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The genetic differences between gallbladder and bile duct cancer cell lines

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Abstract. Biliary tract cancers carry dismal prognoses. It is commonly understood that chromosomal aberrations in cancer cells have prognostic and therapeutic implications. However, in biliary tract cancers the genetic changes have not yet been sufficiently studied. The aim of this study was to clarify the presence of mutations in specific chromosomal regions that are likely to harbor previously unknown genes with a significant role in the genesis of biliary tract cancer. The recently developed bacterial artificial chromosome (BAC) array comparative genomic hybridization (CGH) can facilitate detail analysis with high resolution and sensitivity. We applied this to 12 cancer cell lines of the gallbladder (GBC) and the bile duct (BDC) using a genome-wide scanning array. Cell line DNA was labeled with green colored Cy5 and reference DNA derived from normal human leucocytes was labeled with red colored Cy3. GBC, as well as BDC cell lines, have shown DNA copy number abnormalities (gain or loss). In each of the seven GBC cell lines, the DNA copy number was gained on 6p21.32 and was lost on 3p22.3, 3p14.2, 3p14.3, 4q13.1, 22q11.21, 22q11.23, respectively. In five BDC cell lines, there were DNA copy number gains on 7p21.1, 7p21.2, 17q23.2, 20q13.2 and losses were on 1p36.21, 4q25, 6q16.1, 18q21.31, 18q21.33, respectively. The largest region of gain was observed on 13q14.3-q21.32 (~11 Mb) and of loss on 18q12.2-q21.1 (~15 Mb), respectively. Both GBC and BDC cell lines have DNA copy number abnormalities of gains and/or losses on every chromosome. We were able to determine the genetic differences between gallbladder and bile duct cancer cell lines. BAC array CGH has a powerful potential application in the screening for DNA copy number abnormalities in cancer cell lines and tumors.

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Introduction

Biliary tract (gallbladder and bile duct) cancers carry dismal prognoses. However, few studies exist in the literature regarding the genetic changes in gallbladder and bile duct cancers (1-4). We set out to investigate the genetic changes in biliary tract cancers by bacterial artificial chromosome (BAC) array comparative genomic hybridization (CGH) as an extension of our research conducted in previous studies (5,6). The BAC array CGH was recently developed and is very efficient in identifying chromosomal loss regions, as well as gains, at the mega base level (7). We analyzed genomic changes in 7 gallbladder cancer cell lines and 5 bile duct cancer cell lines.

Materials and methods

Cell lines. As we have previously reported, TGBC1, TGBC2, TGBC14, TGBC24, TGBC44, Mz-ChA1, Mz-ChA2 (5) are gallbladder cancer (GBC) cell lines, and TGBC47, TGBC51, TBCN6 and KMBC are bile duct cancer (BDC) cell lines (6,8,9). The cells were cultured in DMEM with 10% FBS, except the TBCN6 cells, which were cultured in RPMI with 10% FBS. Genomic DNA was extracted using the Genomic tip (Qiagen) according to the manufacturer's instructions.

DNA labeling for BAC array CGH. Test and gender-matched reference DNAs were labeled by random priming in 50 μl reaction volumes containing 0.5 μg of genomic DNA using an array kit (Macrogen). Briefly, a 21 μl pre-mixture was prepared and added to 20 μl of random primer solution (Invitrogen). After denaturing the DNA for 5 min at 100˚C, dNTPs mixture solution was added, leading to a final labeling reaction containing 0.2 mM of dATP, 0.2 mM of dGTP, 0.2 mM of dTTP, 0.1 mM of dCTP, 0.1 mM of Cy3 or Cy5-dCTP (Perkin-Elmer) and 40 U of Klenow fragment (Invitrogen). After labeling, unincorporated nucleotides

