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Review

Chromosomal instability and human hepatocarcinogenesis

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Summary. Recently, many studies have identified losses and gains of several chromosomal loci in human hepatocellular carcinoma (HCC) with fine microsatellite analysis and comparative genomic hybridization. Although distribution of aberrant chromosomal arms differs among HCCs, loss of 1p, 4q, 6q, 8p, 9p, 10q, 13q, 16q and 17p, and gain of 1q, 6p, 8q, 17q and 20q have been recurrently reported, and loss of 4q and 16q seems to occur preferentially in hepatitis B virus-related HCCs. Accumulation of these aberrant chromosomal regions is associated with tumor progression, and some chromosomal aberrations, such as loss of 1p, are frequently identified in well-differentiated HCCs and also detected even in dysplastic nodule and cirrhotic nodule. This evidence suggests that chromosomal instability (CIN) emerges at an early stage during hepatocarcinogenesis and is successively inherent to tumor cells, resulting in acquisition of malignant phenotype. The molecular basis of CIN is beginning to be explored; however, several mechanisms may be involved for CIN of HCC.

Key words: Chromosomal instability, Hepatocellular carcinoma, Cancer-related genes, Mutation, Methylation

Introduction

It is now widely accepted that multiple cancer-related genes are involved in carcinogenesis, and changes in the number of chromosomes in cancer cells may reflect functional loss or gain in these genes, which in turn may contribute to cancer formation and progression. A large number of studies have identified numerous chromosomal aberrations in human hepatocellular carcinomas (HCCs), such as loss and/or gain on several loci of chromosomes (Feitelson et al., 2002; Thorgeirsson and Grisham, 2002). This suggests that the majority of HCC acquire the chromosome instability (CIN) phenotype during the early stages of HCC formation. This review provides an overview of chromosomal aberration at each stage of HCC, and discusses the role and mechanism of the CIN phenotype acquired during human hepatocarcinogenesis.

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Detection of loss and gain of chromosomal arms in HCCs

Mounting evidence suggests that HCC is characterized by multistep progression (Thorgeirsson and Grisham, 2002). Recent genome-wide allelotype studies have detected many chromosomal aberrations in human HCC tissues (Table 1). Among such alterations, the occurrence of allelic imbalance (AI) on 1p, 1q, 4q, 6q, 8p, 8q, 9p, 10q, 13q, 16p, 16q and 17p has been repeatedly reported, on the basis of PCR-based microsatellite analyses, suggesting that tumor-suppressor genes and/or oncogenes, which are essential for human hepatocarcinogenesis, are located on these chromosomal arms (Boige et al., 1997; Nagai et al., 1997; Piao et al., 1998a; Okabe et al., 2000; Laurent-Puig et al., 2001; Wang et al., 2001; Nishimura et al., 2002). A comparison with data from comparative genomic hybridization (CGH) studies, makes it clear that AI of 1p, 4q, 6q, 8p, 9p, 10q, 13q, 16q and 17p is the result of losses and AI of 1q and 8q as gains (Table 2: Marchio et al., 1997, 2000; Kusano et al., 1999; Sakakura et al., 1999; Wong et al., 1999; Guan et al., 2000; Tornillo et al., 2000; Niketeghad et al., 2001). However, the frequency of AI resulting from allelic gain may have been underestimated in PCR-based microsatellite analyses because contamination of non-cancerous cells more readily masks allelic gain than loss. For example, alleles showing imbalance with one paternal and two maternal alleles may well yield an AI index (allelic ratio of cancerous DNA adjusted by that of non-cancerous DNA) of more than 0.5, which has been used as a cut-off value for AI index. In fact, recent CGH studies, which can detect allelic gain as effectively as loss, showed several gains which were not identified as AI with PCR-based microsatellite analyses. These included gains of

6p, 17q and 20q as well as 1q and 8q (Table 2). In addition to CGH studies, we reported that the combination of comparative PCR and microsatellite analysis could be useful for detecting allelic gains (Nishimura et al., 2002). Because previous microsatellite analyses may have overlooked several gains, these new approaches can be expected to be more effective for chromosomal analyses of HCCs.

Chromosomal aberrations and etiological factors of HCCs

There is a wide geographical variation in the incidence of HCC, which has been attributed mainly to geographical differences in the prevalence of etiological factors, such as chronic infection of hepatitis type B (HBV) and type C (HCV), aflatoxin exposure and other types of chronic liver disease. However, the effect of a special etiological factor, such as HBV and HCV, on the formation of alterations in a specific chromosomal arm, remains controversial. Fujimori et al. (1991) reported no significant associations between the integration of HBV and a specific chromosomal aberration. Other reports also showed negative findings for relations between etiological factors and a specific chromosomal alteration, suggesting that HCCs may progress through a similar cascade of molecular events regardless of their viral etiology (Nagai et al., 1997; Sakakura et al., 1999; Tornillo et al., 2000). On the other hand, Laurent-Puig et al. (2001) reported that AI on five chromosomal arms (1p, 4q, 13q, 16p and 16q) and *Axin 1* and *p53* gene mutations were linked to HBV infection. Okabe et al. (2000) used microsatellite analysis and found an association between HBV infection and AI on 13q and 16q. In addition, CGH studies by Marchio et al. (1997) found chromosomal loss of 4q, 16q and 17p in HBV-related HCCs. Wong et al. (2000) reported significant chromosomal loss of 4q, 8p and 16q in HCCs from

Table 1. Summary of genome-wide allelotype studies in human HCC tissues.

1[1]		2	4	5	6		7		8		9		10	11	13	14	16		17		19	20	22	No. of	No. of	Reporter
p	q	q	q	q	p	q	p	q	p	q	p	q	q	p	q	q	p	q	p	q	p	q	q	marker[2]	case[3]	
			44[4]										25	46				36	45					44	46	Fujimori et al. (1991)
44			42			35			60		30				29		40	39	48					275	48	Boige et al. (1997)
51	44	35	52			48	28	28	40	26	33	43	25		53	34	36	31	34	31				195	120	Nagai et al. (1997)
	62		73						64	77			33		40	46		59	46					68	22	Piao et al. (1998a)
			48			36			57	31	38	30			32		41	43	56		33			216	44	Okabe et al. (2000)
33	22		38			29			48		20				31		24	30	45					400	137	Laurent-Puig et al. (2001)
	40[5]		51[6]			43			45	**41**	55				46			52	65					292	60	Wang et al. (2001)[7]
22	**56**			**44**	**33**		**22**		44	**44**									28					400	18	Nishimura et al. (2002)[8]

Summary of the reports for frequencies of AI on each chromosome in human HCC tissues. Fujimori et al. used RFLP markers for these analyses and other reporters adopted PCR-based microsatellite analyses.[1]: Chromosome number. [2]: Number of RFLP or microsatellite markers used in the studies. [3]: Number of HCC cases analyzed. [4]: Percentages of AI on each chromosomal arm described in the reports. [5]: Bolt numbers indicate the percentages of AI which are the result of chromosomal gain. [6]: Underlined numbers denote the percentages of AI which were the result of loss. [7]: Wang et al. (2001) simultaneously examined microsatellite analyses and CGH in the same HCC samples and determined whether chromosomal loss or gain resulted in AI. In this study, all HCC samples were from three regions of Southern China where exposure to HBV and aflatoxin are major risk factors. [8]: Nishimura et al. (2002) also reported the nature of AI with multiplex PCR-based microsatellite analyses. In this study, only well-differentiated HCCs were analyzed.

Table 2 Summary of CGH studies in human HCC tissues.

1[1]		2	4	5	6		7		8		9		10	11	13	14	16		17		19	20	22	No. of	Reporter
p	q	q	q	q	p	q	p	q	p	q	p	q	q	p	q	q	p	q	p	q	p	q	q	case[2]	
30[3]	**58**[4]		70		**33**	37			65	**60**					37		54		51	**33**				50	Marchino et al. (1997)[5]
	78		32						29	**66**			17		37		46		51					41	Kusano et al. (1999)
37	**46**		48	35	**20**	23			28	**31**					20		33		37	**43**				35	Sakakura et al. (1999)[6]
	72		43						37	**48**					37		30			**30**		**37**		67	Wong N. et al. (1999)[7]
36	**66**		40						32	**48**							70		52		42	**20**	28	50	Guan et al. (2000)
19	**56**		25		**38**	22			62	**44**					31	22	16		22	**25**				34	Marchino et al. (2000)[8]
24	**46**		39		**20**	41			44	**41**	24				39					**37**				41	Tornillo et al. (2000)
	57		33			19	**24**			**52**					19		14			**29**		**29**		21	Niketeghad et al. (2001)

[1]: Chromosome number. [2]: Number of HCC cases analyzed. [3]: Percentages of chromosomal alteration on each chromosomal arm described in the reports. Underlined numbers denote the percentages of chromosomal loss. [4]: Bolt numbers indicate the percentages of gain. [5]: In this study, only HBV-related HCCs were analyzed. [6]: In this study, only HCV-related HCCs were analyzed. [7]: In this study, all HCC samples were from Southern China, where chronic HBV infection is the main etiological factor. [8]: In this study, only HCCs from patients serologically negative for both HBV and HCV were analyzed.

