGTCCCAGA	AGCCCAGAAATAAACCCTC	114	in
48	8p21.2	D8S2256	Not listed	GTGTCTTGAGATACTGGTGA	GAGAAATGTCTTTGTTGAGG	101	ni
49	8p21.2	D8S2259	Not listed	TGAAAGCCTGTTTAGAGAGA	CTATTGCCCTGTGTTTTGCC	105	ni
50	8p21.2	D8S1220	Not listed	TTCCGTATACACATGCACCC	TAGCAGCCAGACACAGGAGC	90	ni
51	8p21.1	D8S1445	Not listed	GCAACAGAGCGAGACTCCGTC	AAGCTTACATTCTGGGTGAC	117-139	in
52	8p21.1	D8S2261	Not listed	GTATTTATTCCACAAGCATCTTA	CAACCCCATCAGTCTCTCTAAT	204	ni
53	8p21.1	D8S1444	Not listed	TTCTTCTAGATTTCCTACTA	CATTTGTTAAAAGTACAACC	91	x
54	8p21	D8S2249	Not listed	TCCACCCATTTCAGCCTTTC	CTAAAACATTTAACTTTCATT	101	ni
55	8p21	D8S2248	Not listed	ATACAGGTAGGTGAGGGCAA	TTCTGATGCTCTTCTGGAGT	136	ni
56	8p21	D8S2247	Not listed	CATTGTGGTGGAGGTCGGAG	TTCCCCCATCCCATCTGAG	122	ni
57	8p21	D8S2262	Not listed	ATGTTTTGTTCATGGGTCTTT	AAGAAAAAGGGAAGGGGCAGT	98	ni
58	8p21	D8S339	Not listed	TAGATGTTACCATTTCAC	GATTAGATCTTGGATCAG	162	ni
59	8p21	D8S2245	Not listed	CCTCTTTATCCCACTTTTCAG	CATTTTACGAATATAAGCATCC	138	ni
60	8p21	D8S2244	Not listed	ACAACTAAAGGACTTAAAGG	GACAAGAAAAGAACAAATGG	145	ni
61	8p21	D8S2246	Not listed	TAACCTGTGAATGAGAATAC	TGACAGTTTTGAGAGAATCC	169	ni

[1]ni: noninformative; in: informative.

candidate regions of deletion at 8p.

In this study, LOH at 8p was detected in 56% of informative cases of HCC. However, no allelic loss was found in corresponding hepatocytes including 18 lesions of morphogenetic non-tumor tissues and 14 lesions of cirrhotic liver tissues at any markers, the latter usually considered a

