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Original Article

Genome-wide single nucleotide polymorphism array analysis reveals recurrent genomic alterations associated with histopathologic features in intrahepatic cholangiocarcinoma

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Abstract: Recent studies indicate that genomic alterations (GAs) are associated with many human malignancies. Genome-wide analysis of GAs involved in intrahepatic cholangiocarcinoma (ICC) and association with histopathologic features are limited. To help characterize this relatively rare neoplasm, we collected 32 frozen tissue samples of ICC to study GAs and molecular karyotypes by using single-nucleotide polymorphism array. Recurrent GAs occurring in at least 40% of the patients were further correlated with histopathologic features. Gain of 1q21.3-q23.1 and losses of 1p36.33-p35.3 and 3p26.3-p13 were significantly associated with larger tumor size more than 5 cm in diameter; and loss of 4q13.2-q35.2 with tumor multiplicity. Moreover, losses of 1p36.32-p35.3, 3p26.3-p22.2, 4q13.1-q21.23, 4q31.3-q34.3 and 4q34.3-35.2 were inclined to be associated with high histological grade. As to tumor vascular invasion, gain of 1q21.3-q23.1 and losses of 3p22.1-p12.3 and 4q13.2-q35.2 were significantly associated with tumor vascular invasion. Some regions were concurrently associated with multiple histopathologic characteristics, including loss of 4q13.2-q35.2 associated with larger tumor size, high histological grade and vascular invasion; losses of 1p36.33-p35.3 and 3p26.3-p22.2 with larger tumor size and high histological grade; and gain of 1q21.3-q23.1 with larger tumor size and vascular invasion. Our study indicates that complex chromosomal instability is characteristic of ICC. Detecting crucial GAs will enable risk stratification and development of personalized therapies.

Keywords: ICC, whole genome analysis, SNP array

Introduction

Intrahepatic cholangiocarcinoma (ICC) is relatively infrequent, accounting for 5-15% of primary liver cancer worldwide [1]. The prevalence of ICC shows a wide geographic variation, with increasing incidence in southeast Asia [2]. It accounts for up to 90% of primary liver cancer cases in Thailand due to the extremely high prevalence of chronic liver fluke infestation [3]. Other risk factors include chronic inflammatory biliary disease, hepatolithiasis, biliary malformations, and viral infections [2]. ICC is an aggressive cancer with a high mortality rate. The poor prognosis of ICC is associated with early invasion, widespread metastasis, and lack of effective therapy [4].

American Joint Committee on Cancer (AJCC) 7th edition staging system is now clinically relevant to predict outcomes of ICC patients. For primary tumor (T) classification, the presence of periductal-infiltrating (PI) rather than mass-forming (MF) growth would upgrade a T1 to T4 tumor. Vascular invasion and tumor multiplicity are the parameters used to define T2a and T2b tumors, respectively. These histopathologic features are associated with genomic alterations (GAs) to some degree. Some genome-wide association studies report chromosomal gains of 2q33-qter, 5p14-pter, 7p, 8q22-qter, 13q, 15q, 17q21-q22, 18q12-q21, 19q13.1, and 18q, as well as losses of 1p34-pter, 3p, 4q, 5q11-q14, 6q, 8p, 9p, 16q, 17p, 19p, X and Y by comparative genomic hybridization (CGH) [5-7]. The most limitation is the contaminated normal clone, which may lead to inaccurate estimate of the true copy number state.

Table 1. Histopathologic features of 32 patients with intrahepatic cholangiocarcinoma