Shanghai where HBV infection and aflatoxin exposure are proven risk factors for HCCs, while 1q gain was common to HCCs from different geographical areas. Kusano et al. (1999) showed that gain of 10q was detected exclusively in HCV-related HCCs and amplification of 11q13 occurred preferentially in HBV-related HCCs. These reports show that several researchers have identified commonly occurring associations between losses on 4q and 16q and HBV infection, indicating that these events may be linked to HBV-related carcinogenesis, although the studies differ somewhat as to which chromosomes are affected in HBV-related HCC. However, several problems need further study. First, many HCV-related HCC patients have been found to have occult HBV infection (Cacciola et al., 1999), and the classification of HBV-positive and HCV-positive HCCs is not yet clear. Second, because HBV-related HCC samples sometimes show a low degree of differentiation, the clinicopathological background has to be adjusted prior to analysis (Laurent-Puig et al., 2001). Then to address this issue, the earlier stages of HCC must be analyzed to determine the molecular difference between HBV- and HCV-related carcinogenesis. In addition, although chromosomal aberrations are observed in almost every HCC, no specific ones, those detected in any HCC, have been identified and even nodules arising de novo within one and the same liver have different spectra of chromosomal aberrations in multifocal HCCs (Tsuda et al., 1992; Feitelson et al., 2002). These findings indicate that genetic heterogeneity is one of the characteristics of hepatocarcinogenesis and that there may be many paths for HCC formation even in tumors from a single etiological factor.

Chromosomal aberration during progression of HCC

CIN of HCCs contributes to an increase in the number of chromosomal aberrations during tumor developmental progression. Chromosomal aberrations responsible for tumor progression and metastasis have been analyzed in many studies, especially AI on 4q, 6q, 8p, 13q, 16q or 17p in large and/or undifferentiated HCCs (Feitelson et al., 2002). Allelic loss on 4q and gain of chromosome 4 are reportedly associated with large tumor size (Zimmermann et al., 1997; Bando et al., 1999; Wong et al., 1999), vascular invasion (Zimmermann et al., 1997; Okabe et al., 2000) and poorly-differentiated phenotype (Bando et al., 1999; Okabe et al., 2000). Several studies have shown that 13q loss was linked to undifferentiated phenotype of HCC (Nishida et al., 1992; Kusano et al., 1999; Okabe et al., 2000), vascular invasion (Okabe et al., 2000), intrahepatic metastasis (Okabe et al., 2000), and large tumor size (Nishida et al., 1992). Allelic losses on 16q and/or 17p were detected comparatively frequently in HCC with vascular invasion, intrahepatic metastasis and poorly-differentiated HCC (Tsuda et al., 1990; Nishida et al., 1992; Okabe et al., 2000). The loss of 8p and gain of 20q were also reported to be correlated with vascular invasion, advanced stage and/or large tumor size (Guan et al., 2000; Okabe et al., 2000). These chromosomal aberrations seemed to transform HCC cells into a more aggressive variety, although the role of individual chromosomal aberrations in the acquisition of specific phenotypes, such as tumor metastasis, is still obscure. Qin et al. (1999) used CGH to analyze 10 pairs of primary HCC and their matched metastatic lesions and found that chromosome 8p was deleted in eight metastatic but only three corresponding primary tumors. This finding suggests that loss of 8p may specifically contribute to the development of HCC metastasis. Amplification of 11q13, involving amplification and overexpression of the *cyclin D1* gene, was also associated with aggressive tumor behavior (Nishida et al., 1994; Kusano et al., 1999). On the other hand, interpretation of the role of 8q gain in HCCs seems to be somewhat less straightforward. Kusano et al. (1999) reported that a gain of 8q24 was linked to well-differentiated HCCs, whereas Guan et al. (2000) used CGH analysis to show that a gain of 8q was associated with large tumor size.

The extent of AI in a tumor can be measured in terms of fractional allelic imbalance (FAI) and an increase in AI is reportedly related to an aggressive prognosis and recurrence of colon cancer (Vogelstein et al., 1989; Zhou et al., 2002). In human HCCs, a high number of chromosomal aberrations or a high value of the FAI index correlates inversely with the state of the tumor-cell differentiation (Nishida et al., 1992; Ohsawa et al., 1996; Okabe et al., 2000; Laurent-Puig et al., 2001), and is associated with the presence of vascular invasion or intrahepatic metastasis (Nishida et al., 1992; Okabe et al., 2000). Laurent-Puig et al. (2001) identified significant associations between a high FAI index value and shorter survival of HCC patients. In addition, Chen et al. (2000) analyzed 31 pairs of initial and recurrent HCCs obtained from patients who underwent two consecutive surgeries and showed that initial HCCs, which subsequently relapsed, accumulated more chromosomal aberrations than those which developed *de novo* HCC. Kusano et al. (2002) reported that HCC recurrence was linked to the loss of 13q and aberrations in the number of DNA copies, and that losses of 8p and 13q and amplification at 11q13 were associated with an unfavorable outcome for HCC patients. We also analyzed 49 HCCs with AI on 13q, 16q and 17p, which has been frequently identified with advanced tumors, and demonstrated with the aid of multivariate analysis that the number of chromosomes with AI and AI on 16q can be prognostic factors for metastasis after curative resection of HCCs (Nishida et al., 2002). These findings indicate that an increase in the overall chromosomal aberrations in HCCs may be linked to an unfavorable prognosis, and that the extent of aberrant chromosomal regions could be useful for the prediction of prognosis and the selection of surgical therapy such as liver transplantation for HCC.

Target genes of allelic imbalance in human HCC tissues

Commonly altered chromosomal region

Several studies of HCCs have identified specific altered regions on each chromosome by using high-resolution allelotyping. These reports, summarized in Table 3, showed multiple altered regions even in a single chromosome. Loss of function of tumor-suppressor genes sometimes occurs as a consequence of loss of one allele, which can be detected as AI, in conjunction with inactivation of the other allele induced by mutation, deletion of the gene or promoter methylation on the CpG island. On the other hand, allelic gain may be relevant for the activation of oncogenes because it has been recognized in association with overrepresentation of mapped genes (Virtaneva et al., 2001). For these reasons, the existence of altered chromosomal regions in common is widely assumed to imply the existence of cancer-related genes in or around such loci.

Alteration of the cancer-related genes on each chromosome

As described above, a loss or gain of a chromosome is considered to be associated with inactivation of a tumor-suppressor gene or activation of an oncogene within each chromosomal region. So far, many cancer-related genes in human HCCs have been analyzed for genetic and/or epigenetic alterations. We have summarized the alterations of cancer-related genes on each of the chromosomes analyzed in human HCC tissues in Table 4.

Chromosome 1

Frequent alterations have been reported for HCCs at the distal region of chromosome 1p, alterations of which are mainly observed as a loss (Yeh et al., 1994). Iwata et al. (2000) reported that 89% of HCCs showed hypermethylation of the CpG island and loss of expression of the *14-3-3σ* gene on 1p35, which is known to be responsible for G2 cell-cycle arrest by p53 in response to DNA damage. Hypermethylation of this gene has been identified not only in HCC tissues but also in adjacent non-cancerous tissues, suggesting that this phenomenon is an early event during hepatocarcinogenesis, although it is still a matter of debate as to whether this gene is located within the commonly deleted region of 1p or not.

Alteration of the *p73* gene encoding a protein homologous to p53 on 1p36.3 was analyzed for mutation in human HCCs. AI of *p73* was observed in 20% of HCCs, but PCR-single strand conformation polymorphism (SSCP) analysis of the entire coding region of the *p73* showed no mutation (Mihara et al., 1999). In addition, data published by Tannapfel et al. (1999) identifying a high expression level of p73 in HCC patients with a poor survival prognosis, provide evidence that p73 may not act as a tumor suppressor of HCCs.

The *retinoblastoma (Rb)-interacting zinc finger 1* (*RIZ1*) gene, a member of the nuclear histone/protein

Table 3. Target region of allelic imbalance on each chromosome in human HCC tissues.

CHROMOSOME	TARGET REGION[1]	% OF AI[2]	REPORTER
1p35-36	D1S170	50	Yeh et al. (1994)
1p36.13-36.23	D1S434-D1S436	37	Fang et al. (2000)
4q	D4S1615, D4S1598, D4S620, D4S1566-D4S2979, D4S1617-D4S1545, D4S1537, D4S2920-D4S2954	83	Piao et al. (1998b)
4q21-22, 4q35	D4S1534-D4S2929, D4S2921-D4S2930	74	Bando et al. (1999)
4q22, 4q34, 4q35	D4S395-D4S2986, rs1564498[3]-D4S1584, D4S2943-D4S426	38	Bluteau et al. (2002)[4]
6q23, 6q26-27	D6S977-D6S311, D6S1693-D6S281	50	Koyama et al. (2000)
8p21, 8p22, 8p23	D8S1820-D8S560, D8S261-D8S1790, D8S1706-D8S518	60	Pineau et al. (1999)
9p21, 9p13-q33	D9S1747-D9S1752, D9S166-D9S53	63	Liew et al. (1999)[5]
10q23.3	D10S597-D10S216, D10S216-D10S590	32	Fujiwara et al. (2000)
11p11.2-12	D11S1361-D11S1357	N.R.[6]	Ricketts et al. (2002)
13q12-14, 13q12-13	D13S260-D13S218, D13S126-D13S172	30	Kuroki et al. (1995b)
16p13.13	D16S519-D16S3078	48	Koyama et al. (1999)
16q23.1-24.1	D16S3101-D16S504	63	Balsara et al. (2001)
16q24.1-24.2	D16S534-D16S3091	60	Bando et al. (2000)
16q21, 16q24.2	D16S3106, D16S498	85	Piao et al. (1999)

[1]: Commonly altered chromosomal loci reported. [2]: Number of HCCs with AI/number of total HCCs analyzed. [3]: Single nucleotide polymorphism ID of NCBI. [4]: In this study, association between alcohol intake, high grade of differentiation and loss of the 4q34-35 was described. [5]: In this study, 45 out of 48 HCC cases were positive for HBV-DNA. [6]N.R.: Percentage was not reported.

methyltransferase superfamily, is a candidate tumor-suppressor gene in HCC because it can induce G2 cell-cycle arrest and apoptosis in the HCC cell line (Jiang et al., 1999). Fang et al. (2000) mapped a minimal deleted region in HCC to 1p36.13-p36.23, where the *RIZ* gene is located. Although PCR-SSCP analysis did not show any mutation of the gene, methylation of its promoter CpG island was found in 62% of HCC tissue (Du et al., 2001).

