Hepatocellular and Cholangiolar Carcinoma-Derived Cell Lines Reveal Distinct Sets of Chromosomal Imbalances

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Key Words

Hepatocellular carcinoma · Cholangiocellular carcinoma · Cytogenetics · Chromosomal aberrations · Array CGH · Cell lines

Abstract

Objectives: Hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CC) cell lines are used to analyze the basic mechanisms of carcinogenesis and target therapies. However, it is not yet clear which chromosomal aberrations are to be typically expected in such cell lines. It is also not clear whether there are prerequisites for in vitro growth on the genomic and/or expression level. We therefore analyzed HCC and CC cell lines for typical genetic settings. **Methods:** The HCC cell lines HLE, HLF, Huh7, HepG2 and Hep3b and the CC cell lines EGI1, MzCha1 and TFK-1 were analyzed using high-density arrays for comparative genomic hybridization (aCGH; 244,000 oligonucleotides). Additional fluorescence in situ hybridization analyses were done to confirm the aCGH results and to add information regarding the aneuploidy of cell lines. **Results:** The gain of 1q, in particular q21–22, was detected in all HCC cell lines also as a partial loss of 13q. In

contrast, a loss of 8p in combination with a relative gain of 8q was seen in all CC but no HCC cell lines. Interestingly, a gain of 17q was seen in all cell lines. These aberrations are also well documented for surgical tumor specimens. Besides these imbalances, the cell lines revealed imbalances for 11p, 12p, 14q, 16p, 16q, 21q and 22q, respectively, only rarely seen in surgical tumor specimens. These aberrations could be of importance for the in vitro cultivation of tumor cells. Structural aberrations were accompanied by aneuploidy in 3 of 5 HCC cell lines and 2 of 3 CC cell lines. Ploidy status was not correlated to any of the imbalances mentioned above. **Conclusions:** HCC and CC cell lines revealed characteristic chromosomal imbalances similar to those seen in surgical tumor specimens including chromosomes 1, 8, 13 and 17, respectively. These aberrations are characteristic of the histogenetic origin of the tumor cells. However, the chromosomal imbalances that occurred probably led to the ability of tumor cells to grow in vitro.

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Introduction

Hepatocellular carcinoma (HCC) is the fourth most common malignant cancer, with more than one million patients affected worldwide per year [1, 2]. Large-scale gene expression profiling has provided important insights into the biology of HCC with regard to classification, etiology, survival prediction and identification of signaling pathways that may possibly serve as therapeutic targets [3–7]. Cholangiolar carcinoma (CC) represents about 10–20% of all hepatobiliary tumors [8] with strong geographic variations [9]. Clinical outcome is still poor despite new therapeutic approaches similar to HCC [10].

Cell lines derived from HCC and CC surgical specimens serve as *in vitro* systems to identify candidate genes of carcinogenesis and the effect of drugs and toxins on liver-derived cells. These tumor cells serve also as dynamic systems which can be altered, e.g. by gene silencing. Moreover, fixation artifacts, which are a major problem in formalin-fixed and paraffin-embedded specimens for genetic analyses, are not an issue in cell lines. This can also be said for the admixture of nonneoplastic cells typically seen in surgical tumor specimens.

Despite their frequent use for research purposes, it is not clear whether these cell lines are representative and mirror the mechanisms responsible for malignancy *in vivo*. Indeed, to the best of our knowledge, no systematic molecular cytogenetic analysis has yet been performed for hepatocytic and cholangiolar tumor cell lines in regard to this problem; it is therefore not clear whether or not cell lines in their cytogenetic findings are comparable to surgical tumor specimens grown *in vivo*. It is also not clear if there are mandatory chromosomal imbalances for maintaining tumor cells.

To answer these questions we focused on the detection of chromosomal aberrations in HCC- and CC-derived cell lines using an array-based high density comparative genomic hybridization (aCGH) system. The results were compared with regard to the hepatocellular and cholangiocellular origin of tumor cells analyzed, as well as to the findings reported in the literature for surgical tumor specimens.