Table 2 LOH status for the 8p23.2, 8p23.1, and 8p22 in hepatocellular carcinoma

Case	Age	Sex	St/Mt	Grading	Etiology	D8S264	D8S262	8p23.2	D8S1742	D8S277	D8S1819	D8S503	D8S1130	D8S552	D8S1109	8p23.1	D8S254	D8S261	D8S258	D8S298	8p22	D8S1752	D8S1734	D8S1445	8p21	8p23.2-21
12	60	M	Mt	MD	Alcoholic	ni	ni	ni	ni	ni	ni	ni	○	ni	ni	○	○	○	●	○	●	○	○	○	○	●
11	64	M	St	MD	CH(C)+, LC	ni	ni	ni	○	○	ni	○	○	ni	○	○	○	ni	●	ni	●	○	○	○	○	●
1	42	M	St	PD	CH(B)+	ni	ni	ni	ni	○	○	ni	○	ni	●	●	●	○	ni	ni	●	○	○	○	○	●
2	59	M	St	MD	CH(-)	○	○	○	ni	ni	ni	○	○	ni	●	●	●	○	ni	○	●	ni	○	○	○	●
27	69	M	Mt	PD	CH(C)+	ni	ni	ni	○	○	ni	ni	ni	ni	●	●	○	○	○	ni	○	○	○	○	○	●
26	58	M	St	MD	CH(C)+, LC	○	ni	○	○	○	○	●	○	ni	○	●	○	○	ni	○	○	○	ni	○	○	●
15	31	F	Mt	MD	CH(C)+	ni	ni	ni	ni	ni	ni	●	ni	ni	ni	●	ni	ni	●	ni	●	○	ni	○	○	●
29	73	M	St	WD	CH(C)+	ni	ni	ni	ni	ni	ni	ni	●	ni	○	●	●	ni	●	●	●	○	○	○	○	●
6	72	M	St	MD	CH(C)+	ni	ni	ni	ni	ni	○	ni	●	ni	ni	●	○	ni	●	○	●	○	○	○	○	●
20	65	M	St	WD	Alcoholic, LC	ni	○	○	●	●	●	●	●	●	●	●	●	●	●	ni	●	●	○	○	●	●
8	74	M	Mt	MD	CH(-)	ni	○	○	●	●	ni	ni	●	ni	○	●	●	ni	ni	●	●	●	ni	ni	●	●
7	59	M	Mt	WD	CH(B)+, (C)+	○	○	○	○	●	●	ni	●	ni	ni	●	○	ni	ni	○	○	○	●	●	●	●
19	50	M	Mt	MD	CH(B)+, LC	○	●	●	●	○	ni	●	●	●	○	●	ni	ni	●	○	●	●	●	○	●	●
28	58	M	Mt	MD	CH(B)+	ni	○	○	ni	○	ni	○	●	●	○	●	●	ni	ni	ni	●	ni	○	○	○	●
5	51	M	Mt	MD	CH(B)+	ni	ni	ni	ni	ni	○	ni	●	ni	ni	●	●	ni	ni	ni	●	ni	○	○	○	●
10	71	F	Mt	MD	CH(C)+, LC	ni	ni	ni	○	○	○	●	ni	ni	ni	●	●	○	●	ni	●	○	○	○	○	●
4	57	M	Mt	MD	CH(C)+, LC	○	○	○	ni	●	○	○	○	○	○	●	○	ni	○	○	○	○	○	○	○	●
13	74	M	Mt	MD	CH(-)	ni	ni	ni	○	ni	ni	ni	●	ni	ni	●	ni	ni	ni	ni	ni	○	ni	○	○	●
18	51	M	St	WD	CH(B)+, LC	ni	ni	ni	ni	ni	○	ni	ni	○	ni	○	●	○	ni	ni	●	○	○	○	○	●
16	54	M	Mt	MD	Alcoholic, LC	ni	ni	ni	○	ni	ni	○	○	ni	ni	○	●	ni	○	ni	●	○	ni	○	○	●
25	71	M	St	MD	CH(B)+, (C)+	ni	○	○	○	ni	ni	ni	○	ni	ni	○	ni	ni	ni	ni	ni	○	ni	○	○	○
30	56	M	St	MD	CH(C)+	ni	○	○	○	○	ni	○	ni	ni	○	○	ni	ni	ni	○	○	ni	ni	○	○	○
9	57	M	St	WD	CH(C)+	ni	ni	ni	○	ni	ni	ni	○	ni	ni	○	○	ni	ni	ni	ni	○	○	○	○	○
3	51	M	St	MD	CH(B)+	ni	ni	ni	○	○	○	ni	○	ni	○	○	○	ni	○	ni	○	ni	○	○	○	○
32	54	F	St	MD	CH(B)+, LC	○	ni	○	○	○	ni	○	○	○	○	○	○	○	○	ni	○	○	○	○	○	○
21	67	M	St	WD	CH(C)+, LC	ni	ni	ni	ni	○	ni	ni	ni	ni	ni	○	ni	ni	○	○	○	ni	ni	○	○	○
33	76	F	St	WD	CH(C)+, LC	○	○	○	○	ni	ni	ni	○	ni	○	○	○	ni	ni	○	○	○	○	○	○	○
23	71	M	Mt	MD	CH(-)	ni	ni	ni	ni	ni	ni	ni	○	ni	ni	○	ni	ni	○	ni	○	ni	○	○	○	○
22	65	M	Mt	MD	CH(C)+	ni	ni	ni	ni	ni	○	ni	ni	ni	ni	○	○	ni	ni	○	○	○	○	○	○	○
31	65	F	Mt	MD	CH(C)+, LC	ni	ni	ni	○	ni	○	ni	○	○	○	○	ni	ni	ni	ni	ni	ni	○	○	○	○
17	37	F	Mt	MD	CH(B)+, LC	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni	○	ni	ni	○	ni	ni	○	○	○
24	60	M	Mt	MD	CH(-), LC	ni	○	○	ni	○	ni	ni	ni	ni	○	○	○	ni	○	ni	○	○	○	○	○	○
LOH ●						0	1	1	3	4	2	5	9	3	4	16	10	1	8	2	15	3	2	1	4	20
Informative (32/33)						8	13	16	17	16	12	12	23	7	17	31	23	10	16	13	32	23	23	31	32	32
LOH/Informative (58/264 = 22%)						0%	8%	6%[a]	18%	25%	17%	42%	39%	43%	24%	52%[b]	43%	10%	50%	15%	47%[c]	13%	9%	3%	13%[d]	63%[e]