Chromosomes 3, 5 and 6

Higashitsuji et al. (2000) isolated the gene on 3q28 overexpressed in HCC (gankyrin). It binds to the Rb protein, increasing phosphorylation of Rb and releasing

Table 4. Alteration of cancer-related genes reported in human HCCs.

CHROMOSOME	ALTERED GENE[1]	% OF ALTERATION	TYPE OF ALTERATION	REPORTER
1p35	14-3-3σ	89	CpG methylation	Iwata et al. (2000)
1p36.3	P73	0 (by SSCP for entire coding region)		Mihara et al. (1999)
1p36	RIZ	0 (by SSCP for exon 2-7)		Fang et al. (2000)
		62	CpG methylation	Du et al. (2001)
3p21	CTNNB1[2]	19	mutation	Miyoshi et al. (1998)
3p21.3	RASSF1A	85	CpG methylation	Zhang et al. (2002)[3]
3p22	TGF-ß1RII	44	mutation	Furuta et al. (1999)
3q28	Gankyrin	100	overexpression	Higashitsuji et al. (2000)
5q21	APC	0 (by SSCP for codon 764-1596)		Chen et al. (1998)
6q26-27	M6P/IGF2R	25 (with LOH)4	mutation	De Souza et al. (1995)
		21 (with LOH)	mutation, deletion	Oka et al. (2002)
8p21.3-22	DLC-1	44	LOH	Yuan et al. (1998)
8p23	LPTS	50	Down-regulation	Liao et al. (2000)
8q24	MYC	33	amplification	Kawate et al. (1999)
9p21	P16 ^{INK4A}	48	CpG methylation,	Matsuda et al. (1999)
		68		Azechi et al. (2001)
	P14 ^{ARF}	15	CpG methylation, deletion	Tannapfel et al. (2001)
	P15 ^{INK4B}	64	CpG methylation	Wong I.H.N. et al. (2000)
10q23.3	PTEN	8 (with LOH)	mutation	Fujiwara et al. (2000)
11p11.2	KAI 1	N.R.[5]	Down-regulation	Guo et al. (1998)
11p11.2-12	stSG30184[6]	N.R.	N.R.	Ricketts et al. (2002)
11p15.5	IGF-2	100	AI of expression	Takeda et al. (1996)
11q13	cyclin D1	11	amplification	Nishida et al. (1994)
11q23.2	TSLC-1	80 (with LOH)	CpG methylation, mutation	Kuramochi et al. (2001)
13q12.1	Tg737	0	(by Southern and Norhtern blotting)	Bonura et al. (1999)
13q12.2-14.1	Rb	16 (with LOH)	Mutation	Zhang et al. (1994)
		21	CpG methylation	Roncalli et al. (2002)[7]
13q12-13	BRCA2	5	mutation	Katagiri et al. (1996)
13q34	TFDP1, CUL4A, CDC16	8	amplification	Yasui et al. (2000)
16p13.3	SOCS-1	65	CpG methylation	Yoshikawa et al. (2001)
16p13.3	Axin1	5	mutation, deletion	Satoh et al. (2000)
16q22.1	*CDH1*	67	CpG methylation	Kanai et al. (1997)
16q23	WWOX/FOR	0 (by sequencing of entire coding region)		Yakicier et al. (2001)[8]
17p13.1	P53	32	mutation	Nishida et al. (1993)
17p13.3	HIC-1	90	CpG methylation9	Kanai et al. (1999)
17p13.3	HCCS1	35	mutation	Zhao et al. (2001)
18q21.1	Smad 2, Smad4	3, 6	mutation	Yakicier et al. (1999)
20p11.2	RBBP9/Bog	N.R.	overexpression	Woitach et al. (1998)

[1]: An unabbreviated name of each gene is described in the text. [2]: Putative oncogenes are shown underlined. [3]: In this study, significant association was described betweeen RASSF1A methylation and the level of aflatoxin B1-DNA adduct in HCC. [4]: Twenty-five percent of HCCs with loss of heterozygosity (LOH) on 6q showed mutation of the M6P/IGF2R gene. [5]N.R.: Not reported. [6]: Expressed sequence tag. [7]: In this study, methylation of the p14^{ARF} p15^{INK4B} and p16^{INK4A} genes was also analyzed in HCC and cirrhosis. [8]: In this study, a relationship between 16q23 homozygous deletion of HCC and aflatoxin B1 exposure was described. [9:] In this study, CpG methylation status of the D17S5 locus was analyzed.

the activity of the transcription factor E2F-1. In addition, it accelerates the degradation of Rb and may contribute to HCC formation by destabilizing Rb.

The *isoform A of the human ras-association domain family gene* (*RASSF1A*), cloned from the lung-tumor suppressor locus 3p21.3 is inactivated by promoter methylation in several cancers. Eighty-five percent of HCCs showed methylation of the CpG island promoter of the *RASSF1A*, which was associated with the level of aflatoxin B1-DNA adducts in tumor tissues (Zhang et al., 2002).

Activation mutation of the *ß-catenin* (*CTNNB1*) gene, located on 3p21, has been reportedly identified in 19% of human HCCs (Miyoshi et al., 1998). This type of mutation results in the accumulation of ß-catenin which may translate into the nucleus and act as a transcription factor through binding with high-mobility group box factors of the T-cell factor/lymphocyte enhancer-binding factor family and contribute to tumor formation. The *adenomatous polyposis coli* (*APC*) gene is mapped on 5q21 and is also involved in the Wnt/Wingless signal-transduction pathway as a result of interacting with ß-catenin for initiation of its degradation. However, mutation of the *APC* gene has not been identified in human HCCs (Chen et al., 1998). (Alteration of the Axin1 gene on 16p13.3, which encodes another molecule of the Wnt/Wingless signal-transduction pathway, is described later.)

Loss of the *mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R)* locus, which maps to 6q26-q27, has been frequently detected in human HCC. De Souza et al. (1995) reported the mutation of this gene on the remaining allele in 25% of human HCCs with allelic loss on 6q. The M6P/IGF2R binds the latent complex of transforming growth factor-ß (TGF-ß) and facilitates its activation. Thus, loss of the M6P/IGF2R function decreases activated TGF-ß and increases the extracellular concentration of IGF-II, consequently accelerating cellular proliferation and reducing apoptosis. Oka et al. (2002) also reported that 67% of HCCs and 75% of dysplastic nodules had AI on the *M6P/IGF2R* locus, and 21% of HCCs with AI showed mutation or deletion of this gene, suggesting that haploid insufficiency of this gene was an early event of HCC formation. Gene mutation of the TGF-ß1 type II receptor (TGF-ß1RII) on 3p22 has also been reported in human HCC (Furuta et al., 1999), implying that disruption of the TGF-ß signaling pathway may also be important for hepatocarcinogenesis. (Analysis of Smad 2 and 4, which are also involved in the TGF-ß signaling pathway, is described later).

Chromosomes 8, 9 and 10

Loss of the short arm and/or gain of the long arm of chromosome 8 have been recurrently reported in human HCC (Pineau et al., 1999). Yuan et al. (1998) used representational differential analysis to identify a new gene on 8p21.3-22 frequently deleted in HCC (*deleted in liver cancer-1*: *DLC-1*). *DLC-1* is highly homologous to the rat *p122 RhoGAP* gene and AI on DLC-1 has been detected in 44% of human HCCs. The *liver-related putative tumor suppressor* (*LPTS*) was identified by deletion mapping and positional cloning at 8p23 (Liao et al., 2000). Expression of the *LPTS* gene was reduced or undetectable in HCC and significant growth suppression of the HCC cell line was observed after introduction of the *LPTS* gene. The proto-oncogene *c-myc*, on the other hand, located on the long arm of chromosome 8, was amplified in 33% of HCCs (Kawate et al., 1999).

On the short arm of chromosome 9, two tumor-suppressor proteins, $p16^{INK4A}$ and $p14^{ARF}$, are encoded on the same locus on 9p21 and act as the respective upstream regulators of the Rb-cyclin-dependent kinase 4 (CDK4) and p53 pathway. In addition, the $p15^{INK4B}$ gene, another CDK inhibitor, has also been identified adjacent to the $p16^{INK4A}$. Inactivation of $p16^{INK4A}$ was detected in 48% of HCCs and was mainly induced by hypermethylation of the promoter (Kita et al., 1996; Matsuda et al., 1999; Azechi et al., 2001). Frequent $p15^{INK4B}$ promoter methylation was also identified in 64% of HCCs and may contribute to the disruption of the Rb-CDK4 pathway (Wong et al., 2000). Inactivation of $p14^{ARF}$ was found in 15% of HCCs as a consequence of deletion or promoter methylation (Tannapfel et al., 2001).

