The Influence of Chromosomal Amplification and Deletion on Clinical Characteristics and Prognosis in Patients with Hepatocellular Carcinoma

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The Influence of Chromosomal Amplification and Deletion on Clinical Characteristics and Prognosis in Patients with Hepatocellular Carcinoma

Directed by Professor Byong Ro Kim

The Doctoral Dissertation
Submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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June 2005

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June 2005

Acknowledgements

The author thanks Professor Tae Yeon Jang of Chon buk National University for editorial assistance in English. Also, thanks Professor Woo Jung Lee for statistical analysis and Miss. Jan Dy Lee for medical record review. Professor Hoguen Kim teach CGH to me, help me with a many aspects. I am indebted to director professor Byong Ro Kim for his advice and criticisms during the preperation of this manuscription. Finally, thanks for my parents who give life to me into this world.

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ABSTRACT

The Influence of Chromosomal Amplification and Deletion on Clinical Characteristics and Prognosis in Patients with Hepatocellular Carcinoma

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Hepatocellular carcinoma is a very common and highly malignant tumor, associated mainly with chronic viral hepatitis, cirrhosis of any cause, aflatoxin exposure and ethanol consumption. The study examines chromosomal changes of 37 fresh hepatocellular carcinomas (HCCs) by comparative genomic hybridization (CGH) analysis and analyze the correlation between genetic alteration and prognostic factors. By CGH analysis, frequent chromosomal losses are noted in the chromosomal region of 1p (45.9%), 4q (32.4%), 8p (56.7%), 16p (51.3%), and 16q (54.3%), whereas gains are noted in 1q (67.5%) and 8q (62.1%). The most important genetic alteration impact on 5-year overall survival is 16q ($p<.03$). When it is analyzed for 16q combined with various prognostic factors, α-fetoprotein (AFP) ($p<.028$), tumor size ($p<.037$) and indocyanine green test (ICG_{15min})> 10% ($p<.004$) are significant prognostic factors statistically. Also, it is found that 16p deletion with ICG_{15min} ($p<.049$), 13q deletion with vascular invasion ($p<.022$) and 4q with AFP are significant. As a conclusion, a high frequency of chromosomal arm loss in HCC by CGH analysis are 8p (56.7%), 16q (54.0%), 16p (51.3%), and 1p (45.9%). A high frequency of allelic gain are found on chromosomes 1q (67.5%) and 8q (62.1%). The most important factor in prognosis of hepatocellular carcinoma is a loss of 16q. Losses of 4q and 16q might play important roles in elevation of AFP level. Also, there are poor liver function in case of the losses of 16p, 16q patients group. Otherwise, losses of 16q is concerned with tumor size. Especially, losses of 13q is correlated with vascular invasion and is necessary for the metastais of HCCs. The deletion of 16q, 16p, 13q & 4q can be applied to therapeutic plan on HCC and related to tumor progression and invasiveness of HCC.

Key Words: heptocellular carcinoma, prognosis, comparative genomic hybridization

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I. INTRODUCTION

Hepatocellular carcinoma is one of the most common human malignant tumors, especially in southern and eastern Asia.[1] HCC is the second leading cause of death from cancer in Korea. It is widely accepted that hepatitis B (HBV) or C virus (HCV) infection, subsequent chronic inflammation and hepatocyte regeneration play important roles in the development of HCCs.[2~4] However, the effect of viral infection on hepatocellular transformation remains to be undetermined. Accordingly, it remains unknown whether carcinogenetic process is different in HBV-positive and HCV-postitive livers. HCCs, like many other tumors, is considered to develop and progress as a consequence of an accumulation of genetic alterations.[5] Many investigators have made varying attempts to find genes implicated in hepatocarcinogenesis to construct a genetic pathway in the progression of HCC.[6~9] Although frequent allelic losses at loci of chromosomes 1p, 4q, 5p, 5q, 8p, 10p, 11q, 13q, 16q, and 17p have been researched in HCCs,[10,11] most of these studies fail to provide consistent information concerning genetic changes leading to the evolution of HCCs. Comprehensive analysis is necessary to throughly understand the complicated genetic alterations in malignant tumors. Determination of these comprehensive genetic changes in solid tumors is practically difficult, because the examination of many individual genes by conventional method is laborious and cumbersome. Screening for chromosomal regions with frequent gains and losses is one of the steps toward the identification of genes implicated in the development and progression of tumors. Although karyotyping provides comprehensive information concerning structural aberrations of whole chromosomes, it is highly specialized work and time-concerning even for experienced technicians. Moreover, it is difficult to prepare metaphase spreads from solid tumors such as HCCs. In fact, there are no large scale genetic studies on HCCs as far as we know. Fortunately, comparative genomic hybridization (CGH), a recently developed technology, allows a global analysis

of chromosomal imbalances which may be etiologically relevant or of diagnostic and prognostic importance.

At present, however, there is no available information concerning the relationship between genetic alterations and clinicopathologic characteristics in HCCs. In this study, we wish to know which genetic alternation related with prognostic factors.

II. MATERIAL & METHODS

1. Tumor specimen

Thirty-seven surgically resected HCCs were included for this study. The cases were identified prospectively and consecutively at Yonsei University Severance Hospital between January 1996 and December 2002 for a study of molecular markers for HCCs. Patient information was obtained prospectively without any knowledge of genetic alterations. The macroscopic and microscopic features of the resected specimens are reviewed by an experienced liver pathologist who confirms the diagnosis of HCCs, assesses the presence or absence of vascular invasion, and records the maximal diameter of the tumor. The presence or absence of cirrhosis in the nontumorous part of the resected specimen is also recorded. Cirrhosis is defined as the presence of complete fibrous septa seperating regeneration nodules.[12] Grading of differentiation is performed according to Edmondson and Steiner.[13] According to this classification, 20 HCCs are categorized as well differentiated, 8 HCCs are moderate differentiated, and 9 HCCs are poorly differentiated. Among the 37 HCC patients, 33 (89%) had liver cirrhosis in the non-neoplastic liver. Serum hepatitis B surface antigen is positive in 31 patients (84%) and anti-hepatitis C virus antibody is positive in 2 patients (5%). The selected tissues are stored at -70^0C until DNA extraction is performed. Each tisssue is microdissected in a cryostat to separate the tumor cells from adjacent non-neoplastic tissues. Genomic DNA is performed by the Sodium dodecyl sulphate-proteinase K and phenol-chloroform extraction method.[14]