Case	Age/ sex	Tumor number	Tumor size (cm)	Growth pattern	Capsule	R	Necrosis (%)	VI	NI	Sarcomatoid change	Histology grade	pT*	LN	pStage*
B032	65/F	S	1.5	MF + PI	No	R1	5	No	Yes	No	L	4	No	IVA
B038	50/M	M	8.5	MF	No	R0	30	Yes	Yes	No	H	2b	NA	II
B040	63/M	S	3.0	MF	No	R0	30	No	No	No	L	1	NA	I
B043	42/M	S	5.0	MF	No	R0	5	No	No	No	L	1	NA	I
B044	47/F	S	5.0	MF	No	R0	30	No	No	No	L	1	No	I
B045	48/F	S	9.0	MF	No	R0	10	Yes	Yes	No	H	3	No	III
B046	43/M	S	9.0	MF	No	R0	15	Yes	No	No	L	2a	NA	II
B048	70/M	S	12.0	MF	No	R0	20	No	No	No	H	1	NA	I
B050	66/M	S	8.2	MF	No	R0	20	Yes	Yes	No	H	1	NA	I
B051	80/F	S	3.2	MF	No	R0	15	Yes	No	No	H	1	NA	I
B052	76/M	S	8.0	MF	No	R0	5	Yes	No	No	L	2a	NA	II
B053	57/F	S	7.5	MF	No	R0	10	Yes	No	No	L	2b	No	II
B054	77/F	S	2.5	MF	No	R0	0	No	Yes	No	L	1	No	I
B055	52/F	S	5.0	MF	No	R1	0	Yes	No	No	L	3	Yes	IVA
B056	55/M	M	11.5	MF	No	R0	10	Yes	No	No	L	2b	No	II
B057	40/M	M	10.5	MF	No	R0	15	Yes	Yes	No	L	2b	No	II
B059	63/M	S	9.0	MF + PI	No	R0	15	No	No	No	L	4	No	IVA
B060	41/F	M	11.2	MF	No	R0	10	Yes	No	Yes	H	2b	No	II
B063	51/F	S	11.0	MF	No	R1	10	No	No	No	H	1	NA	I
B065	45/M	S	5.0	MF	No	R0	30	Yes	No	Yes	H	2a	NA	II
B069	46/M	M	8.5	MF	No	R0	30	Yes	No	No	L	3	NA	IVB (M1)
B070	55/F	S	6.0	MF	No	R0	1	Yes	No	No	L	2a	Yes	IVA
B071	51/F	S	5.5	MF	No	R0	5	No	No	No	L	1	No	I
B072	58/M	S	3.5	MF	No	R0	5	Yes	No	No	H	2a	No	II
B076	63/F	S	10.0	MF	No	R0	0	No	No	No	L	1	No	I
B128	47/M	S	4.5	MF + PI	No	R0	0	No	Yes	No	L	4	Yes	IVA
B143	56/M	S	3.0	MF	Partially	R0	20	No	Yes	No	L	1	No	I
B157	60/M	M	6.8	MF + PI	No	R0	0	No	No	No	H	2b	NA	II
B161	54/F	M	7.5	MF + PI	No	R0	10	No	No	No	L	4	No	IVA
B164	71/F	S	5.3	MF + PI	No	R0	5	Yes	No	No	L	1	Yes	IVA
B165	65/M	M	8.0	MF	No	R0	10	No	No	No	H	2b	No	II
B169	74/M	M	8.5	MF + PI	No	R0	20	No	Yes	No	L	4	Yes	IVA

*According to American Joint Committee on Cancer (AJCC, 7th edition). S: solitary: M: multiple; MF: mass-forming type; PI: periductal infiltrating type; R: resection status; VI: vascular invasion; NI: neural invasion; L: low-grade differentiation; H: high-grade differentiation; pT: tumor stage; LN: nodal metastasis; NA: not available; M1: distant metastasis.

Single-nucleotide polymorphism (SNP) arrays are a type of DNA microarray using a pair of probes to detect two allelic loci in the same region of a specific chromosome. SNP arrays can be used to elucidate the genotype of two allelic loci as well as to detect allelic imbalance. By simultaneously detecting fluorescence intensity and genotype, it is possible to determine the percentage of aneuploidy tumor cells among normal and tumor clones, and identify pathologic copy-neutral loss of heterozygosity (CN-LOH). The SNP array-based genome-wide study of ICC is limited. The only one was recently reported by using formalin-fixed paraffin-embedded samples [8]. In this study, 32 frozen tissue samples of ICC were collected for SNP array analysis. The molecular karyotypes, mosaic mixtures of tumor and normal clones, and correlation between GAs and histopathologic characteristics were explored.

Materials and methods

Patient samples

Thirty-two cases of surgically resected ICC with fresh tumor samples were enrolled in this study. The medical records were available and carefully reviewed. Survival time was defined as the time period between the date of the diagnosis and the date of death or last follow-up of the