Frequent loss on chromosome 10q has been reported in HCC and analysis of deletion mapping of 10q has identified commonly deleted regions within the distal part of 10q. One out of 12 HCCs with allelic loss on 10q showed a somatic mutation of the *phosphatase and tensin homolog deleted on chromosome 10 (PTEN) gene,* which encodes a negative regulator of the phosphoinositide 3-kinase/AKT signaling pathway (Fujiwara et al., 2000).

Chromosome 11

Aberration of chromosome 11 in HCC was reported in the form of frequent AI of the short arm and high copy amplification of 11q13. Ricketts et al. (2002) performed deletion mapping of chromosome 11p in HCC and showed that the region of 11p11.2-p12 harbors one or more tumor-suppressor genes. They also identified the expressed sequence tag of stSG30184 as a candidate for a liver tumor suppressor. Guo et al. (1998) reported that the *kangai 1* (*KAI1*) gene on 11p11.2, which was originally discovered in prostate cancer cells and functions in cell-cell and cell-extracellular matrix interactions, was down-regulated in HCC, especially in tumors with metastases. AI for the expression of the *insulin-like growth factor-2* (*IGF-2*) gene mapped at 11p15, which was the result of altered imprinting, was also identified in HCC and may confer a proliferative capability to tumor cells of the liver (Takeda et al., 1996; Schwienbacher et al., 2000). Recently, Kuramochi et al. (2001) reported that the *tumor-suppressor in lung cancer-1* (*TSLC1*) gene on 11q23.2 was inactivated

mainly by promoter hypermethylation in HCC.

We previously reported that high copy amplification and overexpression of the *cyclin D1* gene on 11q13 in HCC was associated with aggressive tumor behavior. In HCC with amplification of *cyclin D1*, the *INT-2* gene was co-amplified, whereas no overexpression of the INT-2 was detected, suggesting that the *cyclin D1* gene is the target of 11q13 amplification in HCC (Nishida et al., 1994).

Chromosome 13

Loss of the 13q region including the *RB* locus has been correlated with loss of the Rb protein in HCC. However, tumor-specific mutation of the Rb gene was detected in only 16% of HCCs with 13q loss (Zhang et al., 1994) and CpG methylation was detected in 21% of HCCs (Roncalli et al., 2002). The *breast cancer 2* (*BRCA2*) gene is another tumor suppressor on 13q, the mutant alleles of which can predispose carriers to breast cancer, but mutation was found in only three of 60 HCCs (Katagiri et al., 1996). Bonura et al. (1999) examined deletion or alteration of the *Tg737* gene on 13q12.1, whose product is involved in liver oval cell proliferation, differentiation and ploidy control, but found no Tg737 genetic alteration in human HCC. Recently, Yasui et al. (2002) identified high copy amplification of the *transcription factor DP-1* (*TFDP1*), *cullin 4A* (*CUL4A*) and *cell-division cycle 16* (*CDC16*) genes in human HCC as targets for amplification at 13q34.

Chromosome 16

Alteration of the Axin1 gene on 16p13.3, whose product is thought to be critical for degrading cytoplasmic ß-catenin, was examined in HCC and 5% of HCCs without the *CTNNB1* mutation was reported to contain the Axin1 mutations (Satoh et al., 2000). The *suppressor of cytokine signaling-1* (*SOCS-1*) gene is also known to be located on 16p13.3. SOCS-1 turns off the cytokine signal transduction through direct interaction with Janus kinase (JAK). Inactivation due to the aberrant methylation of this gene was detected in 65% of human HCCs, suggesting that constitutive activation of the JAK/STAT pathway took place as part of the development of the HCC (Yoshikawa et al., 2001). Kanai et al. (1997) reported reduced expression of the *E-cadherin* (*CDH1*) gene which is located on 16q22.1. Promoter hypermethylation of this gene was detected in 46% of liver tissues with chronic liver disease and 67% of HCCs. E-cadherin plays an important role for cell-cell adhesion and also displays the potential to suppress cell growth and transformation by inhibiting ß-catenin signaling and the Wnt/Wingless pathway. Therefore, it is thought that inactivation of the *CDH1* gene may contribute to HCC formation and loss of intercellular adhesiveness. However, Wei et al. (2002) reported that *E-cadherin* expression was positively correlated with the invasive potential of HCC cells and that it seemed to play diverse and paradoxical roles in HCC. Recently, the *WW domain containing oxidoreductase* (*WWOX*) gene, encoding a protein with two WW domains and a short-chain dehydrogenase domain, has been mapped to 16q23. Deletion or mutation of this gene was reported in primary ovarian cancer, but tumor-specific mutations of this gene have not been detected in HCC (Yakicier et al., 2001).

Chromosome 17

The *p53* gene is mapped to 17p13.1 and 32% of HCCs contain a mutation of this gene. The mutation of p53 is closely associated with loss of 17p (Nishida et al., 1993). In addition, the *hypermethylated-in-cancer-1* (*HIC-1*) gene is located on 17p13.3 and DNA hypermethylation at the *D17S5* locus was detected in 90% of HCCs and 44% of chronic liver disease tissues (Kanai et al., 1999). The *HCC suppressor 1* (*HCCS1*) gene was identified by Zhao et al. (2001) and also mapped to 17p13.3. Deletion or point mutation of *HCCS1* was observed in 35% of HCCs.

Chromosome 18 and 20

The *Smad2 and Smad4* genes, whose products are involved in the TGF-ß signaling pathway, are located on 18q21.1 and a subset of HCC also carry mutations of these genes (Yakicier et al., 1999). The *Rb-binding protein 9/B5T overexpressed gene* (*RBBP9/Bog*) is located on 20p11.2 and is shown to be overexpressed in rat liver epithelial cells resistant to the growth-inhibitory effect of TGF-ß1. It can displace E2F-1 from E2F-1/Rb complex and its overexpression may induce TGF-ß1-resistant phenotype in HCC (Woitach et al., 1998).

Chromosomal aberrations in precancerous lesions

Recent detailed chromosomal analyses of HCC have detected several altered chromosomal loci even in well-differentiated HCCs. For example, Kuroki et al. (1995a) found AI on 1p occurred in 31% of well-differentiated HCCs and 38% of HCCs smaller than 2 cm. Using the microsatellite analysis-comparative PCR combination technique, we also detected several chromosomal gains and losses, including loss of 1p and gain of 1q, in well-differentiated HCCs (Nishimura et al., 2002).

Not only HCC but also precancerous lesions, such as dysplastic nodules or regenerative nodules of liver cirrhosis, are known to harbor several chromosomal aberrations. According to several studies, the FAI index is significantly increased in dysplastic nodules and HCC as compared with regenerative nodules of cirrhosis (Maggioni et al., 2000; Yeh et al., 2001). In addition, AI tended to appear more prevalently in tissues of chronic liver disease with HCC than those without HCC (Kawai et al., 2000), and the FAI index showed a statistically significant increase in cases of cirrhosis who had developed HCC as compared with HCC-free cases

(Roncalli et al., 2000). These observations suggest that accumulation of AI in cirrhosis, dysplastic nodules and HCC is in keeping with a multistep process of carcinogenesis.

Maggioni et al. (2000) studied AI in cirrhosis, dysplastic nodules and HCC, and observed frequent occurrences of AI on 4q and 8p. Kondo et al. (2000) and Yeh et al. (2001) also suggested that AI on 4q, 8p and Xq were early mutations detected in cirrhosis. AI on 1p was also observed in cirrhosis and dysplastic nodules as well as HCC and hypothesized to represent an early event in heptocarcinogenesis (Sun et al., 2001). Roncalli et al. (2000) showed that AI on 1p, 4q, 13q and 18p was confined to cases of cirrhosis that developed HCC. To summarize the findings of these microsatellite analyses of precancerous lesions, AI on 1p, 4q and 8p was identified in several studies. However, the role of 4q and 8p loss in the early step of hepatocarcinogenesis is still unclear because the loss of these chromosomal arms are also more frequently observed in advanced than early HCCs (Guan et al., 2000; Okabe et al., 2000; Freitelson et al., 2002; Kusano et al., 2002). In addition, a loss on the *M6P/IGF2R* locus and deletion of the $p14^{ARF}$ gene has been found in cirrhosis and dysplastic nodules (Yamada et al., 1997; Schlott et al., 2002).