Material and Methods

Cell Lines

HCC cell lines Hep3b and HepG2 were supplied by DSMZ (Braunschweig, Germany). Carcinoma cell lines of cholangiolar differentiation EGI-1 and TFK-1 were also supplied by DSMZ, whereas Mz-Cha-1 came from ATCC (Manassas, Va., USA). Two

of these cell lines (MzChA-1 and TFK-1) were available also as tumor specimens grown in a mouse model (nude mice; NMRI nu/nu). Cell lines HLF, HLE and Huh7 were kindly provided by Prof. Huh (Dept. of Cell Biology, Okayama University).

Cell Cultures

Tumor cell lines were incubated in DMEM with 10% fetal calf serum supplemented with glutamine and penicillin/streptomycin at 37°C in a 5% CO₂ atmosphere in 25-cm² tissue flasks (Oxoid TPP 90025, Oxoid, Pratteln, Switzerland).

Arrays for Comparative Genomic Hybridization

aCGH was performed using a high-density array system with 244,000 oligonucleotides on a chip (Cat. No. G4411B, Agilent, Waldbronn, Germany), covering the complete genome in an average of approximately 5–13 kB. DNA was isolated using the QIAamp DNA Mini kit, (Qiagen Cat. No. 51304), following the supplier's instructions for cultured cells.

Labeling and hybridization were also done as described by the supplier. As a reference, DNA of a female donor was taken (Cat. No. G1521, Promega, Mannheim, Germany). Hybridization was performed for 40 h at 65°C in a hybridization oven (Agilent G2545A).

Validation of aCGH Results by Fluorescence *in situ* Hybridization

Fluorescence *in situ* hybridization (FISH) was performed as described earlier in detail [11]. For all cell lines, 5 centromere- and 10 locus-specific probes were applied (all were supplied by Abbott/Vysis, Baar, Switzerland; see table 2). Locus-specific probes were used to directly confirm the aCGH results. Centromere-specific probes were used to determine the ploidy of tumor cells as this cannot be done by aCGH, due to technical reasons.

Data Analysis

Array images were acquired using a laser scanner (Agilent Scanner System 2605, Agilent). Hybridization spots on a slide were normalized with feature extraction software (version 9.5.3.1.) and processed as described below.

Data were imported to the Genomic Workbench software program version 5.0.14 (Agilent). The search for common aberrations was performed using the ADM1 algorithm based on a t test. The other parameters were set as follows: threshold 6 (the number of spots necessary to indicate an aberration), applied genome hg18 (data set of annotations available at the time of analyses), p-value threshold of 0.05, overlap 0.9 (indicates covering of the complete genome; more information provided on the supplier's website). Aberrations were searched with a resolution of up to 10 kB; those found for less than 3 probes only were not regarded for further analysis.

Results

Overall Findings in HCC Cell Lines

Concordant gains were detected by aCGH in all 5 cell lines for the regions of chromosomes 1, 2, 4, 5, 6, 7, 11, 12, 14, 16, 17, 19, 20, 21 and 22, respectively. Detailed annotations of these aberrations are given in table 1, with ex-

Table 1. Gains and losses occurring in either all HCC cell lines and/or all CC cell lines