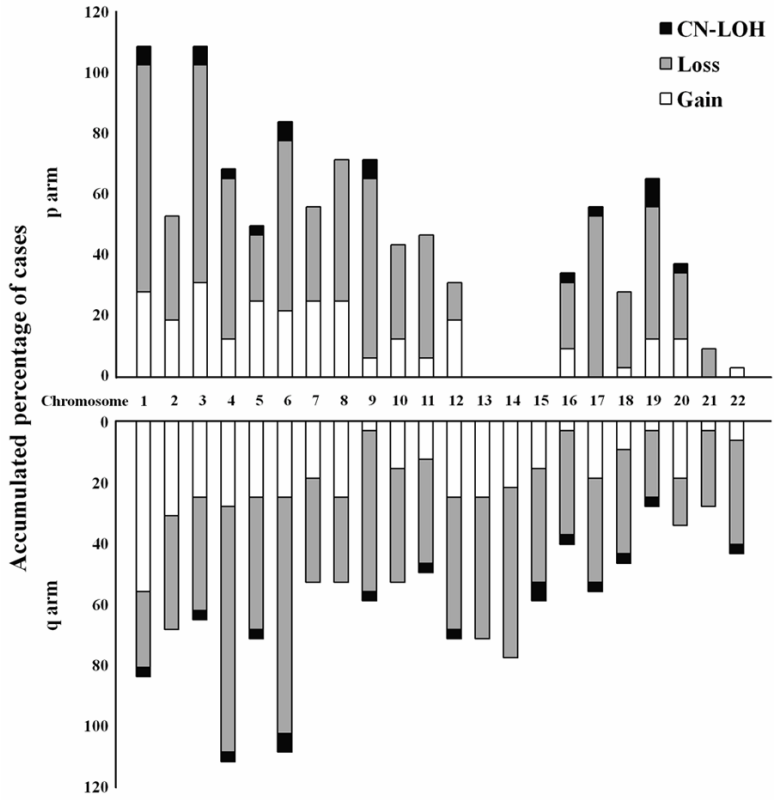


Figure 1. Summary of genomic alterations per chromosome in 32 ICC patients. The figure depicts the percentage of gain (white), loss (gray), and CN-LOH (black) per chromosome.

patients. The hematoxylin and eosin stained sections obtained at the time of diagnosis, and those which were repeated, were reviewed. The AJCC 7th edition staging system was adopted for tumor staging. Institutional review board, in accordance with the Helsinki Declaration, approved this study.

DNA extraction and SNP mapping assay

DNA was extracted from fresh frozen tumor tissues using QIAamp DNA mini kit (Qiagen, Hilden, Germany). DNA concentration and purity were checked by NanoPhotometer (IMPLEN, München, Germany). The 260 nm/280 nm ratios for all DNAs were between 2.2 and 1.8. Molecular karyotyping was performed using Illumina HumanOmni2.5_8 SNP chips (Illumina, Inc., San Diego, CA). SNP array hybridizations were run in the Health Gen-eTech Corp. (Taipei, ROC).

Data analysis

The data were processed in GenomeStudio Genotyping Analysis Module V2011.1.0. The normalized intensity of a subject sample was compared to the reference values derived from 439 normal reference samples. Chromosomal aberrations were determined by both log R ratio ($\log_2^{\text{R subject/R reference}}$) and B allele frequency (BAF). Log R ratios of approximately -1, 0, 0.5, and 1 were classified as monosomy, disomy, trisomy, and tetrasomy, respectively. SNP genotypes were determined by BAF through interpolation from known BAFs of the three canonical clusters (0, 0.5, and 1). Percentage of aneuploid tumor cells was quantified according to the equation reported by Markello et al. [9]. We set a minimum threshold (20%) of detecting mosaic mixtures due to the reliable split of the heterozygote pane of BAF. Types of molecular karyotypes were interpreted according to Jasmine et al. study [10]. To avoid false discoveries of CN-LOH due to non-paired analysis, mosaic mixtures of homozygous and heterozygous genotypes featuring moderate split of BAF without changed copy number were selected.