On the other hand, a CGH study of regenerative nodules of cirrhosis and dysplastic nodules revealed only a few chromosomal aberrations. Three out of six high-grade dysplastic nodules showed loss of 8p and gain of 1q, whereas low-grade dysplastic nodules and regenerative nodules of cirrhosis did not show these aberrations (Tornillo et al., 2002). Wilkens et al. (2002), using nonfluorescent *in situ* hybridization, found that none of the nonmalignant livers they examined contained any aberrant chromosomes. The difference in the frequency of chromosomal aberrations in regenerative nodules between results of microsatellite analyses and those of other methods, may be attributed to the fact that microsatellite analysis can detect more subtle changes than other methods. However, Bluteau et al. (1999) indicated that PCR-based microsatellite genotyping could generate false-positive results with artifacts. In addition, different conditions of sample preservation may affect the efficiency of PCR and cause monoallelic amplification. For example, paraffin-embedded samples, which are widely used in microsatellite analysis of precancerous lesions, contain severely fragmented DNA, which increases the incidence of false-positive detection of AI. Ochiai et al. (2000) analyzed the clonality of precancerous lesions of the liver by using a restriction fragment length polymorphism of an androgen-receptor locus on the X chromosome, and reported that 20% of regenerative nodules of cirrhosis showed a monoclonal pattern. However, they also described that human liver tissue was organized in a mosaic of patches with different X-chromosome inactivation patterns, and that these patches progressively expanded through chronic inflammation. Aoki and Robinson (1989) found only 0.5% of regenerative nodules to be monoclonal with an HBV integration pattern. The monoclonality of regenerative nodules themselves is thus still controversial, so that the assessment of chromosomal aberrations of precancerous lesions needs to be made more accurately.

Several reports suggest that DNA hypermetylation of the CpG island precedes and may cause AI during hepatocarcinogenesis (Kanai et al., 2000; Kondo et al., 2000). Kanai et al. (2000) examined CpG island hypermethylation of the *CDH1* promoter and the allele status at *D16S421*, which is adjacent to the *CDH1* locus. CpG hypermethylation was detected more frequently than AI on the *D16S421* locus in both chronic hepatitis and cirrhosis. Matsumura et al. (2001) also reported that hypermethylation was observed in the early stages of HCC, whereas AI was found in a more advanced stage. Saito et al. (2001) examined the expressions of mRNA of DNA methyltransferases (DNMTs) and DNA methylation status in the same series of HCC. Overexpression of DNMT1 and DNMT3a, hypermethylation of the CpG island of several genes and DNA hypomethylation on pericentromeric satellite regions were detected in chronic hepatitis and liver cirrhosis, suggesting that these phenomena were early events in HCC formation. They also found that there was no significant correlation between the DNMT expression level and DNA methylation status. On the other hand, Lin et al. (2001) reported that the degree of DNA methylation reduction was related to late histological HCC grade and large tumor size. This led them to speculate that genome-wide hypomethylation in HCC was a continuing process that persists throughout tumor growth rather than a historical event occurring only in the precancerous stage, and that overexpression of DNMT might simply be a result of increased cell proliferation. Although the contribution of overexpression of DNMTs to CpG methylation in HCC and the role of genome-wide hypomethylation in hepatocarcinogenesis remain unclear, a recent study demonstrated an association between overexpression of a DNMT3b splice variant, and pericentromeric hypomethylation and aberrant 1q in HCC (Saito et al., 2002).

Molecular mechanism of chromosomal instability in HCC

AI is detected at almost every stage of HCC tissues as well as cell-lines derived from HCC, but the molecular basis of CIN is poorly understood. It is known that CIN can be caused by alterations of molecules which are involved in chromosome condensation, sister-chromatid cohesion, kinetochore structure and function and centrosome/microtubule formation and dynamics, as well as of checkpoint genes that monitor the proper progression of cell cycles (Lengauer et al., 1998). Saeki et al. (2002), using spindle-disrupting agents such as nocodazole and colcemid, reported that five out of the eight HCC cell lines showed an impaired spindle

assembly checkpoint. However, no alterations of mitotic checkpoint genes were found in these cell lines or HCC specimens. On the other hand, failure of the DNA-damage checkpoint is often associated with enhanced mitotic recombination as well as with aberrant chromosome segregation, and several genes involved in DNA-damage have been implicated in human cancer, such as the *BRCA1*, *BRCA2* and *p53* genes (Lengauer et al., 1998). Artandi et al. (2000) found that mice lacking a functional telomerase and p53 developed epithelial cancers with CIN. In these mice, the loss of DNA damage sensors (p53) may allow the abnormal chromosomes to persist, and the cells with such chromosomes to survive and acquire mutations that enhance the potency of the tumor. However, CIN seems to appear early during HCC formation, whereas *p53* mutation does not usually occur until much later. Therefore, although *p53* mutation may exacerbate CIN, it is unlikely to be its primary cause.

Methylation of CpG dinucleotides functions to maintain the stability of chromosome structures while satellite DNA in the heterochromatin is particularly heavily methylated at the CpG island. Wong et al. (2001) identified a strong correlation between hypomethylation of satellite 2 DNA in 1q12 and gain of 1q with a 1q12 breakpoint in human HCC and postulated that hypomethylation altered the interaction between the satellite DNA and chromatin protein, resulting in heterochromatin decondensation, breakage and aberrant 1q formation. Recently, Saito et al. (2002) found that overexpression of DNMT3b4, a splice variant of DNMT3b lacking conserved methyltransferase motifs, significantly correlated with DNA hypomethylation in pericentromeric satellite regions in chronic hepatitis/cirrhosis and HCC. In addition, transfection of human epithelial cells with DNMT3b4 cDNA induced DNA demethylation on satellite 2. These results suggest that overexpression of DNMT3b4 lacking DNA methyltransferse activity results in DNA hypomethylation on satellite 2 in heterochromatin DNA and induces the CIN of chromosome 1 in hepatocytes.

Conclusion

CIN may induce genetic aberration and influence the expression of many cancer-related genes. In consequence, some hepatocytes will acquire immortality, growth advantage and invasiveness. However, identification of cancer-related genes, which are really affected by such chromosomal change and play an important role for hepatocarcinogenesis, may need finer allelotype analysis with a larger number of samples. Advance of such analyses will contribute to the development of new molecular markers that predict behavior of cancer cells and find new therapeutic molecules of HCC.

References

Aoki N. and Robinson W.S. (1989). State of hepatitis B viral genomes in cirrhotic and hepatocellular carcinoma nodules. Mol. Biol. Med. 6, 395-408.

Artandi S.E., Chang S., Lee S.-L., Alson S., Gottlieb G.J., Chin L. and De Pinho R.A. (2000). Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 403, 641-645.

Azechi H., Nishida N., Fukuda Y., Nishimura T., Minata M., Katsuma H., Kuno M., Ito T., Komeda T., Kita R., Takahashi R. and Nakao K. (2001). Disruption of the p16/*cyclin D1*/retinoblastoma protein pathway in the majority of human hepatocellular carcinomas. Oncology 60, 346-354.

Balsara B.R., Pei J., De Rienzo A., Simon D., Tosolini A., Lu Y.Y., Shen F.-M., Fan X., Lin W.Y., Buetow K.H., London W.T. and Testa J.R. (2001). Human hepatocellular carcinoma is characterized by a highly consistent pattern of genomic imbalances, including frequent loss of 16q23.1-24.1. Genes Chromosomes Cancer 30, 245-253.

Bando K., Nagai H., Matsumoto S., Koyama M., Kawamura N., Onda M. and Emi M. (1999). Identification of a 1-cM region of common deletion on 4p35 associated with progression of hepatocellular carcinoma. Genes Chromosomes Cancer 25, 284-289.

Bando K., Nagai H., Matsumoto S., Koyama M., Kawamura N., Tajiri T., Onda M. and Emi M. (2000). Identification of a 1-Mb common region at 16q24.1-24.2 deleted in hepatocellular carcinoma. Genes Chromosomes cancer 28, 38-44.

Bluteau O., Legoix P., Laurent-Puig P. and Zucman-Rossi J. (1999). PCR-Based genotyping can generate artifacts in LOH analyses. Biotechniques 27, 1100-1102.

Bluteau O., Beaudoin J.-C., Pasturaud P., Belghiti J., Franco D., Bioulac-Sage P., Laurent-Puig P. and Zucman-Rossi J. (2002). Specific association between alcohol intake, high grade of differentiation and 4q34-q35 deletions in hepatocelllular carcinomas identified by high resolution allelotyping. Oncogene 21, 1225-1232.

Boige V., Laurent-Puig P., Fouchet P., Flejou J.F., Monges G., Bedossa P., Bioulac-Sage P., Capron F., Schmitz A., Olschwang S. and Thomas G. (1997). Concerted nonsyntenic allelic losses in hyperploid hepatocellular carcinoma as determined by a high-resolution allelotype. Cancer Res. 57, 1986-1990.

Bonura C., Paterlini-Brechot P. and Brechot C. (1999). Structure and expression of Tg737, a putative tumor suppressor gene, in human hepatocellular carcinomas. Hepatology 30, 677-681.

Cacciola L., Pollicino T., Squadrito G., Cerenzia G., Orlando M.E. and Raimondo G. (1999). Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. N. Engl. J. Med. 341, 22-26.

Chen T.-C., Hsieh L.-L., Ng K.-F., Jeng L.-B. and Chen M.-F. (1998). Absence of APC gene mutation in the mutation cluster region in hepatocellular carcinoma. Cancer lett. 134, 23-28.

Chen Y.-J., Yeh S.-H., Chen J.-T., Wu C.-C., Hsu M.-T., Tsai S.-F., Chen P.-J. and Lin C.-H. (2000). Chromosomal changes and clonality relationship between primary and recurrent hepatocellular carcinoma. Gastroenterology 119, 431-440.

De Souza A.T., Hankins G.R., Washington M.K., Orton T.C. and Jirtle R.I. (1995). M6P/IGF2R gene is mutated in human hepatocellular carcinomas with loss of heterozygosity. Nat. Genet. 11, 447-449.