Chromosome	Chromosome band	Gained region				Chromosome	Chromosome band	Gained region			
		start	stop	size	probes n			start	stop	size	probes n
Gains											
1							q12.3	61,135,690	65,530,381	4,394,692	622
HCC	p36.33	836,343	3,070,895	2,234,553	300						
	q21.2	144,112,595	150,290,477	6,177,883	488		q12.3	65,558,691	65,591,699	33,009	8
	q22	151,689,698	155,102,486	3,412,789	486		q12.3	65,607,044	69,994,848	4,387,805	487
	q23.2-24.1	159,307,849	159,758,930	451,082	77						
	q32.2	198,544,050	204,835,991	6,291,942	680	12					
	q32.2	209,723,576	211,369,675	1,646,100	167	HCC	p13.33	192,203	3,443,729	3,251,527	361
	q42.12	221,318,190	229,887,283	8,569,094	777		p13.33	6,227,581	7,249,423	1,021,843	158
	q42.2	232,809,040	234,814,928	2,005,889	175		p13.1	31,375,253	32,716,802	1,341,550	125
CC	q42.3	246,794,322	246,875,192	80,871	12		p12.1	47,265,452	56,560,248	9,294,797	1,319
2							q13.13	107,420,123	112,882,430	5,462,308	579
HCC	p22.1	42,844,630	47,734,656	4,890,027	468		q13.2	119,012,615	124,383,410	5,370,796	605
	p16.3	53,563,088	55,893,540	2,330,453	216		q14.1	130,868,078	131,928,007	1,059,930	127
	p16.1	60,584,878	62,292,786	1,707,909	170	14					
	p14	64,651,967	65,783,515	1,131,549	105	HCC	q31.1	21,719,393	24,042,410	2,323,018	337
	p14	69,218,676	74,795,481	5,576,806	609	16					
	p11	84,919,087	86,135,288	1,216,202	148	HCC	p13.12	15,022,821	22,666,085	7,643,265	579
	q36.3	216,601,776	217,266,211	664,436	84		p11.2	28,144,761	31,458,436	3,313,676	388
	q36.3	218,397,811	220,461,168	2,063,358	299	17					
	q37.1	231,685,211	233,431,082	1,745,872	209	HCC	p13.3	585,856	8,318,441	7,732,586	983
	q37.1	233,437,021	234,508,466	1,071,446	119		p11.2	16,204,925	19,230,693	3,025,769	278
	q37.3	238,402,477	239,146,299	743,823	84		p11.2	22,978,686	28,360,521	5,381,836	618
	q37.3	241,000,846	242,366,051	1,365,206	151		q11.2	31,102,705	33,210,431	2,107,727	193
4							q11.2-qter	33,241,958	78,154,619	44,912,662	4,380
HCC	p16.3	418,532	4,281,963	3,863,432	399	CC	q21.2	40,812,631	40,926,354	113,724	13
	p16.1	6,276,325	9,681,373	3,405,049	330		q21.32-qter	47,566,333	78,638,667	31,072,335	2,796
	p14	38,984,514	39,644,620	660,107	82	19					
5						HCC	p13.3	601,828	623,033	21,206	5
HCC	p15.33	580,352	1,274,466	694,115	77		p13.3	1,977,231	2,074,547	97,317	15
	q13.1	65,471,109	65,481,190	10,082	2		q13.12-13.32	38,068,451	46,538,616	8,470,166	937
	q23.1	115,248,273	115,461,904	213,632	21		q13.32-q13.43	46,547,693	63,017,721	16,470,029	2,028
6						20					
HCC	p21.1	30,372,509	44,290,990	13,918,482	1,675	HCC	p13	44,096	132,255	88,160	17
	p21.1	44,309,561	44,391,933	82,373	18		p13	137,865	25,821,265	25,683,401	2,218
7							p11.1	26,023,584	26,097,070	73,487	3
HCC	p22.3	314,709	2,985,284	2,670,576	286		q11.1-13.31	29,297,070	55,179,441	25,882,372	2,357
	p22.2	4,527,086	6,743,967	2,216,882	258		q13.33	60,039,625	62,184,038	2,144,414	304
	p13	43,614,252	45,151,855	1,537,604	204	21					
8						HCC	q22.3	43,069,742	43,773,885	704,144	100
CC	q13.2-qter	67,548,433	146,265,046	78,716,614	6,078		q22.3	43,979,106	45,896,575	1,917,470	309
11						22					
HCC	p15.5	310,683	3,905,365	3,594,683	418	HCC	q11.1-13.1	16,614,966	37,683,716	21,068,751	2,513
	p15.4	8,663,051	9,824,020	1,160,970	140		q13.1	37,718,469	39,344,767	1,626,299	220
	p15.2	16,732,726	18,688,187	1,955,462	225						
	p11.12	46,099,012	48,001,739	1,902,728	220						
	q12.1	56,815,117	57,350,051	534,935	81						
	q12.3	60,634,009	61,114,631	480,623	62						

Table 1 (continued)