A total of 32 informative liver cancer cases were analyzed for LOH at the sixteen microsatellite markers. The number of informative cases and frequency of LOH was shown at the bottom. St: solitary tumor; Mt: multiple tumor; WD: well differetiation; MD: moderately differretiation; PD: poorly differentiation; CH: chronic hepatitis; LC: liver cirrhosis. ● LOH; ○ retention of heterozygosity; ni: noninformative. Significant different from the average, 52% *vs* 22%, [b]$P = 0.0008$; 47% *vs* 22%, [c]$P = 0.004$; 63% *vs* 22%, [e]$P = 0.007$; No statistically significant different from the average, 6% *vs* 22%, [a]$P = 0.207$; 13% *vs* 22%, [d]$P = 0.257$.

premalignant liver lesion. Our result suggests that allelic loss at 8p is an important event in the initiation or promotion of HCC.

Furthermore, among the informative regions 8p23.2, 8p23.1, 8p22, and 8p21, allelic loss was significantly more frequent at 8p23.1 and 8p22 than at 8p23.2 and 8p21 on both sides of the loci. Several minimal regions adjacent to frequently deleted markers were also identified, such as *D8S277, D8S503, D8S1130,* and *D8S552* at 8p23.1, and *D8S254* and *D8S258* at 8p22. On the basis of the minimal regions of overlapping deletions at 8p, we identified two sites, 8p23.1 and 8p22, possibly containing TSGs involved in human liver carcinogenesis. That is to say, the commonly deleted regions were restricted to 8p23.1-22 suggesting that the key genes exist in two distinct regions that might be closely related to the carcinogenesis of

Table 3 Distribution of LOH frequency at 8p in hepatocellular carcinoma cases by clinicopathological variables