Du Y., Carling T., Fang W., Piao Z., Sheu J.C. and Huang S. (2001). Hypermethylation in human cancers of the RIZ1 tumor suppressor gene, a member of a histone/protein methyltransferase superfamily. Cancer Res. 61, 8094-8099.

Fang W., Piao Z., Simon D., Sheu J.C. and Huang S. (2000). Mappping of a minimal deleted region in human hepatocellular carcinoma to 1p36.13-p36.23 and mutational analysis of the RIZ(PRDM2) gene

localized to the region. Genes Chromosomes Cancer 28, 269-275.

Feitelson M.A., Sun B., Tufan N.L.S., Liu J., Pan J. and Lian Z. (2002). Genetic mechanisms of hepatocarcinogenesis, Oncogene 21, 2593-2604.

Fujimori M., Tokino T., Hino O., Kitagawa T., Imamura T., Okamoto E., Mitsunobu M., Ishikawa T., Nakagama H., Harada H., Yagura M., Matsubara K. and Nakamura Y. (1991). Allelotype study of primary hepatocellular carcinoma. Cancer Res. 51, 89-93.

Fujiwara Y., Hoon D.S., Yamada T., Umeshita K., Gotoh M., Sakon M., Nishisho I. and Monden M. (2000). PTEN/MMAC1 mutation and frequent loss of heterozygosity identified in chromosome 10q in a subset of hepatocellular carcinomas. Jpn. J. Cancer Res. 91, 287-292.

Furuta K., Misao S., Takahashi K., Tagaya T., Fukuzawa Y., Ishikawa T., Yoshioka K. and Kakumu S. (1999). Gene mutation of transforming growth factor ß1 type II receptor in hepatocellular carcinoma. Int. J. Cancer 81, 851-853.

Guan X.-Y., Fang Y., Sham J.S.T., Kwong D.L.W., Zhang Y., Liang Q., Li H., Zhou H. and Trent J.M. (2000). Recurrent chromosome alterations in hepatocellular carcinoma detected by comparative genomic hybrization. Genes Chromosomes Cancer 29, 110-116.

Guo X.-Z., Freiss H., Di Mola F.F., Heinicke J.-M., Abou-Shady M., Graber H.U., Baer H.U., Zimmermann A., Korc M. and Büchler M. (1998). KAI1, a new metastasis suppressor gene, is reduced in metastatic hepatocellular carcinoma. Hepatology 28, 1481-1488.

Higashitsuji H., Itoh K., Nagao T., Dawson S., Nonoguchi K., Kido T., Mayer R.J., Arii S. and Fujita J. (2000). Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. Nat. Med. 6, 96-99.

Iwata N., Yamamoto H., Sasaki S., Itoh F., Suzuki H., Kikuchi T., Kaneto H., Iku S., Ozeki I., Karino Y., Satoh T., Toyota J., Satoh M., Endo T. and Imai K. (2000). Frequent hypermethylation of CpG island and loss of expression of the 14-3δ gene in human hepatocellular carcinoma. Oncogene 19, 5298-5302.

Jiang G., Liu L., Buyse I.M., Simon D. and Huang S. (1999). Decreased RIZ1 expressoin but not RIZ2 in hepatoma and suppressoin of hepatoma tumorigenicity by RIZ1. Int. J. Cancer 83, 541-546.

Kanai Y., Ushijima S., Hui A.-M., Ochiai A., Tsuda H., Sakamoto M. and Hirohashi S. (1997). The E-cadherin gene is silenced by CpG methylation in human hepatocellular carcinoma. Int. J. Cancer 71, 355-359.

Kanai Y., Hui A.-M., Sun L., Ushijima S., Sakamoto M., Tsuda H. and Hirohashi S. (1999). DNA hypermethylation at the D17S5 locus and reduced HIC-1 mRNA expression are associated with hepatocarcinogenesis. Hepatology 29, 703-709.

Kanai Y., Ushijima S., Tsuda H., Sakamoto M. and Hirohashi S. (2000). Aberrant DNA methylation precedes loss of heterozygosity on chromosome 16 in chronic hepatitis and liver cirrhosis. Cancer Lett. 148, 73-80.

Katagiri T., Nakamura Y. and Miki Y. (1996). Mutations in the BRCA2 gene in hepatocellular carcinomas. Cancer Res. 56, 4575-4577.

Kawai H., Suda T., Aoyagi Y., Isokawa O., Mita Y., Waguri N., Kuroiwa T., Igarashi M., Tsukada K., Mori S., Shimizu T., Suzuki Y., Abe Y., Takahashi T., Nomoto M. and Asakura H. (2000). Quantitative evaluation of genomic instability as a possible predictor for development of hepatocelular carcinoma: Comparison of loss of heterozygosity and replication error. Hepatology 31, 1246-1250.

Kawate S., Fukusato T., Ohwada S., Watanuki A. and Morishita Y. (1999). Amplificcation of c-myc in hepatocellular carcinoma: Correlation with clinicopathologic features, proliferative activity and p53 overexpression. Oncology 57, 157-163.

Kita R., Nishida N., Fukuda Y., Azechi H., Matsuoka Y., Komeda T., Sando T., Nakao K. and Ishizaki K. (1996). Infrequent alterations of the $p16^{INK4A}$ gene in liver cancer. Int. J. Cancer 67, 176-180.

Kondo Y., Kanai Y., Sakamoto M., Mizokami M., Ueda R. and Hirohashi S. (2000). Genetic instability and aberrant DNA methylation in chronic hepatitis and cirrhosis – a comprehensive study of loss of heterozygosity and microsatellite instability at 39 loci and DNA hypermethylation on 8 CpG islands in microdissected specimens from patients with hepatocellular carcinoma. Hepatology 32, 970-979.

Koyama M., Nagai H., Bando K., Ito M., Moriyama Y. and Emi M. (1999). Localization of a target region of allelic loss to a 1-cM interval on chromosome 16p.13.13 in hepatocellular carcinoma. Jpn. J. Cancer Res. 90, 951-956.

Koyama M., Nagai H., Bando K., Matsumoto S., Tajiri T., Moriyama Y. and Emi M. (2000). New target region of allelic loss in hepatocellular carcinomas within a 1-cM interval on chromosome 6p23. J. Hepatol. 33, 85-90.

Kuramochi M., Fukuhara H., Nobukuni T., Kanbe T., Maruyama T., Ghosh H.P., Pletcer M., Isomura M., Onizuka M, Kitamura T., Sekiya T., Reeves R.H. and Murakami Y. (2001). TSLC1 is a tumor-suppressor gene in human non-small-cell lung cancer. Nat. Genet. 27, 427-430.

Kuroki T., Fujiwara Y., Tsuchiya E., Nakamori S., Imaoka S., Kanematsu T. and Nakamura Y. (1995a). Accumulation of genetic changes during development and progression of hepatocellular carcinoma: Loss of heterozygosity on chromosome arm 1p occurs at an early stage of hepatocarcinogenesis. Genes Chromosomes Cancer 13, 163-167.

Kuroki T., Fujiwara S., Nakamori S., Imaoka S., Kanematsu T. and Nakamura Y. (1995b). Evidence for the presence of two tumour-suppressor genes for hepatocellular carcinoma on chromosome 13q. Br. J. Cancer 72, 383-385.

Kusano N., Shiraishi K., Kubo K., Oga A., Okita K. and Sasali K. (1999). Genetic aberrations detected by comparative genomic hybridization in hepatocellular carcinomas: Their relationship to clinicopathological features. Hepatology 29, 1858-1862.

Kusano N., Okita K., Shirahashi H., Harada T., Shiraishi K., Oga A., Kawauchi S., Furuya T. and Sasaki K. (2002). Chromosomal imbalances detected by comparative genomic hybridization are associated with outcome of patients with hepatocellular carcinoma. Cancer 94, 746-751.

Laurent-Puig P., Legoix P., Bluteau O., Belghiti J., Franco D., Binot F., Monges G., Thomas G., Bioulac-Sage P. and Zucman-Rossi J. (2001). Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 120, 1763-1773.

Lengauer C., Kinzler K.W. and Vogelstein B. (1998). Genetic instabilities in human cancers. Nature 396, 643-649.

Liao C., Zhao M., Song H., Uchida K., Yokoyama K.K. and Li T. (2000). Identification of the gene for a novel liver-related putative tumor suppressor at a high-frequency loss of heterozygosity region of chromosome 8p23 in human hepatocellular carcinoma. Hepatology 32, 721-727.

Lin C.-H., Hsieh S.-Y., Sheen I.-S., Lee W.-C., Chen T.-C., Shyu W.-C. and Liaw Y.-F. (2001). Genome-wide hypomethylation in hepatocellular carcinogenesis. Cancer Res. 61, 4238-4243.

Liew C.T., Li H.M., Lo K.W., Leow C.K., Lau W.Y., Hin L.Y., Lim B.K., Lai P.B.S., Chan J.Y.H., Wang X.-Q., Wu S. and Lee J.C.K. (1999). Frequent allelic loss on chromosome 9 in hepatocellular carcinoma. Int. J. Cancer 81, 319-324.

Maggioni M., Coggi G., Cassani B., Bianchi P., Romagnoli S., Mandelli A., Borzio M., Colombo P. and Roncalli M. (2000). Molecular changes in hepatocellular dysplastic nodules on microdissected liver biopsies. Hepatology 32, 942-946.