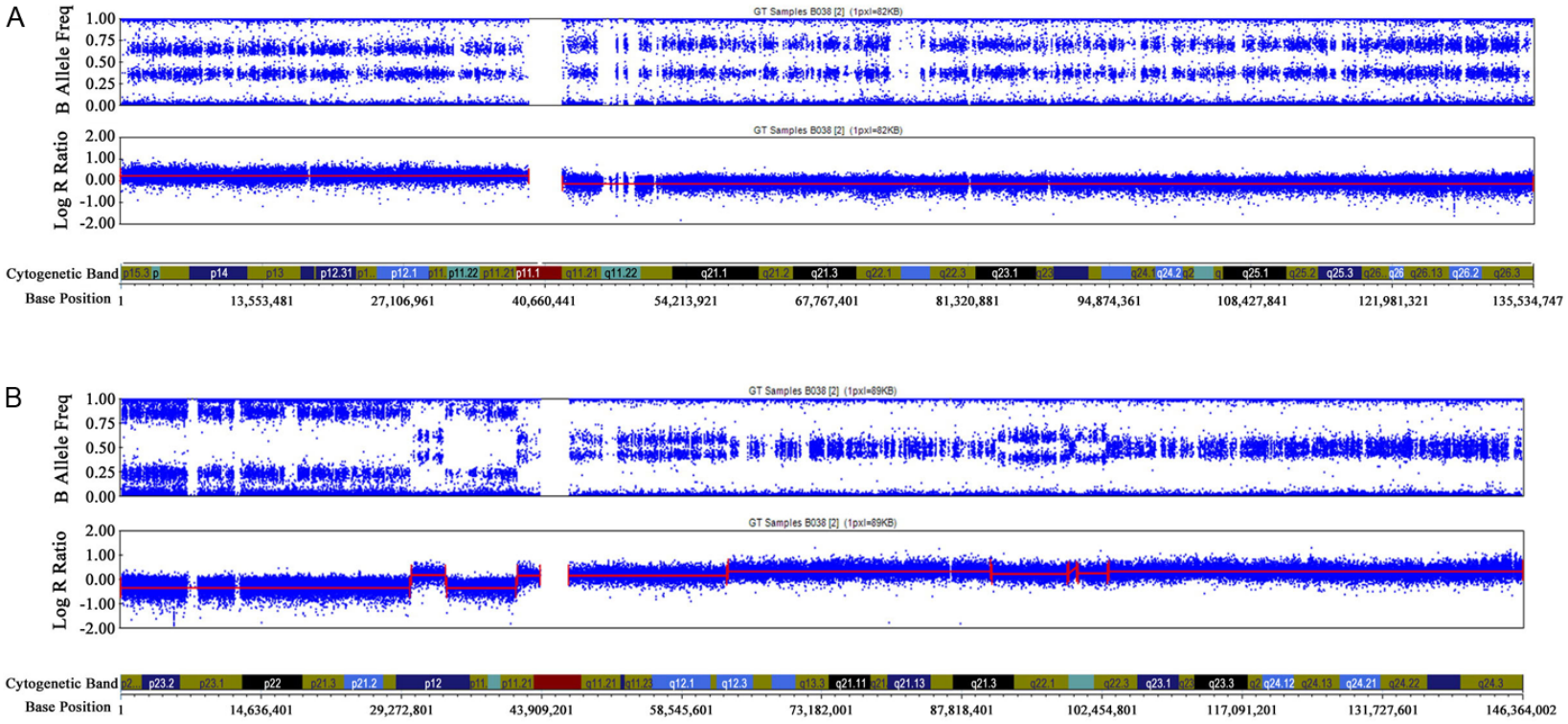
Statistical analysis

All statistical analyses were performed using SPSS for windows 11.0 software (SPSS Inc. Chicago, IL). The significance of association between recurrent GAs and histopathologic variables were determined by Chi-square and Fisher exact test. A *P* value of 0.05 or less was considered statistically significant.

Results

Patient histopathologic characteristics

Table 1 summarizes pertinent histopathologic findings. Subjects included 18 men and 14 women with a median age of 55.5 years (range, 40-80; mean, 57.2 years). No patient was treated with chemotherapy before surgery. Seventeen (53%) of the 32 patients revealed local recurrence at a median follow-up period of 5.1 months (range, 1.4-50.9). Seventeen (53%)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B Allele Freq
1.00
0.75
0.50
0.25
0.00
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Log R Ratio
2.00
1.00
0.00
-1.00
-2.00
Cytogenetic Band
Base Position
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27,106,961
40,660,441
54,213,921
67,767,401
81,320,881
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108,427,841
121,981,321
135,534,747
B
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0.25
0.00
GT Samples B038 [2] (1pxl=89KB)
Log R Ratio
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1.00
0.00
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Cytogenetic Band
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58,545,601
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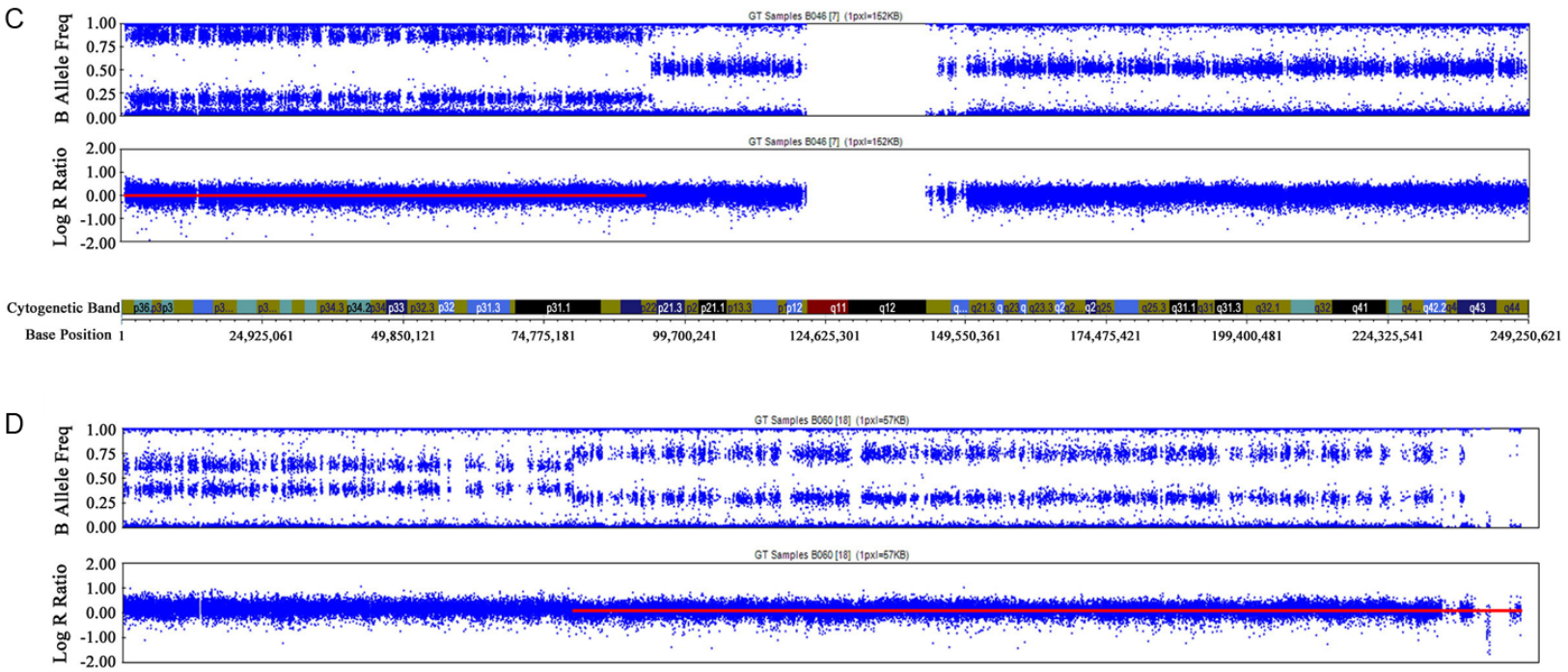