2. Comparative genomic hybridization analysis

Genomic DNA samples from tumors are labeled with Spectrum Green deoxyuridine triphosphate (dUTP) (Vysis Inc., Downers Grove, IL), and normal reference genomic DNA was labeled with Spectrum Red dUTP (Vysis) using the nick translation technique. Labeled tumor and reference DNA (200~400ng), as well as 10 μg of unlabeled human cobalt uptake protein (Cot-1 DNA) (Vysis) are dissolved in 10μl of hybridization buffer (50% formamide, 10% dextran sulfate, and 2x standard saline citrate) and denatured at 37^0C on denatured normal metaphase spreads. After hybridization for 3 days, the slides

are washed and counterstained with 4',6-diamino-2-phenylindole dihydrocholoride (DAPI) in antifade solution. CGH hybridizations were analyzed using an Olympus fluorescent microscope and the Cytovision image analysis system (Applied Imaging, Sunderland, Tyne & Wear, UK). Three digital images (DAPI, Spectrum Green, and Spectrum Red) were acquired from 10 to 20 metaphases in each hybridization. DNA of normal male and DNA from tumor cell lines with known aberrations are used as control test DNA. Green-to-red intensity ratio profiles are calculated for each chromosome and threshold values defining gains and losses are set at 1.25 and 0.75, respectively. High level increase in copy number (amplicon) is defined as a ratio of tumor/reference greater than 1.5. Schematic diagraphic discription is shown in Figure 1.

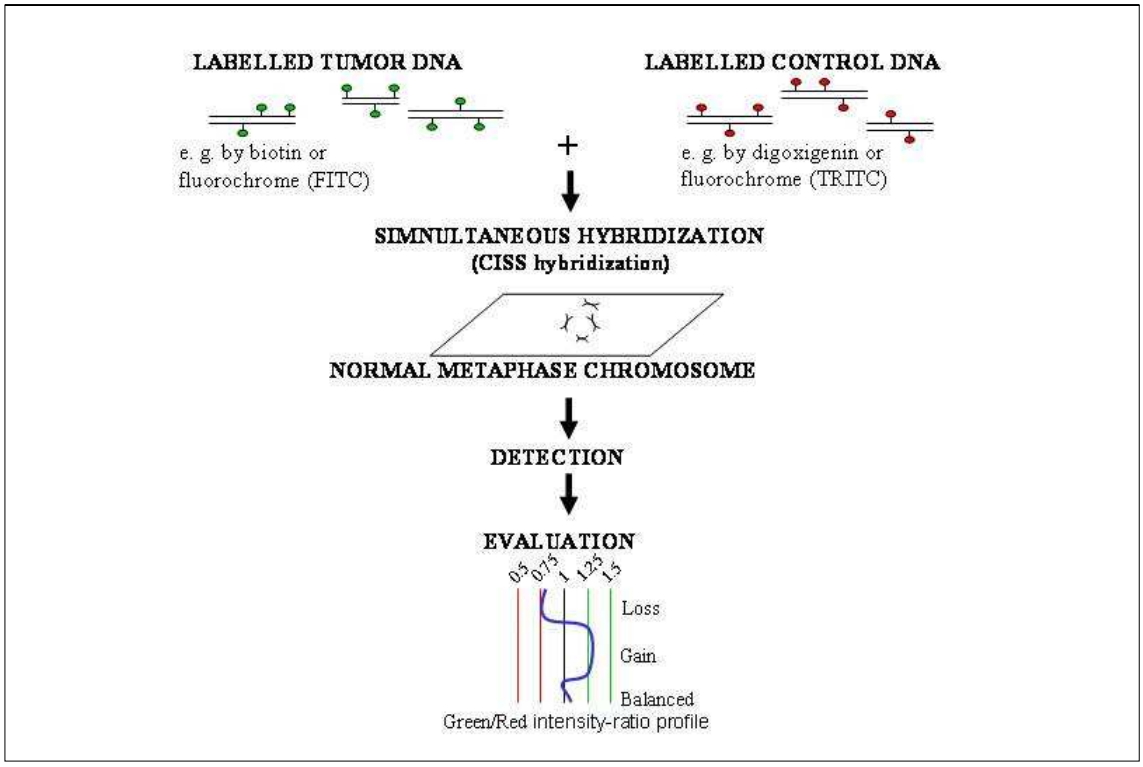

Figure 1. Schematic diagram of comparative genomic hybridization method.

3. Analysis of patient's clinicopathologic features & Statistical Analysis

We review clinical recording chart of 37 patients as follows; HBV infection, AFP level (preoperation), ICG_{15min}, tumor size, vascular invasion, and tumor stage. Statistical analysis is performed using SPSS package version 10.0 statistical software, USA. The Kaplan-Meier method is used to calculate survival rates and log-rank test is used to analyze differences. Total DNA copy number aberrations, whether gains or losses, differences in proportions between the groups are analyzed the chi-square test. For all statistical tests, a probability p value of less than 0.05 is considered significant.

III. RESULTS

This study included 37 patients of 31 males and 6 females, whose average age is 51 years (range 16~66). Average follow-up period is 51 months. Serum AFP levels ranged from less than 10 ng/ml(within reference range) in 21 patients(56.8%). The disease stage of the HCCs is classified according to modified Union International Contre le Cancer (UICC)[15]. One case (3%) is stage I ($T_1N_0M_0$), 16 (45%) as stage II ($T_2N_0M_0$), 11 (30%) as stage III ($T_3N_0M_0$), 8 (22%) as stage IV ($T_{1\sim4}N_0M_1$ or $T_4N_0M_0$). Table 1 summarizes the patients' characteristics, histopathologic differentiation, tumor size, vascular invasion, AFP, ICG_{15min}, Stage and CGH results. Fluorescence photomicrograph of hepatocellular carcinoma of case 1 of CGH analysis is shown in Figure 2.