Chromosome	Chromosome band	Gained region			
		start	stop	size	probes n
Losses					
3					
CC	p21.1	60,357,546	60,420,986	63,441	9
	p21.1	60,437,660	60,595,171	157,512	18
4					
HCC	q22.1-28.3	90,267,740	133,126,058	42,858,319	2,990
6					
CC	q25.3	158,997,666	159,011,514	13,849	3
	q25.3	162,263,463	162,627,793	364,331	39
8					
CC	p23.1	12,275,978	12,285,664	9,687	2
9					
HCC	p24.3	1,146,909	1,898,135	751,227	56
	p23	9,067,723	12,203,822	3,136,100	171
	p21.3	22,271,486	25,899,546	3,628,061	160
	p21.1	28,607,433	28,735,187	127,755	16
	p13.3	30,347,026	30,425,535	78,510	5
13					
HCC	q14.12	52,551,793	53,879,567	1,327,775	50
	q21.1-21.3	55,111,599	62,308,736	7,197,138	358
	q21.3-22.2	62,338,814	75,553,983	13,215,170	829
	q22.2-31.3	75,612,338	90,614,492	15,002,155	772
14					
HCC	q11.1	82,884,987	87,124,061	4,239,075	165
18					
HCC	q12.1	35,382,981	39,656,201	4,273,221	234
	q21.1	47,077,902	49,595,012	2,517,111	186
	q21.32	59,793,733	63,780,372	3,986,640	196

amples in figures 1–4. Losses were seen for the regions of chromosomes 4, 9, 13, 14 and 18. Aberrations spanned a length of from 21 up to 44,913 kB (from 5 up to 4,380 spots on the array).

FISH analysis was done to confirm the aCGH results. Overall, there was a close correlation of FISH and aCGH results; findings were in concord in 48 of 50 analyses performed with locus-specific probes (table 2). By applying centromere-specific probes we found a polyploidy, indicated by a mean of >2.50 signals/nucleus, in 3 of 5 HCC cell lines, i.e. HLE, Huh7 and Hep3b.

We compared these aberrations found by aCGH with those obtained in surgical specimens and reported by Moynzadeh et al. [12]. Overall, there was a clear agree-

ment between imbalances seen in these specimens and in the cell lines of our study; however, the percentage of cases of specific aberration differed. Whereas in the surgical specimens the number of affected cases was maximally about 55%, aberrations were seen in part in all our samples (table 3).

Overall Findings in CC Cell Lines

Gains were detectable in all 3 cell lines for chromosomes 1, 5, 8 and 17, respectively. Losses in all 3 cell lines were seen for parts of chromosomes 3, 6 and 8, respectively. The shortest aberration spanned 10 kB (2 spots) and the longest covered 78,716,614 kB (6,078 spots) as listed in table 1.

These results were confirmed by FISH, using the locus-specific probes in 28 of 30 analyses. Polyploidy was seen in 2 of 3 cell lines (EG11 and MzCha-1).

Our data were compared to those given for surgical tumor specimens in the progenetix database analyzed by conventional and/or aCGH. In this database, the most frequent aberrations occurring in at least 30% of cases were losses for 1p and 6q, and gains for 8q, 17q and 20q, respectively. In our CC cell lines we were able to confirm gains for chromosomes 8q and 17q, and a loss of 6q, respectively (table 3).

Based on the two main tasks of our study, we separated these findings into 2 main groups.

(1) Aberrations of hepatocellular or cholangiocellular origin:

(a) detectable in all HCC cell lines but in none of the CC cell lines

(b) detectable in all CC cell lines but in none of the HCC cell lines

(c) occurring in all HCC and CC cell lines

(2) Aberrations occurring in all HCC or CC cell lines but rarely reported in surgical tumor specimens (<5%).

Within these groups we found chromosomal imbalances as follows:

Group 1a

In all HCC cell lines, gains of chromosomal material were seen for the long arm of chromosome 1 including bands q21, q22, q23.2-24.1, q32.2, q42.12 and q42.2, respectively (fig. 1). In addition, 1p demonstrated a gain for the region p36.33.