Figure 2. A. Mosaic mono-allelic amplification and deletion in chromosome 10. Approximately 70% of the cells have mono-allelic amplification in 10p15.3-p11.1. Constituting genotypes are AAA, AAB, ABB, and BBB (BAF: 0/0.37/0.63/1, CN = 2.7). Approximately 50% of the cells have mono-allelic deletion in 10q11.1-q26.3. Constituting genotypes are A and B (BAF: 0/0.38/0.72/1, CN = 1.5). B. Mosaic bi-allelic amplification in chromosome 8. The tumor cells have bi-allelic amplification in 8q12.2-q21.3 and 8q22.3-q24.3. Constituting genotypes are AAAA, ABAB, and BBBB (BAF: 0/0.48/0.52/1, CN = 2.7-2.8). C. Mosaic CN-LOH in chromosome 1. Approximately 70% of the cells show BAF splitting without CN change in 1p26.33-p22.1. Constituting genotypes are AA and BB (BAF: 0/0.2/0.9/1, CN = 2). The entire chromosome 1q arm is normal. D. Mono-allelic amplification superimposed on the CN-LOH in chromosome 2. The tumor cells show amplification with a large split of the heterozygote pane in BAF plot. Constituting genotypes are AAA and BBB (BAF: 0/0.3/0.8/1, CN = 2.2-2.3).

Table 2. Association of recurrent genomic regions with tumor size and tumor number

Gain	Cytoband	Size (Mb)	Characteristic		*P*
			Tumor size		
	1q21.3-q23.1	3	< 5 cm	> 5 cm	
	No: 56.3% (N = 18)		50.0% (9/18)	50.0% (9/18)	0.039
	Yes: 43.8% (N = 14)		14.3% (2/14)	85.7% (12/14)	
Loss	Cytoband	Size (Mb)	Characteristic		*P*
			Tumor size		
	1p36.33-p35.3	28	< 5 cm	> 5 cm	
	No: 43.8% (N = 14)		57.1% (8/14)	42.9% (6/14)	0.022
	Yes: 56.3% (N = 18)		16.7% (3/18)	83.3% (15/18)	
	3p26.3-p13	73.6	< 5 cm	> 5 cm	
	No: 56.3% (N = 18)		55.6% (10/18)	44.4% (8/18)	0.005
	Yes: 43.8% (N = 14)		7.1% (1/14)	92.9% (13/14)	
			Tumor number		
	4q13.2-q35.2	119.5	Single	Multiple	
	No: 59.4% (N = 19)		89.5% (17/19)	10.5% (2/19)	0.011
	Yes: 40.6% (N = 13)		46.2% (6/13)	53.8% (7/13)	

patients had distant metastasis at a median follow-up period of 9.9 months (range, 2.1-32.3): retroperitoneal lymph nodes (53%), lung (35%), and peritoneal carcinomatosis (12%). The median follow-up period was 26.6 months (range, 2.8-86.9). The overall 2-, 3-, and 5-year survival rates for these 32 cases were 34%, 28%, and 16%, respectively.