Marchio A., Meddeb M., Pineau P., Danglot G., Tiollais P., Bernheim A. and Dejean A. (1997). Recurrent chromosomal abnormalities in hepatocellular carcinoma detected by comparative genomic hybridization. Genes Chromosomes Cancer 18, 59-65.

Marchio A., Pineau P., Meddeb M., Terris B., Tiollais P., Bernheim A., Dejean A. (2000). Distinct chromosomal abnormality pattern in primary liver cancer of non-B, non-C patients. Oncogene 19, 3733-3738.

Matsuda Y., Ichida T., Matsuzawa J., Sugimura K. and Asakura H. (1999). P16I^{NK4A} is inactivated by extensive CpG methylation in human hepatocellular carcinoma. Gastroenterology 116, 394-400.

Matsumura T., Makino R. and Mitamura K. (2001). Frequent down-regulation of E-cadherin by genetic and epigenetic changes in the malignant progression of hepatocellular carcinoma. Clin. Cancer Res. 7, 594-599.

Mihara M., Nimura Y., Ichimiya S., Sakiyama S., Kajikawa S., Adachi W., Amano J. and Nakagawara A. (1999). Absence of mutation of the p73 gene localized at chromosome 1p36.3 in hepatocellular carcinoma. Br. J. Cancer 79, 164-167.

Miyoshi Y., Iwao K., Nagasawa Y., Aihara T., Sasaki Y., Imaoka S., Murata M., Shimano T. and Nakamura Y. (1998). Activation of the ß-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Cancer Res. 58, 2524-2527.

Nagai H., Pineau P., Tiollais P., Buendia M.A., Dejean A. (1997). Comprehensive allelotyping of human hepatocellular carcinoma. Oncogene 14, 2927-2933.

Niketeghad F., Decker H.J., Caselmann W.H., Lund P., Geissler F., Dienes H.P. and Schirmacher P. (2001). Frequent genomic imbalances suggest commonly altered tumour genes in human hepatocarcinogenesis. Br. J. Cancer 85, 697-704.

Nishida N., Fukuda Y., Kokuryu H., Sadamoto T., Isowa G., Honda K., Yamaoka Y., Ikenaga M., Imura H. and Ishizaki K. (1992). Accumulation of allelic loss on arms of chromosomes 13q, 16q and 17p in the advanced stages of human hepatocellular carcinoma. Int. J. Cancer 51, 862-868.

Nishida N., Fukuda Y., Kokuryu H., Toguchida J., Yandell D.W., Ikenaga M., Imura H. and Ishizaki K. (1993). Role and mutational heterogeneity of the p53 gene in hepatocellular carcinoma. Cancer Res. 53, 368-372.

Nishida N., Fukuda Y., Komeda T., Kita R., Sando T., Furukawa M., Amenomori M., Shibagaki I., Nakao K., Ikenaga M and Ishizaki K. (1994). Amplification and overexpressoin of the *cyclin D1* gene in aggressive human hepatocellular carcinoma. Cancer Res. 54, 3107-3110.

Nishida N., Fukuda Y., Komeda T., Ito T., Nishimura T., Minata M., Kuno M., Katsuma H., Ikai I., Yamaoka Y. and Nakao K. (2002). Prognostic impact of multiple allelic losses on metastatic recurrence in hepatocellular carcinoma after curative resection. Oncology 62, 141-148.

Nishimura T., Nishida N., Itoh T., Kuno M., Minata M., Komeda T., Fukuda Y., Ikai I., Yamaoka Y. and Nakao K. (2002). Comprehensive allelotyping of well-differentiated human hepatocellular carcinoma with semiquntitative determination of chromosomal gain or loss. Genes Chromosomes Cancer 35, 329-339.

Ochiai T., Urata Y., Yamano T., Yamagishi H. and Ashihara T. (2000). Clonal expansion in evolution of chronic hepatitis to hepatocellular carcinoma as seen at an X-chromosome locus. Hepatology 31, 615-621.

Ohsawa N., Sakamoto M., Saito T., Kobayashi M. and Hirohashi S. (1996). Numerical chromosome aberrarions in hepatocellular carcinoma detected by fluorescence *in situ* hybridization. J. Hepatol. 25, 655-662.

Oka Y., Waterland R.A., Killian J.K., Nolan C.M., Jang H.-S., Tohara K., Sakaguchi S., Yao T., Iwashita A., Yata Y., Takahara T., Sato S., Suzuki K., Masuda T. and Jirtle R.L. (2002). M6P/IGF2R tumor suppressor gene mutated in hepatocellular carcinomas in Japan. Hepatology 35, 1153-1163.

Okabe H., Ikai I., Matsuo K., Satoh S., Momoi H., Kamikawa T., Katsura N., Nishitai R., Takeyama O., Fukumoto M. and Yamaoka Y. (2000). Comprehensive allelotype study of hepatocellular carcinoma: Potential differences in pathways to hepatocellular carcinoma between hepatitis B virus-positive and -negative tumors. Hepatology 31, 1073-1079.

Piao Z., Park C., Park J.-H. and Kim H. (1998a). Allelotype analysis of hepatocellular carcinoma. Int. J. Cancer 75, 29-33.

Piao Z., Park C., Park J.-H. and Kim H. (1998b). Deletion mapping of chromosome 4q in hepatocellular carcinoma. Int. J. Cancer 79, 356-360.

Piao Z., Park C., Kim J.J. and Kim H. (1999). Deletion mapping of chromosome 16q in hepatocellular carcinoma. Br. J. Cancer 80, 850-854.

Pineau P., Nagai H., Prigent S., Wei Y., Gyapay G., Weissenbach J., Tiollais P., Buendia M.-A. and Dejean A. (1999). Identification of three distinct regions of allelic deletions on the short arm of chromosome 8 in hepatocellular carcinoma. Oncogene 18, 3127-3134.

Qin L.-X., Tang Z.-Y., Sham J.S.T., Ma Z.-C., Ye S.-L., Zhou X.-D., Wu Z.-Q., Trent J.M. and Guan X.-Y. (1999). The association of chromosome 8p deletion and tumor metastasis in human hepatocellular carcinoma. Cancer Res. 59, 5662-5665.

Ricketts S.L., Garcia N.F., Betz B.L. and Coleman W.B. (2002). Identification of candidate liver tumor suppressor genes from human 11p11.2-p12. Genes Chromsomes Cancer 33, 47-59.

Roncalli M., Bianchi P., Grimaldi G.C., Ricci D., Laghi L., Maggioni M., Opocher E., Borzio M. and Coggi G. (2000). Fractional allelic loss in non-end-stage cirrhosis: Correlations with hepatocellular carcinoma development during follow-up. Hepatology 31, 846-850.

Roncalli M., Bianchi P., Bruni B., Laghi L., Destro A., Di Gioia S., Gennari L., Tommasini M., Malesci A. and Coggi G. (2002). Methylation framework of cell cycle inhibitors in cirrhosis and associated hepatocellular carcinoma. Hepatology 36, 427-432.

Saeki A., Tamura S., Ito N., Kiso S., Matsuda Y., Yabuuchi I., Kawata S. and Matsuzawa Y. (2002). Frequent impairment of the spindle assembly checkpoint in heptocellular carcinoma. Cancer 94, 2047-2054.

Saito Y., Kanai Y., Sakamoto M., Saito H., Ishii H. and Hirohashi S. (2001). Expression of mRNA for DNA methyltransferases and methyl-CpG-binding proteins and DNA methylation status on CpG islands and pericentromeric satellite regions during human

hepatocarcinogenesis. Hepatology 33, 561-568.

Saito Y., Kanai Y., Sakamoto M., Saito H., Ishii H. and Hirohashi S. (2002). Overexpression of a splice variant of DNA methyltransferase 3b, DNMT3b4, associated with DNA hypomethylation on pericentromeric satellite regions during human hepatocarcinogenesis. Proc. Natl. Acad. Sci. USA 99, 10060-10065.

Sakakura C., Hagiwara A., Taniguchi H., Yamaguchi T., Yamagishi H., Takahashi T., Koyama K., Nakamura Y., Abe T. and Inazawa J. (1999). Chromosomal aberrations in human hepatocellular carcinomas associated with hepatitis C virus infection detected by comparative genomic hybridization. Br. J. Cancer 80, 2034-2039.

Satoh S., Daigo Y., Furukawa Y., Kato T., Miwa N., Nishiwaki T., Kawasoe T., Ishiguro H., Fujita M., Tokino T., Sasaki Y., Imaoka S., Murata M., Shimano T., Yamaoka Y. and Nakamura Y. (2000). AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat. Genet. 24, 245-250.

Schlott T., Scharf J.G., Gorzel C., Middel P. and Spring H. (2002). Cirrhotic livers reveal genetic changes in the MDM2-P14^{ARF} system of cell cycle regulators. Brit. J. Cancer 86, 1290-1296.

Schwienbacher C., Gramantieri L., Scelfo R., Veronese A., Calin G.A., Bolondi L., Croce C.M., Barbanti-Brodano G. and Negrini M. (2000). Gain of imprinting at chromosome 11p15: A pathogenetic mechanism identified in human hepatocarcinomas. Proc. Natl. Acad. Sci. USA 97, 5445-5449.

Sun M., Eshleman J.R., Ferrell L.D., Jacobs G., Sudilovsky E.C., Tuthill R., Hussein M.R. and Sudilovsky O. (2001). An early lesion in hepatic carcinogenesis: Loss of heterozygosity in human cirrhotic livers and dysplastic nodules at the 1p36-p34 region. Hepatology 33, 1415-1424.