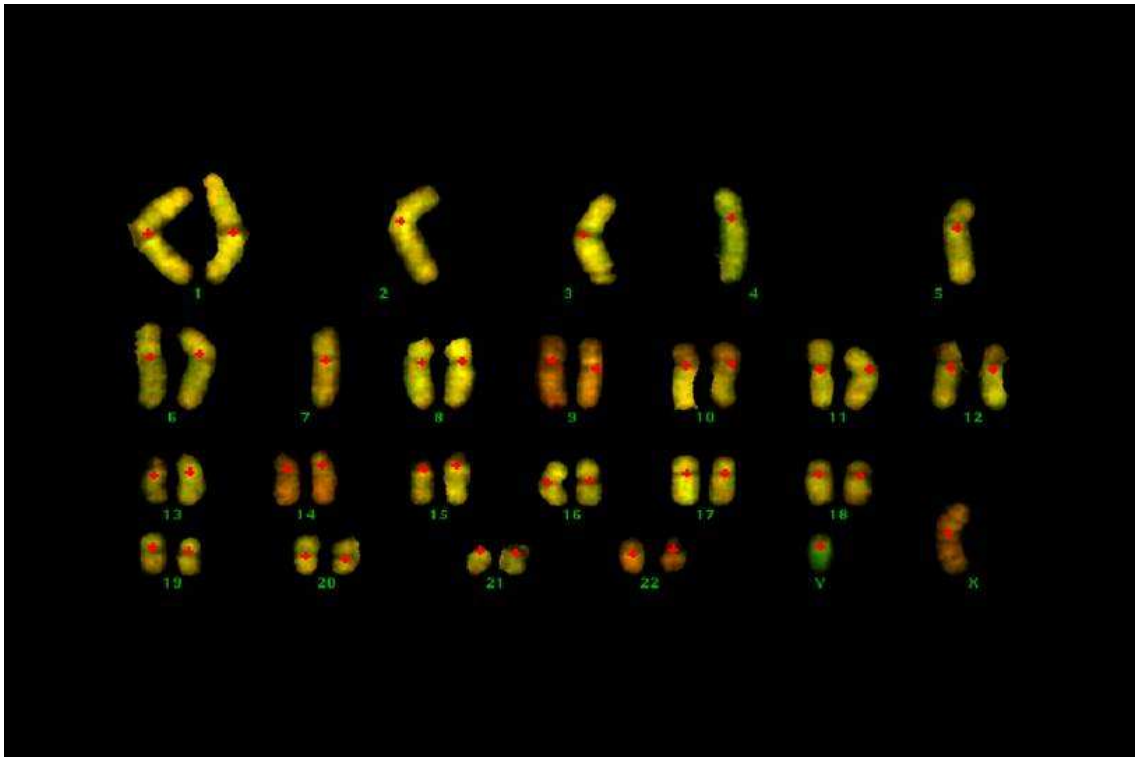

Figure 2. Fluorescence of photomicrograph showing the result of a CGH analysis of the hepatoceullar carcinoma of case 1.

A schematic summary of all chromosomal copy number aberrations is shown in Figure 3, 4. The chromosomal losses are more frequent than the gains. All patients show chromosomal loss in at least one chromosomal arm. The frequency of chromosomal losses is summarized in Table 2. A high frequency of chromosomal arm loss in HCC by CGH analysis is 8p (56.7%), 16q (54.0%), 16p (51.3%), and 1p (45.9%). Moderate frequency loss is detected at 4q (32.4.%), 14q (24.3%), and 17p (21.6%). The frequency of chromosomal loss on the other chromosomes was less than 20%.

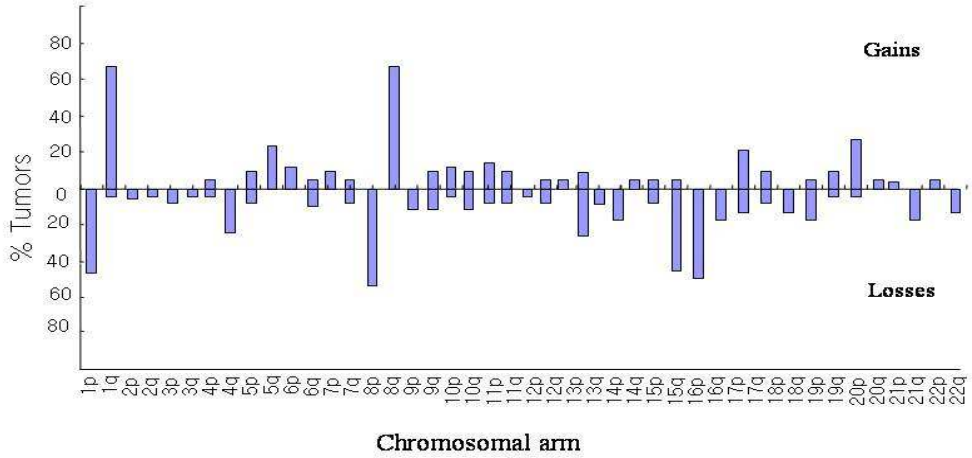

Figure 3. The rate of chromosomal loss and gain observed on a designated 37 non-acrocentric chromosomal arms of HCC in graphic form. Each bar represents the percentage of loss (lower) or gain (upper) of a chromosomal arm