SNP array analysis

The average genotyping call rates were 98.18% ± 1.67% (range, 94.56-99.83%). GAs was identified in all the patients. A total of 1227 GAs (autosomes only) were detected with a median of 37.5 per case (range, 0-80): 369 gains with a median of 8.5 per case (range, 0-32), 818 losses with a median of 24.5 per case (range, 0-74), and 40 CN-LOH with a median of 0 per case (range, 0-12). The most common mosaic mixtures of tumor and normal clones were 20% trisomy/disomy for gain, 70% monosomy/disomy for loss, and 30% homozygous/heterozygous genotypes for CN-LOH. In a total of 1227 GAs, we further clarified 299 regions (24.4%) of mono-allelic amplification, 56 regions (4.6%) of mono-allelic amplification superimposed on CN-LOH, and 14 regions (1.1%) of bi-allelic amplifications for gain; 807 regions (65.8%) of mono-allelic deletions, and 11 regions (0.9%) of homozygous deletions for loss; and 40 regions (3.3%) of CN-LOH. The sizes of GAs ranged from 0.01 to 243.2 Mb, with a median of 4.09 Mb (range, 0.04-243.2) in gain, 20.98 Mb (range, 0.01-243.2) in loss, and 31.78 Mb (range, 0.07-144.6) in CN-LOH. The frequencies of chromosome changes (**Figure 1**) and representative types of identified GAs are illustrated (**Figure 2**).

Detection of recurrent regions and target genes associated with histopathologic characteristics

To delineate minimal overlapping regions, we narrowed down the size of the recurrent GAs occurring in at least 40% of the patients. A total of 13 gains and 288 losses were evaluated and correlated with histopathologic characteristics.

Recurrent genomic regions associated with tumor size and tumor number

Table 2 summarizes the associations between recurrent gains and losses, and tumors larger than 5 cm in diameter and tumor number. Gain of 1q21.3-q23.1 was significantly associated with tumor size larger than 5 cm. Some candidate oncogenes correspond to the GAs within or near to the region of 1q21.3-q23.1, including *IL6R, TPM3, HAX1, NTRK1* and *DDR2* (**Table 5**). Moreover, losses of 1p36.33-p35.3 and 3p26.3-p13 were inclined to be associated with larger tumor size more than 5 cm in diameter. Some candidate tumor suppressor genes (TSGs) correspond to these two GAs. *TP73* is located within the region of 1p36.33-p35.3, and *VHL, PPARG, PLCD1, RASSFIA, BAP1, HYAL1, ACY1, FHIT* and *FOXP1* were within the region of 3p26.3-p13 (**Table 5**). As to tumor number, loss of 4q13.2-q35.2 was significantly related to tumor multiplicity with candidate TSG, *LARP7*, located at this region (**Table 5**).

Recurrent genomic regions associated with histological grade

Losses of 1p36.32-p35.3, 3p26.3-p22.2, 4q13.1-q21.23, 4q31.3-q34.3 and 4q34.3-35.2 were significantly associated with high

Table 3. Association of recurrent genomic regions with histological grade

Loss	Cytoband	Size (Mb)	Characteristic		*P*
			Histological grade		
	1p36.32-p35.3	24	Low-grade	High-grade	
	No: 37.5% (N = 12)		91.7% (11/12)	8.3% (1/12)	0.018
	Yes: 62.5% (N = 20)		50.0% (10/20)	50.0% (10/20)	
	3p26.3-p22.2	36.8	Low-grade	High-grade	
	No: 43.8% (N = 14)		92.9% (13/14)	7.1% (1/14)	0.005
	Yes: 56.2% (N = 18)		44.4% (8/18)	55.6% (10/18)	
	4q13.1-q21.23	24.9	Low-grade	High-grade	
	No: 53.1% (N = 17)		88.2% (15/17)	11.8% (2/17)	0.006
	Yes: 46.9% (N = 15)		40.0% (6/15)	60.0% (9/15)	
	4q31.3-q34.3	25.6	Low-grade	High-grade	
	No: 53.1% (N = 17)		94.1% (16/17)	5.9% (1/17)	< 0.001
	Yes: 46.9% (N = 15)		33.3% (5/15)	66.7% (10/15)	
	4q34.3-q35.2	10.4	Low-grade	High-grade	
	No: 53.1% (N = 17)		88.2% (15/17)	11.8% (2/17)	0.006
	Yes: 46.9% (N = 15)		40.0% (6/15)	60.0% (9/15)	

histological grade (**Table 3**). *TP73* at 1p36.3; and *VHL, PPARG* and *PLCD1* within the region of 3p25.3-p21.3 are corresponding candidate TSGs (**Table 5**).

Recurrent genomic regions associated with tumor vascular invasion

Table 4 summarizes the association between GAs and tumor vascular invasion. Gain of 1q21.3-q23.1, which was related to larger tumor size, was also significantly associated with tumor vascular invasion. Moreover, tumors with losses of 3p22.1-p12.3 and 4q13.2-q35.2 tended to vascular invasion. Some candidate TSGs correspond to these two GAs, including *PLCD1, RASSFIA, BAP1, HYAL1, ACY1, FHIT* and *FOXP1* located within the region of 3p22-p14, and *LARP7* at 4q25.