Clinicopathological Variables	D8S 264	D8S 262	8p23.2	D8S 1742	D8S 277	D8S 1819	D8S 503	D8S 1130	D8S 552	D8S 1109	8p23.1	D8S 254	D8S 261	D8S 258	D8S 298	8p22	D8S 1752	D8S 1734	D8S 1445	8p21	8p23.2-21
Tumor size (mm)																					
> 50	0/4	0/4	0/5 (0%)	0/6	1/5	0/6	2/4	4/12	0/1	3/8	10/14 (71%)	4/10	0/5	3/7	1/6	6/13 (46%)	0/10	0/10	0/15	0/15 (0%)	10/15 (67%)
< 50	0/3	1/7	1/8 (13%)	3/11	3/11	2/6	3/7	5/11	3/6	1/9	6/17 (35%)	6/13	1/7	5/9	1/7	9/16 (56%)	3/11	2/14	1/15	4/15 (27%)	10/17 (59%)
Tumor number																					
St	0/2	0/3	0/5 (0%)	1/9	1/8	1/5	2/6	3/12	1/3	3/10	6/15 (41%)	9/18	1/6	4/7	1/7	4/13 (31%)	3/20	2/18	1/24	4/24 (17%)	17/24 (71%)
Mt	0/5	1/10	1/10 (10%)	2/7	3/8	1/6	3/6	6/11	2/4	1/7	10/16 (63%)	1/4	0/4	4/9	1/6	5/13 (38%)	0/2	0/5	0/7	0/7 (0%)	2/7 (29%)
Growth Pattern																					
Eg	0/8	1/13	1/15 (7%)	3/16	4/13	2/11	4/11	9/22	3/7	3/15	14/28 (50%)	8/20	1/8	7/14	2/13	13/26 (50%)	3/20	2/21	1/29	4/29 (14%)	18/29 (62%)
Ig	0/1	0/1	0/1 (0%)	0/1	0/3	0/2	1/1	0/1	0/0	1/2	2/5 (40%)	2/2	0/2	1/2	0/0	2/2 (100%)	0/2	0/2	0/2	0/2 (0%)	2/2 (100%)
Formation of capsule																					
Fc-	0/6	1/8	1/10 (10%)	2/3	1/4	1/4	2/3	4/7	3/4	2/5	5/6 (83%)	3/4	1/3	3/3	0/3	5/5 (100%)	2/5	1/5	0/5	2/5 (40%)	5/5 (100%)
Fc+	0/2	0/6	0/6 (0%)	1/14	3/12	1/8	3/9	5/17	0/3	2/12	11/25 (44%)	7/18	0/7	5/13	2/10	10/23 (43%)	1/18	1/18	1/26	2/26 (8%)	15/26 (58%)
Infiltration to capsule																					
Fc-Inf-	0/6	1/8	1/10 (10%)	1/5	1/7	0/7	3/5	3/8	0/4	2/8	7/11 (64%)	3/10	0/5	4/9	0/3	7/15 (47%)	1/10	1/12	0/15	1/15 (7%)	10/15 (67%)
Fc-Inf+	0/1	0/3	0/3 (0%)	1/8	1/3	0/2	1/4	3/10	0/0	1/5	5/13 (38%)	5/9	0/3	3/5	2/7	6/11 (55%)	1/10	0/8	0/13	1/13 (8%)	7/13 (54%)
Septal formation																					
Sf-	0/0	0/3	0/3 (0%)	1/7	2/6	1/7	3/5	3/8	1/4	2/5	7/13 (54%)	5/9	0/5	5/9	1/4	7/15 (47%)	1/12	0/12	0/16	1/16 (6%)	9/16 (56%)
Sf+	0/6	1/9	1/10 (10%)	2/8	1/5	0/3	1/4	3/10	1/1	2/7	5/12 (42%)	4/10	0/3	3/6	1/6	7/11 (64%)	2/9	1/9	0/12	2/12 (17%)	8/12 (67%)
Grading																					
WD	0/3	0/3	0/4 (0%)	1/4	2/4	2/4	1/2	4/6	1/4	1/2	4/8 (50%)	3/6	1/2	3/5	1/5	3/7 (43%)	1/7	1/7	1/7	2/7 (29%)	4/7 (57%)
MD	1/7	0/9	1/13 (8%)	2/13	2/12	1/9	4/12	5/17	1/12	3/6	11/21 (52%)	6/13	1/8	5/11	1/13	10/18 (56%)	2/12	1/13	0/21	2/21 (10%)	13/21 (62%)
PD	0/0	0/1	0/1 (0%)	0/1	0/3	0/1	0/1	1/3	2/3	0/1	2/3 (67%)	1/2	1/3	0/1	0/0	1/2 (50%)	0/2	0/2	0/2	0/2 (0%)	2/2 (100%)
pT																					
pT1	0/1	0/2	0/2 (0%)	0/2	0/1	0/1	0/1	1/2	0/3	0/2	0/5 (0%)	2/6	0/1	2/3	1/2	3/6 (50%)	0/6	0/6	0/6	0/6 (0%)	3/6 (50%)
pT2	1/7	0/7	1/10 (10%)	2/6	1/7	1/3	4/6	4/5	2/5	3/3	7/11 (64%)	5/8	1/4	5/7	0/4	7/11 (64%)	2/8	1/9	0/11	2/11 (18%)	8/11 (73%)
pT3	0/2	0/2	0/3 (0%)	1/4	2/2	1/3	0/2	3/8	1/5	0/1	5/8 (63%)	2/4	0/1	0/2	1/4	2/5 (40%)	1/5	1/5	1/8	2/8 (25%)	5/8 (63%)
pT4	0/0	0/0	0/0 (0%)	0/0	0/1	0/1	0/0	0/1	1/1	0/0	1/1 (100%)	1/1	0/2	0/0	0/0	1/2 (50%)	0/1	0/1	0/2	0/2 (0%)	1/2 (50%)
pN																					
pN-	1/8	0/10	1/12 (8%)	3/7	3/7	2/4	3/5	6/9	1/8	0/1	5/12 (42%)	4/9	0/0	6/7	2/7	8/13 (62%)	3/11	2/10	1/13	4/13 (31%)	9/13 (69%)
pNx	0/0	0/3	0/3 (0%)	0/6	1/5	0/4	1/4	2/9	2/6	1/5	6/12 (50%)	2/4	0/6	1/5	0/1	2/4 (50%)	0/5	0/5	0/7	0/7 (0%)	4/7 (57%)
pM																					
pM-	1/6	0/11	1/13 (8%)	2/7	1/5	1/2	3/5	3/10	3/8	2/4	6/14 (43%)	3/7	1/5	5/9	1/6	6/11 (55%)	2/10	1/11	0/13	2/13 (15%)	7/13 (54%)
pMx	0/0	0/0	0/0 (0%)	0/0	2/3	0/2	0/1	2/2	0/2	1/1	3/4 (75%)	5/6	0/1	0/1	0/0	5/7 (71%)	1/5	0/5	0/8	1/8 (13%)	7/8 (88%)
Vascular infiltration																					
V-	0/7	1/10	1/13 (8%)	2/10	3/10	1/8	4/9	6/13	2/6	2/9	9/20 (45%)	6/15	0/5	7/13	0/7	11/20 (55%)	2/16	2/18	1/21	3/21 (14%)	14/21 (67%)
V+	1/7	0/2	1/9 (11%)	1/7	1/6	1/4	1/3	3/10	1/1	2/8	7/11 (64%)	4/6	1/5	1/3	2/6	4/7 (57%)	1/6	0/5	0/9	1/9 (11%)	5/9 (56%)
Bile duct infiltration																					
B-	0/10	1/13	1/17 (6%)	3/18	4/15	2/11	4/13	10/24	3/9	3/17	15/28 (54%)	7/18	2/9	8/15	2/12	11/24 (46%)	2/19	2/20	1/27	3/27 (11%)	16/27 (59%)
B+	0/1	0/1	0/2 (0%)	0/3	0/3	1/4	1/3	0/4	1/3	1/4	3/4 (75%)	3/5	1/5	0/1	0/4	4/5 (80%)	1/4	0/4	0/5	1/5 (20%)	4/5 (80%)
Liver cirrhosis																					
LC-	0/2	0/6	0/6 (0%)	1/8	2/7	1/6	1/4	7/14	1/1	3/8	11/18 (61%)	6/11	0/4	4/7	2/8	9/16 (56%)	1/10	1/13	1/18	2/18 (11%)	12/18 (67%)