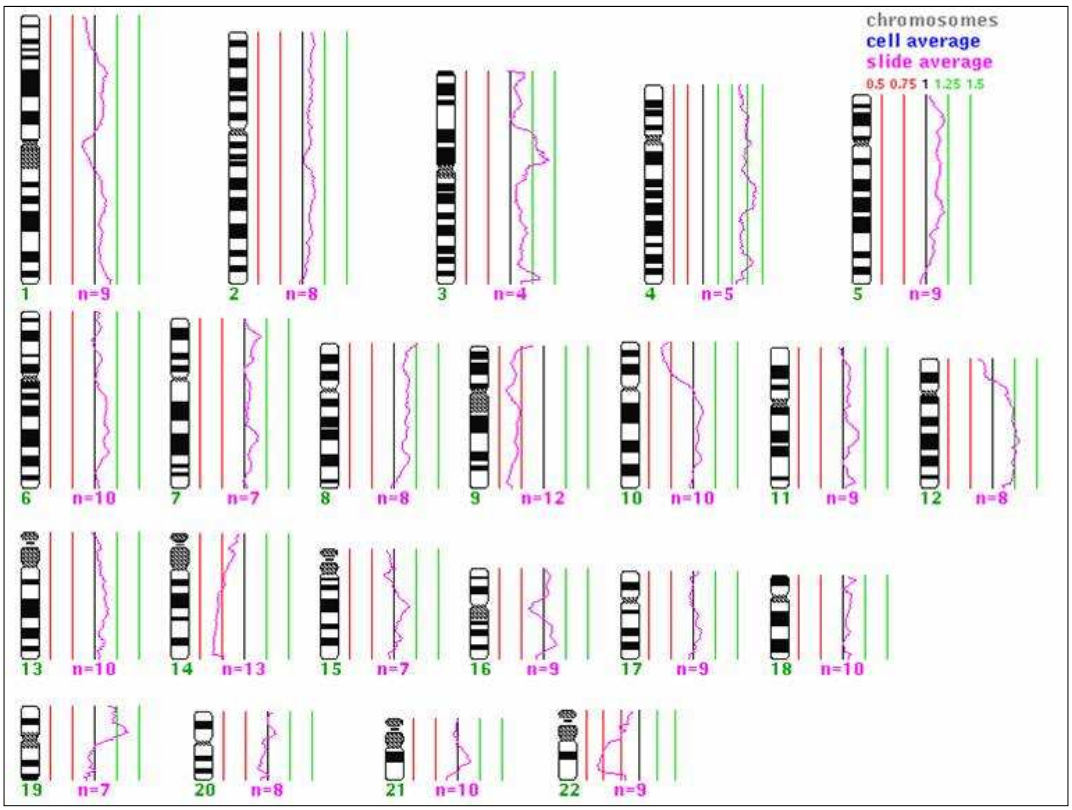

Figure 4. The CGH analysis on primary HCCs. Green lines represent gains and red lines highlight losses. The intensity ratio of a balanced copy number is 1.0 (central vertical line). The left-side shift indicates underrepresentations (value 0.75) while right-side shift indicates overrepresentations (value 1.25). Primary HCC shows gains of 3p, 19p, and high copy number amplication of 4p, 4q, Losses are detected on 10p, 14q, 22q.

Table 1. Summary of Clinical Data and Chromosomal Aberration Detected by CGH from 37 HCCs

Case N0.	Sex/Age	Virus	Differentiation	Size	Stage	AFP	ICG_{15min}	Vascular invasion	Follow up	CGH result	
										Gain	Loss
1	M/52	-	M	9cm	III	6.7	8	-	89	1q, 6p	8p,
2	M/48	B	M	5cm	IV	2	18	+	64	5q, 8q,	1p, 4q, 8p, 16p, 16q
3	F/62	B	W	1cm	II	2	-	-	68	8q	8p, 17p
4	M/57	B	M	0.5cm	I	8595	12	-	10	8q	4q, 8p, 14q, 16p, 16q
5	M/45	B	M	5cm	II	1518	17.1	-	43	1q, 8q, 20p	1p, 4q, 8p, 14q, 16p,16q,
6	M/31	B	P	6cm	III	97.1	13	+	110	1q, 17q	4q, 8p, 13q,16p, 16q, 17p
7	M/54	-	M	8cm	II	133	17.4	-	12		1p, 4q, 8p, 16p, 16q,
8	M/49	B	W	4.8cm	II	44.2	12.28	-	39	1q	8p, 16q
9	M/52	B	W	4cm	II	3.3	10.8	-	14	1q,6p, 8q, 20p	4q, 8p, 13q, 16q
10	M/50	B	P	12cm	III	6575	15.8	+	2	1q, 8q	8p, 13q
11	M/66	B	P	8cm	II	523	12.41	-	30	8q, 20p	8p, 16p, 16q
12	M/59	B	P	8cm	III	4.1	16	+	29	1q, 20p	8p, 16p, 16q
13	M/41	B	M	5cm	III	710	8.07	-	32	1q, 6p, 17q	1p, 4q, 8p, 14q
14	M/66	B	W	5cm	III	96.8	12.5	-	53	1q, 6p, 8q	1p, 4q, 8p, 14q, 16p, 17q
15	M/60	-	W	3.5cm	II	2535	7.8	-	20	1q, 8q, 20p	4q, 8p, 16q
16	M/51	B	W	3.5cm	II	3	16	+	23	1q, 5q, 8q	8p, 13q, 14q, 16q
17	M/54	B	W	6cm	II	2.2	2.74	-	40	1q, 8q, 17q	8p
18	M/65	C	W	2.5cm	IV	40	25	+	34	1q, 5q, 8q, 17q	8p, 14q, 16q, 17p
19	F/25	B	W	5cm	II	309.1	8.3	-	42	1q, 8q	4q, 8p
20	M/41	B	W	2cm	II	2	14.7	-	106	8p	
21	M/53	B	P	5cm	III	2361	5.4	-	15	1q, 5q, 8q	1p, 8p, 16p, 16q, 17p
22	M/55	B	W	8cm	III	4.9	9.5	-	175	1q, 5q, 17q	16p
23	M/58	C	W	5cm	II	8.3	7.24	-	89	1q, 5q, 6p, 8q	1p, 13q, 14q, 17p
24	M/62	B	W	2.7cm	II	2.2	12.09	-	86	5q	1p, 16p
25	M/49	B	W	3.3cm	II	499	17.6	-	18	8q	1p,16p, 16q
26	M/53	B	W	3cm	II	2.6	10.1	-	49	1q, 20p	1p, 16p, 16q
27	M/50	B	W	14cm	IV	30000	2.3	+	106		1p, 16p, 16q
28	F/53	B	P	8cm	III	3.9	7.4	-	93	8q	
29	M/42	B	M	2.5cm	III	361	12.32	-	48	1q, 17q	1p, 16p, 16q
30	M/16	B	P	3.5cm	II	2	5.73	-	14	1q, 8q	1p, 4q
31	M/53	B	W	8.5cm	III	2	4.3	-	80	1q, 5q, 8q	1p, 16p, 16q, 17p
32	M/57	B	W	5cm	II	5.9	44.3	-	6	1q, 8q	
33	F/66	B	P	6cm	II	3.8	7.5	-	18		1p, 16p
34	M/62	-	W	7cm	II	2.2	8.6	-	70	1q, 6p	
35	M/45	B	W	7cm	IV	6.64	11.8	+	33		16q
36	M/38	B	P	10cm	IV	4.21	11.9	+	41	1q, 8q, 17q	1p, 4q, 13q, 16p
37	F/64	B	M	3.5cm	II	2011	6.3	-	39	1q, 8q	1p, 14q, 16p, 16q

M: male; F: female; B: hepatitis B virus; C: hepatitis C virus; W: well differentiated; M: moderate differentiated; P: poorly differentiated; -: no invasion; +: invasion.