Recurrent genomic regions associated with multiple histopathologic features

Furthermore, some regions were concurrently associated with multiple histopathologic characteristics. Loss of 4q13.2-q35.2 was significantly associated with larger tumor size, high histological grade and vascular invasion; losses of 1p36.33-p35.3 and 3p26.3-p22.2 with larger tumor size and high histological grade; and gain of 1q21.3-q23.1 with larger tumor size and vascular invasion (**Tables 2**-**4**).

Selected oncogenes and tumor suppressor genes located at recurrent regions

The genes located at the amplified and deleted regions were identified from the Miller et al. study of gene expression in ICC compared with non-cancerous bile duct [11], and UCSC Genome Browser website (http://genome.ucsc.edu). The shortest overlapping regions of GAs and gene expressions are summarized in Supplementary Table 1. The most frequent GAs were 1q21.3-q22 (50%) for gain; and 3p21.31-p14.2 (69%) and 4q13.2 (69%) for loss. The associated genes were involved in regulation of cell cycle, apoptosis transcription, cellular signaling, cytoskeletal structure, extracellular matrix, and cellular adhesion. **Table 5** summaries the candidate oncogenes and TSGs corresponding to the GAs associated with histopathologic features.

Discussion

In this study we interpreted molecular karyotypes of ICC, which were exclusively recognized by SNP arrays, but not commonly identified by CGH arrays. For the first time, to the best of our knowledge, molecular karyotypes of ICC were clarified. We further delineated some recurrent regions correlated with histopathologic features. The corresponding genes affected in these regions include oncogenes and TSGs, which are dysregulated in a broad array of cancers, and are associated with tumor progression and adverse outcome.

GAs is associated with some histopathologic parameters. A tumor size more than 5 cm had been considered a prognostic variable in the AJCC 6th edition, but it was removed from the AJCC 7th edition because of the lack of statistical significance in relation to survival upon multivariate analysis [12, 13]. According to the AJCC 7th edition, vascular invasion and tumor multiplicity are prognostic variables used to discriminate pT1 from pT2a, and diagnose

Table 4. Association of recurrent genomic regions with tumor vascular invasion

Gain	Cytoband	Size (Mb)	Characteristic		*P*
			Vascular invasion		
	1q21.3-q23.1	3	Absent	Present	
	No: 56.2% (N = 18)		66.7% (12/18)	33.3% (6/18)	0.037
	Yes: 43.8% (N = 14)		28.6% (4/14)	71.4% (10/14)	
Loss	Cytoband	Size (Mb)	Characteristic		*P*
			Vascular invasion		
	3p22.1-p12.3	34.1	Absent	Present	
	No: 50% (N = 16)		68.8% (11/16)	31.2% (5/16)	0.038
	Yes: 50% (N = 16)		31% (5/16)	68.8% (11/16)	
	4q13.2-q35.2	119.5	Absent	Present	
	No: 56.2% (N = 18)		72.2% (13/18)	27.8% (5/18)	0.006
	Yes: 43.8% (N = 14)		21.4% (3/14)	78.6% (11/14)	

T2b, respectively [14, 15]. In the current study gain of 1q21.3-q23.1, and losses of 3p22.1-p12.3, and 4q13.2-q35.2 were significantly associated with vascular invasion, and loss of 4q13.2-q35.2 with tumor multiplicity. These data may not only provide insight into tumorigenesis, but may also assist in predicting the tumor stage and promoting the development of adjuvant therapy in ICC.

We identified some GAs previously reported in ICC of either Asian or European origin [5-7, 11, 16]. The most frequent gains were 1q, 2q, 5p, 7p, 8q, and 13q; and the corresponding losses were 1p, 3p, 4q, 6p, 6q, 8p, 9p, and 17p with a variable proportion of samples in a particular chromosome region [5-7, 11, 16]. Losses at 4q and 13q, which are frequently observed chromosomal alterations in high-grade hepatocellular carcinoma (HCC) [5, 17], were commonly detected and associated with vascular invasion and poorly differentiated tumors in our study. Gain at 1q, frequently identified and associated with early events in HCC [5, 6, 18], is the most common amplification in ICC. The frequent change at 1q gain in our study (52%) was similar to the report by Homayounfar et al. (42%) [5]. Our previous study exploring GAs in combined HCC-CC also revealed high amplification at 1q in both components [19], not surprising given that ICC share GAs with HCC to some degree. The role of 1q amplification in ICC will be further explored by using a larger sample size.