In 13q, we found chromosome bands q14.11-q31.3 to be a commonly lost region in all HCC cell lines (fig. 3). Surprisingly, none of the CC cell lines demonstrated this aberration. As a loss of 13q is reported to be correlated to a low differentiation of carcinoma cells, we looked for the

Table 2. FISH analysis was done for locus-specific probes to confirm the results of aCGH

	1p36/ TP73	1q25/ ABL2	6q23 MYB	8q24/ CMYC	13q14/ Rb1	17q11.2/ Her2	19p13 MAN2B1	19q13 CRX	20q12 D20S108	20q13.2
HLE	yes!	yes	yes	yes	yes	yes	yes	yes	yes	yes
Huh7	yes	yes	yes	no!	yes	yes	yes	yes	yes	yes
HLF	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
HepG2	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Hep3b	yes	yes	no	yes	yes	yes	yes	yes	yes	yes
EGI-1	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
MzCha-1	yes	yes	yes	yes	yes	yes	yes	no	yes	yes
TFK-1	yes	yes	yes	yes	yes	yes	yes	yes	yes	no
	CEP1	CEP3	CEP7	CEP8	CEP17	Mean number of signals	Poliploidy			
HLE	1.10	3.58	2.34	3.35	4.07	2.89	yes			
Huh7	2.00	2.61	2.78	*	2.98	2.59	yes			
HLF	1.05	2.09	*	2.00	3.97	2.28	no			
HepG2	2.03	2.14	2.95	1.96	2.32	2.28	no			
Hep3b	3.64	3.21	3.08	3.19	3.07	3.24	yes			
EGI-1	2.84	3.20	2.15	3.98	3.80	3.19	yes			
MzCha-1	2.83	*	3.69	2.80	3.62	3.23	yes			
TFK-1	1.96	2.08	1.97	2.01	1.98	2.00	no			

Concordant results were seen in 48 of 50 analyses done in HCC cell lines and in 28 of 30 hybridizations in CC cell lines. In addition, centromeric probes were used to define ploidy status. A clearly increased number of chromosomes with more than 2.5 signals/nucleus was revealed in 3 of 5 HCC cell lines and in 2 of 3 CC cell lines.

! = aCGH result confirmed/not confirmed by FISH. * no data obtained for this experiment.

Table 3. The most frequently occurring chromosomal imbalances in surgical specimens of HCC and CC occurring in at least 20% of tumor samples

Chromosomal region	Surgical specimens		HCC cell lines	
	gain/loss	% of cases with aberration	corresponding results	number of cell lines with aberration
1q21.1-q44	gain	>55	yes	5 (100%)
4q21.1-q35	loss	>30	yes	5 (100%)
8p21.1-p22	loss	>35	yes	4 (80%)
8q22.1-q24.3	gain	>45	yes	4 (80%)
CC cell lines				
1p36-pter	loss	>30	no	1 (20%)
6qcen-qter	loss	>35	yes	3 (100%)
8q11.2-qter	gain	>30	yes	3 (100%)
17p13	loss		yes	3 (100%)
17qcen-qter	gain	>30	yes	3 (100%)
20q?-qter	gain	>30	yes	2 (66%)

Specimens were from [12] and the Progenetix data base, with an incidence of at least 20%. These aberrations were also seen in the cell lines of our study but with a higher percentage of affected samples.

Fig. 1. Findings of aCGH for chromosome 1. A very specific finding was that easily detectable gains of parts of 1q are seen in all HCC cell lines. The mean number of chromosomes in a nucleus (listed in table 2) is given below the name of the cell lines. There was no correlation of this mean number and the type of cell line (HCC vs. CC) or any of the structural aberrations found by aCGH.

histological differentiation of CC cell lines Mz-ChA-1 and TFK-1. Cell line Mz-ChA-1 revealed a moderate-to-low differentiation and TFK-1 a low differentiation in the histological examination (fig. 5).

Group 1b

Chromosomal imbalances seen only in CC cell lines but in none of the HCC cell lines were not detectable. However, for chromosome 8, a combination of imbalances occurred in CC cell lines only. There was a gain of 8q spanning 8q13-qter for all 3 samples (fig. 2). This gain was

accompanied by a loss of the remaining chromosome, i.e. 8p in 2 CC cell lines. The difference in the signal ratio of the loss of 8p and the gain of 8q was at least 0.5, indicating a relative gain of 8q of at least 1 additional copy. For cell line TFK-1, a loss of 8p was not indicated by a deviation of the signal ratio line and FISH results did not reveal aberrant counts for this region; however, signal ratios for 8p and 8q differed from values described for the other 2 cell lines.