The frequency of chromosomal gain is summarized in Table 2.

Table 2. Genetic alterations by CGH in HCCs

Loss(%)		Gain(%)	
1p	45.9	1q	67.5
4q	32.4	5q	21.6
8p	56.7	6p	16.2
13q	18.9	8q	62.1
14q	24.3	17q	18.9
16p	51.3	20p	16.2
16q	54.0		
17p	21.6		

A high frequency of allelic gain was found on chromosomes 1q (67.5%) and 8q (62.1%). Other chromosomal arms have chromosomal gain frequency of less than 20%. It is to evaluate the correlation between the change occurring on each chromosomal arm and clinicopathologic characteristics. Five-year overall survival rate of 37 patients is 43.0% (Fig. 5), and AFP is the most strong impact prognostic factor ($p<.004$) among prognostic factors (HBs Ag, ICG_{15min}, Size, vascular invasion).

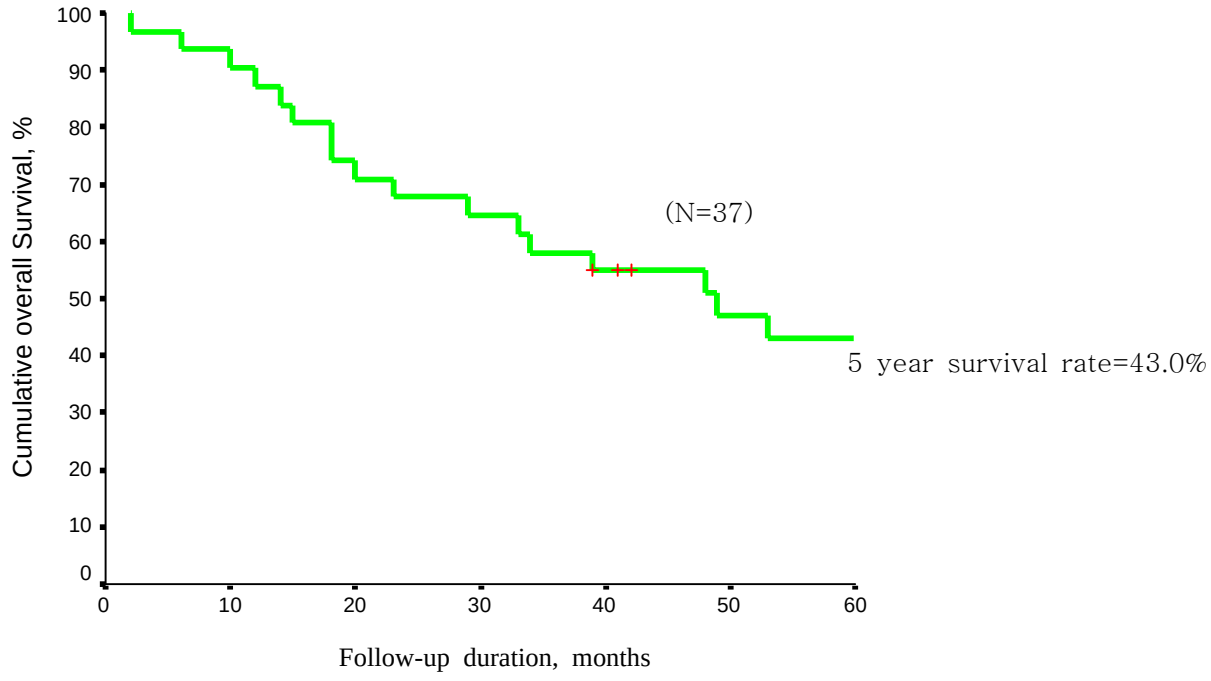

Fig. 5. 5-year survival rate of 37 hepatoceullular carcinomas.

When cumulative overall survival according to the normal or elevation of AFP is analyzed, the case of elevated AFP shows poor survival ($p<.004$) (Fig. 6).

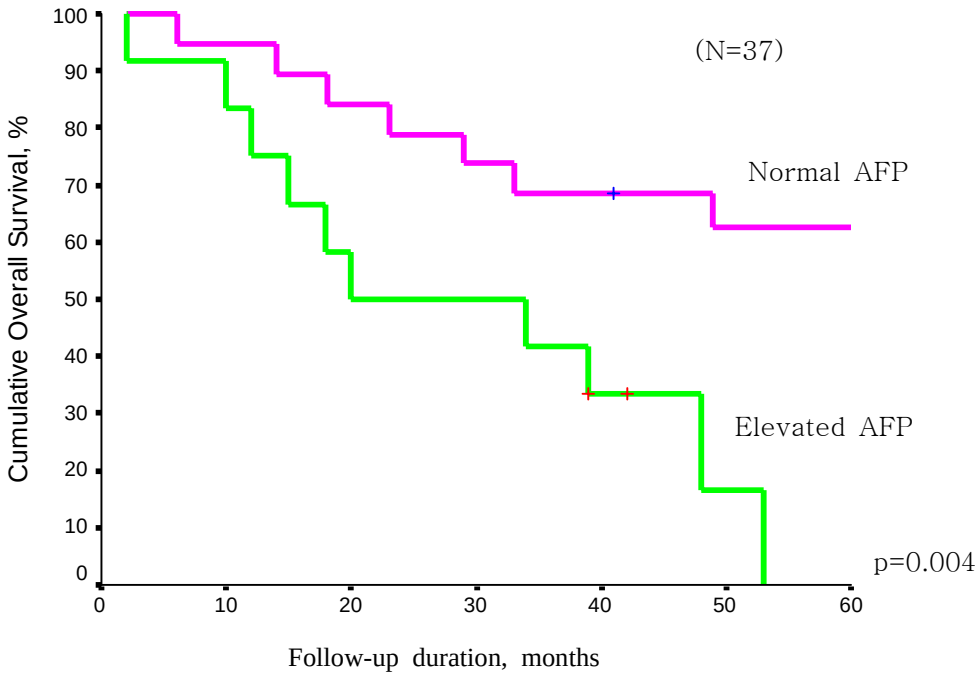

Fig. 6. Overall survival of hepatocellular carcinomas according to AFP level.