Takeda S., Kondo M., Kumada T., Koshikawa T., Ueda R., Nishio M., Osada H., Suzuki H., Nagatake M., Washimi O., Takagi K., Takahashi T., Nakao A. and Takahashi T. (1996). Allelic-expression imbalance of the insulin-like growth factor 2 gene in hepatocellular carcinomas and underlying disease. Oncogene 12, 1589-1592.

Tannapfel A., Wasner M., Krause K., Geissler F., Katalinic A., Hauss J., Mossner J., Engeland K. and Wittekind C. (1999). Expression of p73 and its relation to histopathology and prognosis in hepatocellular carcionoma. J. Natl. Cancer Inst. 91, 1154-1158.

Tannapfel A., Busse C., Weinans L., Benicke M., Katalinic A., Geiber F., Hauss J. and Wittekind C. (2001). INK4a-^{ARF} alterations and p53 mutations in hepatocellular carcinomas. Oncogene 20, 7104-7109.

Thorgeirsson S.S. and Grisham J.W. (2002). Molecular pathogenesis of human hepatocellular carcinoma. Nat. Genet. 31, 339-346.

Tornillo L., Carafa V., Richter J., Sauter G., Moch H., Minola E., Gambacorta M., Bianchi L., Vecchione R. and Terracciano L.M. (2000). Marked genetic similarities between hepatitis B virus-positive and hepatitis C virus-positive hepatocellular carcinomas. J. Pathol. 192, 307-312.

Tornillo L., Carafa V., Sauter G., Moch H., Minola E., Gambacorta M., Vecchione R., Bianchi L. and Terracciano L.M. (2002). Chromosomal alterations in hepatocellular nodules by comparative genomic hybridization: High-grade dysplastic nodules represent early stages of hepatocellular carcionoma. Lab. Invest. 82, 547-553.

Tsuda H., Zhang W., Shimosato Y., Yokota J., Terada M., Sugimura T., Miyamura T. and Hirohashi S. (1990). Allele loss on chromosome 16 associated with progression of human hepatocellular carcionoma. Proc. Natl. Acad. Sci. USA 87, 6791-6794.

Tsuda H., Oda T., Sakamoto M. and Hirohashi S. (1992). Different pattern of chromosomal allele loss in multiple hepatocellular carcinomas as evidence of their multifocal origin. Cancer Res. 52, 1504-1509.

Virtaneva K., Wright F.A., Tanner S.M., Yuan B., Lemon W.J., Caligiuri M.A., Bloomfield C.D., de La Chapelle A. and Krahe R. (2001). Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. Proc. Natl. Acad. Sci. USA 98, 1124-1129.

Vogelstein B., Fearon E.R., Kern S.E., Hamilton S.R., Presinger A.C., Nakamura Y. and White R. (1989). Allelotype of colorectal carcinomas. Science 244, 207-211.

Wang G., Zhao Y., Liu X., Wang L., Wu C., Zhang W., Liu W., Zhang P., Cong W., Zhu Y., Zhang L., Chen S., Wan D., Zhao X., Huang W. and Gu J. (2001). Allelic loss and gain, but not genomic instability, as the major somatic mutation in primary hepatocellular carcinoma. Genes Chromosomes Cancer 31, 221-227.

Wei Y., Van Nhieu J.T., Prigent S., Srivatanakul P., Tiollais P. and Buendia M.-A. (2002). Altered expression of E-cadherin in hepatocellular carcinoma: Correlations with genetic alterations, ß-catenin expression, and clinical features. Hepatology 36, 692-701.

Wilkens L., Bredt M., Flemming A., Mengel M., Klempnauer J., Kreipe H. and Flemming P. (2002). Detection of chromosomal aberrations in well-differentiated hepatocellular carcinoma by bright-field *in situ* hybridization. Mod. Pathol. 15, 470-475.

Woitach J.T., Zhang M., Niu C.-H. and Thorgeirsson S.S. (1998). A retinoblastoma-binding protein that affects cell-cycle control and confers transforming ability. Nat. Genet. 19, 371-374.

Wong N., Lai P., Lee S.-W., Fan S., Pang E., Liew C.-T., Sheng Z., Lau J.W.-Y. and Johnson P.J. (1999). Assessment of genetic changes in hepatocellular carcinoma by comparative genomic hybridization analysis; Relationship to disease stage, tumor size, and cirrhosis. Am. J. Pathol. 154, 37-43.

Wong I.H.N., Lo Y.M.D., Yeo W., Lau W.Y. and Johnson P.J. (2000). Frequent p15 promoter methylation in tumor and peripheral blood from hepatocellular carcinoma patients. Clin. Cancer Res. 6, 3516-3521.

Wong N., Lai P., Pang E., Fung L.-F., Sheng Z., Wong V., Wang W., Hayashi Y., Perlman E., Yuna S., Lau J.W.-Y. and Johnson P.J. (2000). Genomic aberrations in human hepatocellular carcinomas of differing etiologies. Clin. Cancer Res 6, 4000-4009.

Wong N., Lam W.-C., Lai P.B.-S., Pang E., Lau W.-Y. and Johnson P.J. (2001). Hypomethylation of chromosome 1 heterochromatin DNA correlates with q-arm copy gain in human heptocellular carcinoma. Am. J. pathol. 159, 465-471.

Yakicier M.C., Irmak M.B., Romano A., Kew M. and Ozturk M. (1999). Smad2 and Smad4 gene mutations in hepatocellular carcinoma. Oncogene 18, 4879-4883.

Yakicier M.C., Legoix P., Vaury C., Gressin L., Tubacher E., Capron F., Bayer J., Degott C., Balabaud C. and Zucman-rossi J. (2001). Identification of homozygous deletions at chromosome 16p23 in aflatoxin B1 exposed hepatocellular carcinoma. Oncogene 20, 5232-5238.

Yamada T., De Souza A.T., Finkelstein S. and Jirtle R.L. (1997). Loss of the gene encoding mannose 6-phosphate/insulin-like growth factor II receptor is an early event in liver carcinogenesis. Proc. Natl. Acad. Sci. USA 94, 10351-10355.

Yasui K., Arii S., Zhao C., Imoto I., Ueda M., Nagai H., Emi M. and Inazawa J. (2002). TFDP1, CUL4A, and CDC16 identified as targets for amplification at 13q34 in hepatocellular carcinomas. Hepatology

35, 1476-1484.

Yeh S.-H., Chen P.-J., Chen H.-L., Lai M.-Y., Wang C.-C. and Chen D.-S. (1994). Frequent genetic alterations at the distal region of chromosome 1p in human hepatocellular carcinomas. Cancer Res. 54, 4188-4192.

Yeh S.-H., Chen P.-J., Shau W.-Y., Chen Y.-W., Lee P.-H. Chen J.-T. and Chen D.-S. (2001). Chromosomal allelic imbalance evolving from liver cirrhosis to hepatocellular carcinoma. Gastroenterology 121, 699-709.

Yoshikawa H., Matsubara K., Qian G.S., Jackson P., Groopman J.D., Manning J.E., Harris C.C. and Herman J.G. (2001). SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellar carcinoma and shows growth-suppression activity. Nat. Genet. 28, 29-35.

Yuan B.-Z., Miller M.J., Keck C.L., Zimonjic D.B., Thorgeirsson S.S. and Popescu N.C. (1998). Cloning, characterization, and chromosomal localization of a gene frequently deleted in human liver cancer (DLC-1) homologous to rat RhoGAP. Cancer Res. 58, 2196-2199.

Zhang X., Xu H.-J., Murakami Y., Sachse R., Yashima K., Hirohashi S., Hu S.-X., Benedict W.F. and Sekiya T. (1994). Deletions of chromosome 13q, mutations in retinoblastoma 1, and retinoblastoma protein state in human hepatocellular carcinoma. Cancer Res. 54, 4177-4182.

Zhang Y.-J., Ahsan H., Chen Y., Lunn R.M., Wang L.-Y., Chen S.-Y., Lee P.-H., Chen C.-J. and Santella R.M. (2002). High frequency of promoter hypermethylation of RASSF1A and p16 and its relationship to aflatoxin B1-DNA adduct levels in human hepatocellular carcinoma. Mol. Carcinog. 35, 85-92.

Zhao X., Li J., He Y., Lan F., Fu L., Guo J., Zhao R., Ye Y., He M., Chong W., Chen J., Zhang L., Yang N., Xu B., Wu M., Wan D. and Gu J.R. (2001). A novel growth suppressor gene on chromosame 17p13.3 with a high frequency of mutation in human hepatocellular carcinoma. Cancer Res. 61, 7383-7387.

Zhou W., Goodman S.N., Galizia G., Lieto E., Ferraraccio F., Pignatelli C., Purdie C.A., Piris J., Morris R., Harrison D.J., Paty P.B., Culliford A., Romans K.E., Montgomery E.A., Choti M.A., Kinzler K.W. and Vogelstein B. (2002). Couting alleles to predict recurrence of early-stage colorectal cancers. Lancet 359, 219-225.

Zimmermann U., Feneux D., Mathey G., Gayral F., Franco D. and Bedossa P. (1997). Chromosomal aberrations in hepatocellular carcinomas: Relationship with pathological features. Hepatology 26, 1492-1498.

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