HCC cell lines also demonstrated aberrations of chromosome 8. Four of the HCC cell lines revealed a

Fig. 2. aCGH for chromosome 8 revealed imbalances of chromosome arms 8p and 8q, giving a clue for i(8q). In contrast, aberrations of chromosome 8 in HCC cell lines were more variable.

nearly complete loss or gain of the chromosome. In cell line Hep3b, a loss of major parts of the chromosomes was seen, interrupted by some areas of gain in the long arm. There was, however, no consistent loss of 8p in comparison to 8q.

Group 1c

The only chromosomal aberration found in both kinds of cell lines and in all samples was 17q+ spanning bands q22-qter (fig. 4). In HCC cell lines, as seen for larger parts of 17p, the more proximal chromosomal bands up to q12

were also gained. However, gain of 17p was not complete, but interrupted for regions p11 and p13-qter in 4 samples. In contrast, in CC, a loss of 17p was seen in 2 samples (EZI-1, MzCha-1). For TFK-1, a loss of 17p was not found (confirmed by FISH); however, there was also an imbalance in the sense of a relative loss of 17p in comparison to 17q.

Group 2

Within this group we collected chromosomal imbalances that were seen in all HCC or CC cell lines, but reported in less than 5% of surgical tumor specimens in the

Fig. 3. Chromosomal imbalances of chromosome 13 focused on 13q. aCGH detected a region spanning from q14.12 to q31.3. This aberration was not seen in CC cell lines. However, histological examination of in vivo grown tumor cells of 2 of the CC cell lines showed a moderate differentiation of the carcinoma, as depicted in figure 5.

literature. By this criterion, partial or complete gains of 11p, 12p, 14q, 16p, 16q, 21q and 22q were included in this group.

Discussion

Cell lines are an important system for evaluating pharmacological and toxicological effects in humans [13]. They often also reveal the basic mechanisms of tumor growth and development and are a frequently used source

for research purposes [14]. Moreover, they represent a pure population of tumor cells without an admixture of inflammatory cells or fibroblasts, for example, typically seen as a desmoplastic reaction in solid tumors. This decreases the threshold for the detection of tumor-specific alterations such as genomic imbalances; however, cell lines of solid tumors are difficult to establish. This observation is still poorly understood and it is questionable whether there is a bias for successfully maintaining such cells in vitro. We therefore looked for chromosomal imbalances that part HCC cells from CC cells and/or for aberrations

Fig. 4. Chromosome 17 was the only chromosome aberrant in all cell lines. In particular, the long arm of this chromosome was always affected. However, as seen for chromosome 8, the pattern of aberrations varied. Nevertheless, gain of chromosomal material distal to q21.32 was detected in all samples.

probably needed for the successful in vitro culture of tumor cells.

Chromosomal imbalances parting HCC cell lines from CC cell lines were taken together in group 1. Here, we found gains of 1q at first. This was expected since these aberrations are reported most frequently in surgical tumor specimens with 57% of cases affected by gains of 1q21-2 as shown in a meta-analysis of 785 samples using conventional CGH analysis [12], and in 90% of samples using aCGH [15]. Similar reports have been made for the regions more distal on 1q including q23, q32 and q42, re-

spectively. We therefore assume these aberrations to be a basic event in carcinogenesis in surgical tumor samples as well as in cell lines of hepatocellular origin.

A further aberration seen in all HCC cell lines but in none of the CC cell lines was the loss of 13q14-q31.1. Losses of 13q have been reported in 27% of surgical specimens of HCC as a sign of dedifferentiation [12]. The loss is also seen in the context of increasing chromosomal instability [16, 17]. Deletion of 13q has been reported in other solid tumors, but with a frequency of less than 5% [18]. A recent study reported a loss of 13q occurring in CC

Fig. 5. Tumor specimens of cell lines MzChA-1 and TFK-1 grown in nude mice (kindly provided by N. Malek). For both cell lines, no loss of 13q was found and in both tumor samples a moderate (but not low) differentiation was seen.