Univariate analysis on chromosomal aberrations is performed for the potential factors that are associated with patient survival. 16q chromosomal loss is significantly correlated with 5-year overall survival (Table 3) (Fig. 7), but other chromosomal abberations are not significant statistically.

Table 3. Genetic alterations impact on 5-year overall survival

Loss		Gain	
Gene	p-value	Gene	p-value
1p	0.73	1q	67.5
4q	0.16	5q	21.6
8p	0.46	6p	16.2
13q	0.90	8q	62.1
14q	0.80	17q	18.9
16p	0.95	20p	16.2
16q	0.03		
17p	21.6		

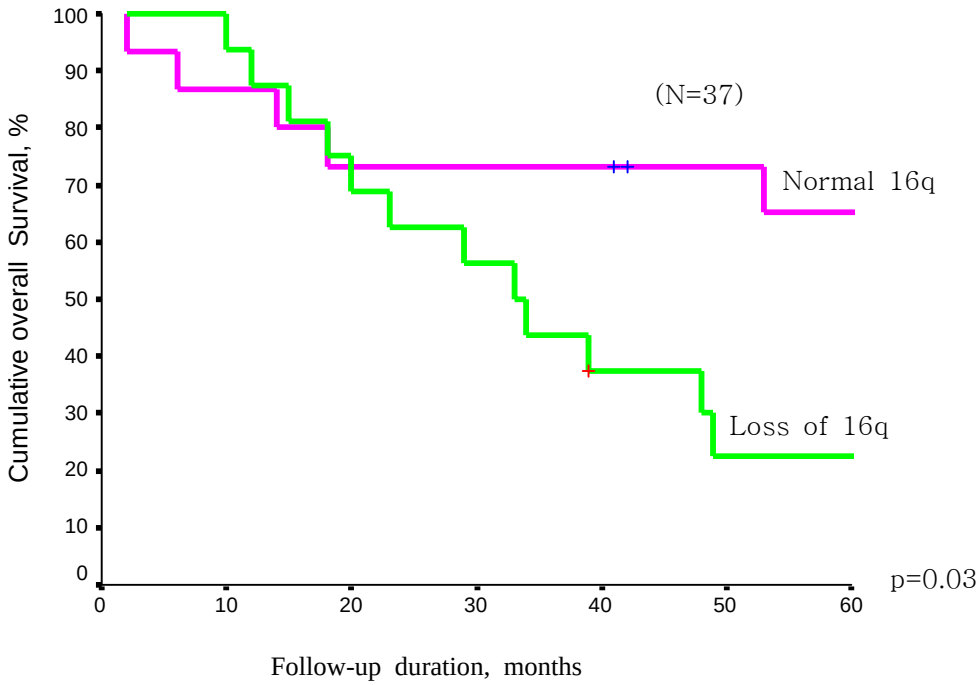

Fig. 7. Overall survival according to the loss of 16q.

As a result of efforts to find out relationship genetic alterations with prognostic factor statistically, 4q and AFP ($p<0.048$) (Table 4), 16q and AFP ($p<0.028$) (Table 5), 16p and ICG_{15min} ($p<0.049$) (Table 6), 16q and ICG_{15min} ($p<0.004$) (Table 7), 16q and size ($p<0.037$) (Table 8), 13q and vascular invasion ($p<0.022$) (Table 9) and are correlated with prognostic factors of HCCs.

Table 4. Correlation 4q and α-fetoprotein

		AFP level		Total
		> 7 IU/ml	≤ 7 IU/ml	
4q	Loss count	8	4	12
	(%)	(66.7%)	(33.3%)	(100.0%)
	Normal count	8	17	25
	(%)	(32.0%)	(68.0%)	(100.0%)
Total	Count	16	21	37
	(%)	(43.2%)	(56.8%)	(100.0%)

p< 0.048

Table 5. Correlation 16q and α-fetoprotein

		AFP level		Total
		> 7 IU/ml	≤ 7 IU/ml	
16q	Loss count	12	8	20
	(%)	(60.0%)	(40.0%)	(100%)
	Normal count	4	13	17
	(%)	(23.5%)	(76.5%)	(100%)
Total	Count	16	21	37
	(%)	(43.2%)	(56.8%)	(100%)

p< 0.028

Table 6. Correlation 16p and ICG_{15min}

		ICG_{15min}		Total
		> 10 %	≤ 10 %	
16p	Loss count	14	5	19
	(%)	(73.7%)	(26.3%)	(100.0%)
	Normal count	7	10	17
	(%)	(41.2%)	(58.8%)	(100.0%)
Total	Count	21	15	36
	(%)	(58.3%)	(41.7%)	(100.0%)

p< 0.049

Table 7. Correlation 16q and ICG_{15min}

		ICG_{15min}		Total
		> 10 %	≤ 10 %	
16q	Loss count	16	4	20
	(%)	(80%)	(20.0%)	(100%)
	Normal count	5	11	16
	(%)	(31.3%)	(68.8%)	(100%)
Total	Count	21	15	36
	(%)	(58.3%)	(41.7%)	(100%)

p< 0.004

Table 8. Correlation 16q and tumor size.

		Size		Total
		> 5 cm	≤ 5 cm	
16q	Loss count	14	6	20
	(%)	(70%)	(30.0%)	(100.0%)
	Normal count	6	11	17
	(%)	(35.3%)	(64.7%)	(100.0%)
Total	Count	20	17	37
	(%)	(52.6%)	(47.3%)	(100.0%)

p<0.037

Table 9. Correlation 13q and vascular invasion

		Vascular invasion		Total
		Yes	No	
13q	Loss count	4	2	6
	(%)	(66.7%)	(33.3%)	(100.0%)
	Normal count	5	26	31
	(%)	(41.2%)	(58.8%)	(100.0%)
Total	Count	9	28	37
	(%)	(24.3%)	(75.7%)	(100.0%)

$p < 0.022$

IV. DISCUSSION

Extensive studies have been made to elucidate the genetic process of hepatocyte carcinogenesis.[16~9] However, genes relevant to the development and progression of HCCs have hardly been identified. The current molecular cytogenic study, CGH, revealed that some chromosomal imbalances were significantly associated with pathologic findings and prognosis of patients with HCCs. Although there are published articles concerning CGH analysis of HCCs,[20~22] this article is report to our knowledge that cytogenetic changes detected by CGH are potentially useful for the estimation of biologic characteristics including the prognosis of patients with HCCs.