LC+	0/5	1/5	1/7 (14%)	2/9	2/9	1/6	4/8	2/9	2/6	1/9	5/13 (38%)	4/10	1/6	4/9	0/5	6/13 (46%)	2/11	1/11	0/14	2/14 (14%)	8/14 (57%)
Chronic hepatitis																					
CH-	0/1	0/3	0/4 (0%)	2/3	2/3	1/1	1/2	3/7	1/1	2/4	4/8 (50%)	4/6	1/3	2/5	1/3	5/7 (71%)	2/6	0/5	0/8	2/8 (25%)	6/8 (75%)
CH+	0/6	1/7	1/9 (11%)	1/13	2/13	1/11	4/9	6/16	2/6	2/13	12/23 (52%)	6/17	0/7	6/11	1/10	10/22 (45%)	1/17	2/19	1/24	2/24 (8%)	14/24 (58%)

Eg: expansive growth; Ig: infiltrative growth; St: solitary tumor; Mt: multiple tumor; LC: liver cirrhosis; CH: chronic hepatitis.

HCC. Our results are consistent with previously reported patterns of molecular change in HCC and other epithelial tumors. No statistically significant differences were detected in the candidate regions 8p23.1 and 8p22 between the frequency of LOH and any clinicopathologic characteristics, including etiological factors considered to contribute to tumorigenesis, and malignant factors usually important to the subsequent progression of tumors. These results led us to the hypothesis that loss of 8p is not essential for the subsequent development or progression of HCC.

Moreover, with respect to the results of allelotyping, several genes, such as angiopintin 2 (ANGPT2), AGPAT5, LOC648814, DEFB 137 and DEFB 136, LONRF1, and FLJ36980, which were adjacent to the candidate markers D8S277, D8S503, D8S1130, D8S552, and D8S1109 at 8p23.1, respectively, were analyzed for somatic mutations or expression by single nucleotide polymorphisms (SSCPs) and the reverse transcription polymerase chain reaction (RT-PCR) methods. However, no significant mutation or absence of expression of these adjacent genes was found (data not shown), indicating that alterations of those genes may not be closely related to the carcinogenesis[16-19,26]. Several new candidate cancer-susceptibility genes at 8p22, such as deleted in breast cancer 2 (DBC2), leucine zipper tumor suppressor 1 (LZTS1), and deleted in liver cancer 1 (DLC1), and mitochondrial tumor suppressor 1 (MTUS1) have been cloned[27-30]. We have analyzed these genes in the same HCC samples, but a somatic mutation or absence of expression of these candidate genes is rare in Japanese patients (data not shown), indicating that these well-known candidate genes are not the main targets of the observed LOH at 8p22. Although no significant genetic alterations were detected in HCC in the present study, it could not be denied that they had already had some epigenetic change during the pre-cancer stage or earlier in the carcinogenesis. Although detailed data have not been published, the present results strongly suggest that other unknown genes in the region 8p22-23.1 play an important role in HCC. Further studies are needed to identify critical oncogenes or TSGs, including those in 8p22-23.1. Our results should be useful for identifying the targets of deletion at 8p.

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