[19]; however, conventional CGH was used with a much lower resolution of approximately 1 MB. Moreover, the authors did not include which part of the chromosome arms were affected but only listed aberrations as a loss or gain of complete arms, which is not precise. As 13q deletion is reported as a sign of dedifferentiation, we wondered whether the CC cell lines not bearing this aberration were of low differentiation or not. We therefore conducted a histological examination, by placing tumor cells into a mouse model. This was done successfully for 2 cell lines. Both of them revealed a moderate differentiation after in vivo growth. We therefore assume that where there is not a loss of 13q, this is in the context of a preserved capability of tumor cells to differentiate, rather than it being a characteristic of cholangiolar differentiation.

Imbalances of chromosome 8 were also helpful in differentiating HCC cell lines from CC cell lines. A gain of 8q13-qter occurred in all CC cell lines. In HCC cell lines, a part of 8q was gained in 3 of 5 cell lines only. Interestingly, in CC cell lines, the gain of 8q was accompanied by a loss of at least one copy of 8p in relation to 8q. This could be discussed as an isochromosome 8q [i(8q)]. This kind of aberration is the second most common isochromosome in human neoplasia and is frequently found in solid tumors such as adenocarcinoma (of the lung 22%, colon 15%, stomach 12%) and malignant melanoma (17%) [20]. It is frequently seen in acute myeloid leukemia

and myelodysplastic syndrome and data on malignant melanoma also suggest it is a secondary rather than a primary aberration [20]. No data are available to suggest which of the aberrations, gain of 8q or loss of 8p has the greater effect on tumor progression. To date, gain of 8q seems to be the more important abnormality. This is not in full accordance with earlier reports demonstrating i(8q) in a large number of HCC cell lines and in correlation with a dedifferentiation and chromosomal instability [11].

Loss of the short and gain of the long arm of a chromosome were also seen for chromosome 17. Indeed, this chromosome was the only one revealing the same aberration in all HCC and CC samples, i.e. gain of q21-qter. However, the regions spanned by this aberration were different, with HCC cell lines having a gain of 17q including the centromeric region and for some cases also parts of 17p. In CC cell lines, 17q+ was seen in varying extents with a common gained region starting at q21. Interestingly, in all CC cell lines, the gain of 17q was accompanied by a relative loss of 17p. We do not assume this finding to be an isochromosome i(17q), since the typical breaking region of i(17q) spans an interval of approximately 240 kB in the Smith-Magenis syndrome common deletion region in 17p11.2 [21]. Losses of 17p were not seen in HCC cell lines. Indeed, 4 of 5 cell lines revealed a gain of 17p interrupted by a not-gained region spanning from 17p12 to p13.2.

Besides these aberrations occurring frequently in our cell lines and in surgical specimens, we found HCC cell-line imbalances to be reported only rarely in the latter. These included gains of 11p, 12, 14q, 16p, 16q, 21q and 22q. Imbalances of these chromosomes are also rarely (<10%) found to occur in other solid tumors with the exception of chromosomes 12 and 16; 12p is gained in tumors of the female and male genital tract [22] and 16q is altered by losses and not gains in lobular carcinoma and lobular carcinoma in situ [23, 24]. It is assumed that E-Cadherin is lost as a consequence of this aberration leading to the development of the lobular phenotype. Loss of 16q in such cases is frequently accompanied by a gain of 16p.

Taking these findings together, there are chromosomal imbalances found in surgical specimens of HCC/CC as well as in the cell lines. In part, these aberrations are typical for hepatic and/or cholangiolar differentiation. There are also imbalances detectable in cell lines prob-

ably necessary for ongoing tumor cell growth in vitro. These aberrations are found rarely in the surgical tumor specimens. However, it is not clear why they occur. It may be speculated that the imbalances could already be in the tumor cells from which the cell lines are derived, in which case it would have to be assumed that these aberrations are helpful for the tumor cells to maintain in vitro. On the other hand, these aberrations could occur as secondary events, giving a growth advantage for the tumor cells in vitro. The question which of these alternatives applies could only be answered by the culture of cloned cells obtained directly from surgical tumor specimens.

In conclusion, carcinoma cell lines of hepatic and cholangiolar origin can be separated by aberrations of chromosomes such as 1, 8 and 13. There are, nevertheless, aberrations shared by all cell lines such as 17q+. It will be of interest to investigate the implications of these aberrations in further detail.

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