The present study represents the genome-wide investigation on the genetic imbalance in HCC in relation to tumor size, AFP, ICG_{15min}, and vascular invasion. It is found through the CGH analysis that there are frequent chromosomal losses on 8p (56.7%), 16q (54.0%), and 16p (51.3%), but chromosomal gains are most prevalent on 1q (67.5%), 8q (62.1%). These findings in this study are in general agreement with previous loss of heterozygosity and CGH results. For example, gain of 1q, 8q, and 20q and loss of 16q, 17p, 4q, 1p, and 8p have been detected as frequent chromosomal alterations in HCCs in at least one of the previous CGH studies.[23,4] But, amplifications at 11q, 12q, 20 chromosome are known in HCCs,[25,26] a copy number gains of chromosome 7, 12, 19, 21, and Y are identified in Hong Kong hepatocellular carcinoma line.[27] This difference suggests that these aberrations are accumulated during tumor progression.[28]

Gain of 1q is the most frequent change in HCC, involving 58~78% of the previous studies. About 10% of these showed high-copy-number amplification. In this study, gain of 1q involving 67.5% of the tested cases is the most frequent change in HCCs. This observation together with the finding of an amplicon on 1q21~22, indicates the likehood of important proto-oncogenes residing in this region. According to the Genome Data Base, 1q21 habors the gene that encodes the human mRNA for hepatoma-derived growth factor. The enhanced expression of this gene could be associated with the

paracrine and/or autocrine activity that supports tumor growth. CGH studies on soft tissue sarcomas, osteosarcoma, bladder cancer,[29] breast cancer,[30] and the Ewing family of tumors have also reported the presence of a recurring 1q21~22 amplicon.[31~33] Amplification of the flagellar basal body rod protein(FLG) and small proline-rich protein (SPRR3) genes, also located on 1q21, have been identified in several sarcoma cell lines.[34] An increased expression of CACY (calcyclin) and CAPL (calcium protein, murine placental homologue) of the S-100 family calcium-binding proteins have been mapped to the same region and implicated in tumor progression and metastasis.[35]

Amplification of the distal region of the long arm of chromosome 8 is frequently seen in a variety of solid tumors, leukemias and lymphomas, and MYC a major protooncogene being involved in over 80% of neoplasias.[36] Gains at 8q24 are recurrent in both HCC cell lines and primary tumors and most likely involves the c-MYC gene.[20] The importance of the c-MYC gene in hepatocarcinogenesis has been firmly shown both in human tumors and in a transgenic mouse model. Coexpression of c-MYC and transforming growth factor-α enhances the development of HCC in transgenic mice through the disruption of the pRb/EF2 (retinoblastoma tumor suppressor protein/transcriptional regulatory protein) pathway. In addition, transforming growth factor-α may function as a survival factor for neoplastic cells and thereby accelerate the neoplastic process.[37,38] Deregulation of c-MYC gene expression mediated by chromosome translocations and viral integration is very common in cancer.[39]

CGH study on HCC that the deletion of chromosomes 8p might contribute to the development of HCC metastasis.[40] Several candidate tumor suppressor genes have been mapped to 8p including DLC-1(deleted in liver cancer) (8p21.3~22)[41] and FEZ1 (fasciculation and elongation protein zeta 1) (8p22).[42] In this study, the rate of loss of 8p is 56.7%, it can conclude that 8p might harbor one or more tumor suppressor genes that are important in HCC progression especially in the tumor metastasis, as well as other kinds of cancers, although 8p is not significant correlation with prognostic factors.

Genetic alterations successively emerge in individual tumor cell because of genetic instability inherent to tumor cells. Advanced tumors show more malignant characteristics on tumor cells. It is postulated that the identification of genetic changes linked to malignant characteristics of tumor cells allows us to estimate the prognosis of each patient with high precision.[43] In HCCs, losses of 8p, 16q, and 16p, and gains of 1q and 8q, although they are not independent prognostic factors, are associated with poor prognosis. Loss of heterozygosity on chromosome 16 often coexists with deletions on chromosome 4.[44] Furthermore, deletion mapping suggested that there may be two tumor suppressor genes on chromosome 16q and one putative suppressor gene located in the region 4q26~27, all of which may play a role in the aggressive phenotype of HCC.[45,46] In HCCs, inactivation of 16p has been reported, but the principal inactivation

mechanisms are quite diverse. The 16p gene is a cell-related gene encoding a 16p protein that binds competitively to cyclin-dependant kinase 4 protein(Cdk 4) and thereby inhibits the interaction of Cdk 4 with cyclin D1 to stimulate passage through the G_1 phase of the cell cycle.[47] The disruption of 16p-mediated cell cycle control seems to play a role in hepatocarcinogenesis because inactivation of the 16p gene has been reported in HCCs.[48~50] This study shows that loss of 16q is significantly related with impacting on overall 5-year survival ($p<.004$) among genetic alteration genes, that is, decreased five year survival rate in 16q deletion patients.

In general, the conventional TNM staging classification[51,52] is less widely used in HCCs than other malignant tumors because the prognosis is related to the state of the underlying liver disease as much as the extent of the tumor itself. It is compared bewteen the pattern of genetic alterations and 16 cases of T_2 and 11 cases of T_3, respectively, and it is found that there is no significant difference except a higher incidence of 1q gain in stage III. Rather, tumor size or the presence of vascular invasion in conjunction with measurement of the underlying liver function may be better prognostic parameters of HCCs.