The most commonly deleted regions in the present study were at chromosome 3p, which has also been identified as a common event in many other cancers, including lung cancer [20, 21], colon cancer [22], and esophageal squamous cell carcinoma [23]. Loss of 3p26.3-p13 was significantly correlated with tumor size; 3p26.3-p22.2 with histological grade; and 3p22.1-p12.3 with vascular invasion in ICC. Several candidate TSGs were identified that are associated with the inhibition of tumor cell growth and induction of apoptosis. Down-regulation of *FHIT* at 3p14.2 and *CACNA2D3* at 3p21.1 is associated with the pathogenesis of lung cancer and poor prognosis of colon and esophageal cancers [20, 24]. Loss of 3p25-pter, involving *VHL* and *ATG7*, is significantly associated with distant metastasis and poor survival in colon cancer [22]. Another frequently deleted region was 1p36, which was associated with tumor size, and histological grade in our study. Loss of 1p36 may contribute to the carcinogenesis and pathogenesis of infected liver fluke-related CC [25]. The putative TSGs include *RIZ, p73, DFFB*, *CASP9*, *PAX7*, and *ID3*. LOH of *p73* was reported in 54.5-75.6% of cases of ICC [16, 25]. Other reported regions containing TSGs were also commonly identified in our study. *p53*, located at 17p13.1, is implicated in the tumorigenesis of ICC. LOH of *p53* was reported at a frequency of 30% [26]. Up to 77% of ICC were found to contain *p53* mutations when examining all exons of *p53* [27]. The 9p21 gene cluster, harboring candidate TSGs $p16^{INK4a}/p14^{ARF}$ and $p15^{INK4b}$, were involved in 37-50% of ICC by LOH or promoter hypermethylation [16, 28]. The number of amplifications was less frequent than deletions. Activation of the oncogenes *KRAS*, *MYC*, *ERBB2*, *MET*, *BRAF*, *IDH*, and *EGFR* is triggered by point mutation or protein overexpression rather than genomic amplification [29-33].

In this study, non-paired tumor samples were concerned with CN-LOH analysis. False discovery of CN-LOH may occur in un-paired studies [34]. However, coexisted tumor and normal clones, featuring mosaic mixtures of homozygous and heterozygous genotyping ca-

Table 5. Selected oncogenes and tumor suppressor genes located at recurrent regions associated with histopathologic features

Gene Symbol	Description	Cytoband	Function	HF
IL6R	Interleukin 6 receptor	1q21	Acute phase response	TS, VI
TPM3	Tropomyosin 3	1q21.2	Muscle contraction	TS, VI
HAX1	HCLS1 associated protein X-1	1q21.3	Protein binding	TS, VI
NTRK1	Heurotrophic tyrosine kinase, receptor, type 1	1q21-1q22	Protein phosphorylation	TS, VI
DDR2	Discoidin domain receptor tyrosine kinase 2	1q23.3	Cell-matrix adhesion	TS, VI
TP73	Tumor protein p73	1p36.3	Apoptosis	TS, HG
VHL	von Hippel-Lindau tumor suppressor	3p25.3	Protein ubiquitination	TS, HG
PPARG	Peroxisome proliferator-activated receptor gamma	3p25	Regulation of transcription	TS, HG
PLCD1	Phospholipase C, delta 1	3p22-3p21.3	Calcium ion binding	TS, VI
RASSFIA	Ras association (RalGDS/AF-6) domain family member 1	3p21.3	Cell cycle arrest	TS, VI
BAP1	BRCA1 associated protein-1	3p21.31-3p21.2	Regulation of cell proliferation	TS, VI
HYAL1	Hyaluronoglucosaminidase 1	3p21.3-p21.2	Regulation of cell proliferation	TS, VI
ACY1	Aminoacylase 1	3p21.1	Proteolysis	TS, VI
FHIT	Fragile histidine triad	3p14.2	DNA replication	TS, VI
FOXP1	Forkhead box P1	3p14.1	Transcriptional repressor	TS, VI
LARP7	La ribonucleoprotein domain family, member 7	4q25	RNA processing	VI, TN

Italics: selected oncogenes in amplified regions; bold italics: selected tumor suppressor genes in deleted regions. HF: histopathologic feature; HG: histologic grade; TN: tumor number; TS: tumor size; VI: vascular invasion.

lls, make detection of CN-LOH confident. Cancers with homozygous mutations of candidate genes in CN-LOH regions have been frequently reported [35, 36]. Further detailed characterization of these CN-LOH regions may facilitate the discovery of molecular pathologic lesions and understanding of tumorigenesis in ICC.

In summary, we reported molecular karyotypes and recurrent GAs of ICC. Specific recurrent regions were significantly associated with vascular invasion and tumor multiplicity. These findings may help risk stratification of ICC and development of personalized cancer treatment strategies.

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Disclosure of conflict of interest

None.

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