We compared the genetic alterations of 16q with total 37 cases with > 5 cm and ≤ 5 cm tumor. Loss of 16q with > 5 cm tumor is 14 cases, loss of 16q with ≤ 5 cm tumor is 6 cases, normal 16q with > 5 cm tumor is 6 cases, normal 16q with ≤ 5 cm tumor is 11 cases. It shows that high genetic losses of 16q are significantly found in large HCCs. Guan et al[22] studied the association between the incidence of chromosomal alteration and tumor size. The incidence of gain 20q was obviously increased in large tumors. Gain of 8q and loss of 8p showed significant difference between small size and large size tumors. This various high genetic losses of 8q, 16q, 20q could be explained by genetic alterations which accumulated during tumor progression.[29~30]

By analyzing the relationship between genomic alterations and AFP in Table 4 and 5, losses of 16q in increase of AFP is 14 cases, loss of 16q in normal of AFP is 6 cases, normal 16q in increase of AFP is 6 cases, and normal of 16q in normal of AFP is 11 cases. And loss of 4q in increase of AFP is 8 cases, loss of 4q in normal AFP is 4 cases, normal 4q in increase of AFP is 8 cases, and normal 4q in normal AFP is 17 cases. There is statistically a significant relationship between 4q and 16q and AFP. AFP is considered as prognostic factor based on chromosomal alteration. AFP concentration is correlate with differentiation of HCC and tumor size. It can be explained that two tumor suppressor genes is on chromosome 16q and one putative suppressor gene located in the region 4q26~27.

When the relationship between ICG_{15min} and 16q and 16p, respectively, studies, loss of 16q in > 10% ICG_{15min} is 16 cases, loss of 16q in ≤ 10% ICG_{15min} is 4 cases,

normal 16q in > 10% ICG_{15min} is 5 cases, normal 16q in ≤ 10% ICG_{15min} is 11 cases. In addition, the cases who has loss of 13q with vascular invasion are more than patients with no vascular invasion. This is a new detection in this study.

In summary, We document that HCC development and progression involve multiple genetic alterations which were 8p, 16p, 16q, 1q, 8q. The frequent gain and loss of chromosomal regions identified in this study may represent candidate regions for potential oncogenes and tumor suppressor genes, respectively. Correlations between genetic alterations and poor prognostic factors are shown significantly in groups of 16q and 4q deletion with AFP, 16q and 16p deletion with ICG_{15min}, 16q deletion with tumor size (>5 cm), 13q deletion with vascular invasion. Especially, this study shows that the 5-year survival rate of 16q deletion patients decreases.

V. CONCLUSION

The most important factor in prognosis of hepatocellular carcinoma is a loss of 16q. The chromosomal losses are more frequent than the gains. All patients show chromosomal loss in at least one chromosomal arm. A high frequency of chromosomal arm loss in HCC by CGH analysis is 8p (56.7%), 16q (54.0%), 16p (51.3%), and 1p (45.9%). A high frequency of allelic gain was found on chromosomes 1q (67.5%) and 8q (62.1%). AFP is the most strong impact prognostic factor ($p<0.004$) among prognostic factors (HBs Ag, ICG_{15min}, Size, vascular invasion). Losses of 4q and 16q might play important roles in elevation of AFP level. Also, there are poor liver function in case of the losses of 16p, 16q patients group. Otherwise, losses of 16q is concerned with tumor size. Especially, losses of 13q is correlated with vascular invasion and is necessary for the metastais of HCCs. Losses of 16p, 16q, 13q, 4q can be applied to therapeutic plan in hepatocellular carcinoma and their deletions are related with tumor progression and invasiveness of hepatocellular carcinoma.

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ABSTRACT(IN KOREAN)

간세포암환자의 특정 염색체의 변형과 임상양상 상관관계

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채 윤 석

간암은 매우 흔한 암종이며 발생하면 예후가 좋지 않은 암종이다. 간암의 발생은 만성적인 B형 혹은 C형 간염바이러스 감염과, 술이나 아플라톡신과 같은 독성에 장시간 노출되어 발생한 간경화가 원인이 되는 것으로 생각하고 있다. 또한 간염바이러스 감염과 여러 가지 발암물질에 노출되면 간세포 유전자의 손실 (deletion), 증폭 (duplication) 및 전좌 (translocation)등의 염색체이상이 확인되었으며 유전자의 변형이 종양의 발생과 진행에 관련이 있기 때문에 이러한 유전자를 찾는 것이 암을 이해하고 치료하는데 있어 매우 중요하다. 비교유전자교잡법 (Comparative Genomic Hybridization)은 한번의 교잡(hybridization)을 통하여 종양 유전자의 복제수 변화(손실 또는 증폭)을 정상 염색체에 mapping하여 확인 할 수 있다. 따라서 본 연구에서는 CGH를 통한 간암의 유전자 변형을 찾아내고 이를 바탕으로 간암의 예후인자인 α-fetoprotein, ICG_{15min}, Tumor size, TACE, Multiplicity, Capsular invasion, Vascular invasion, Satellite nodule등과 연관지어 분석함으로써 어떠한 유전자가 예후인자와 관계가 있고 그 예후인자는 예후인자로써 의미가 있는지 알아보고자 하였다. 1996년 1월부터 2002년 12월까지 37예의 간암을 대상으로 CGH를 하였다. 그리고 의무기록을 열람하여 해당환자의 임상적 특징을 후향적으로 조사 하였다. CGH 결과 가장 흔한 염색체 손실은 8p (56.7%), 16q (54.3%), 16p (51.3%), 1p (45.9%), 그리고 4q(32.4%) 순이었다. 반면 획득은 1q (67.5%), 8q (62.1%) 이었다. 5년 생존율과 가장 관련이 있는 유전자 이상은 16q 손실이었다.($p<.03$) 그리고 16q 유전자 손실과 예후인자들과의 통계학적 상관관계는 α-fetoprotein ($p<.028$), 종양크기 ($p<.037$), 그리고 $ICG_{15min} > 10\%$ ($p<.004$)등이 통계학적으로 의미가 있었다. 그 외 13q 유전자 손실과 혈관침범 ($p<.022$), 그리고 4q 손실과 α-fetoprotein ($p<.049$)이 의미가 있었다.

결론적으로 간암의 발생과 예후에 가장 관련된 유전자 변형은 16q 이며, 13q 유전자 손실이 있는 간암은 혈관침범 가능성이 높아 예후도 좋지 않을것으로 생각되며 여러 유전자중 4q 손실은 간암의 가장 중요한 예후인자인 α-fetoprotein 증감과 관련이 깊은 것으로 생각된다. 그러므로 16q, 16p, 13q, 4q 손실은 간암의 치료방향의 설정 및 예후 예측에 유용할 것으로 생각된다.

핵심되는말: 간암, 예후인자, 비교유전자교잡법