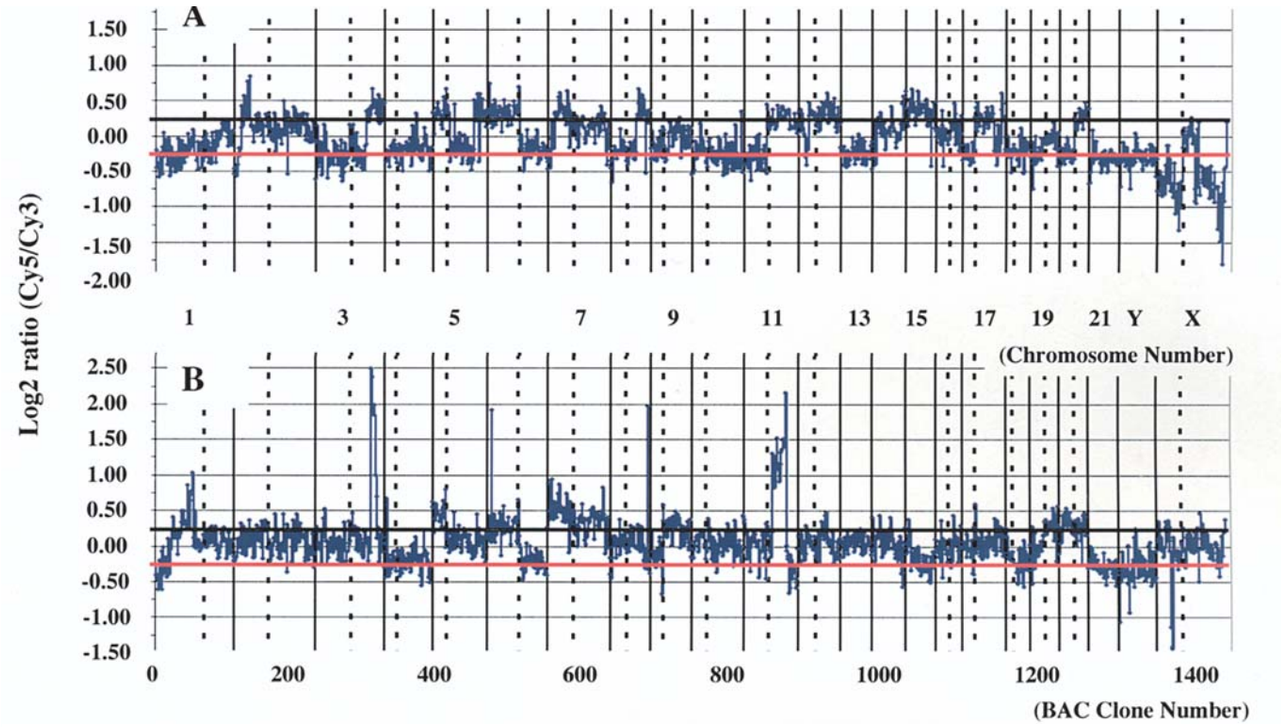

Figure 1. BAC array CGH profiles of gallbladder and bile duct cancer cells. The green line represents the cut-off level of the gain region and the red line represents the cut-off level of the loss region. BAC clones with a copy number ratio >0.3 were considered to be gained and those with a ratio ≤0.3 were considered to be lost. The vertical solid line and dotted line represent the boundary and the centromere of each chromosome. (A) and (B) represent TGBC24 (gallbladder cancer) and TBCN6 (bile duct cancer) cell lines, respectively.

were removed by using the QIAquick PCR purification kit (Qiagen).

BAC array hybridization, imaging and data analysis. Labeled test and reference DNAs were mixed with solution B of the array kit (Macrogen) and ethanol-precipitation. The pellet was dissolved in 80 μl of Solution C and 8 μl of solution D. Probes were denatured for 5 min at 73˚C, and incubated at 37˚C for 60 min to allow the blocking of repetitive sequences. The array slide was provided by Macrogen MAC Array™ KARYO 1,400 and pre-hybridized for 30 min at room temperature (RT). After two rinses of 10 sec each in sterilized distilled water and isopropanol, the slides were dried by centrifugation. The hybridization-to-wash procedure was performed by Hybristation (Genomic Solutions) automatically. Briefly, hybridization was performed under a 22x40 mm cover slip with incubation for 72 h at 37˚C. The slides were washed at 46˚C for 15 min in 50% formamide and took turns at 2X SSC, 2X SSC and 0.1% SDS at 46˚C for 30 min, PN buffer at RT for 15 min, and finally 2X SSC at RT for 5 min. After washing, the slides were dehydrated by 70, 85, and 100% ethanol at RT for 1 min each, followed by centrifugation drying. The arrays were scanned using GenePix4000A (Axon Instrument). The Mac Viewer software (Macrogen) was used to locate spots automatically on the Cy3 and Cy5 image acquisitions and to calculate fluorescence ratios. The Mac Viewer software automatically analyzed and summarized the results as follows: (i) Averaged the ratios of the replicates and calculated the standard deviation, (ii) rejected individual spot data based upon several criteria (including weak fluorescent signals), (iii) adjusted the Cy5/Cy3 ratios such that the ratios of the normal genomic regions were always equal to 0, despite variations in dye labeling efficiency, and (iv) plotted data relative to the position of the clones on human genome (according to July 2003 UCSC cartography).

Statistical analysis. Significant difference comparisons of the frequencies of chromosomal imbalances between GBC cell line and BDC cell lines were assessed using two-sided Fisher's exact test. P-values <0.05 were considered significant, unless otherwise specified. Fisher's exact test was carried out with Stat View J software version 5 (SAS Institute).

Results

Gallbladder cancer cell lines. Different profiles of genomic copy-number abnormalities are demonstrated on various chromosomal regions in each of the GBC cell lines (Fig. 1A). All chromosomes in each of the seven GBC cell lines had DNA copy number abnormalities. Gain regions were on 1p, 1q, 2p, 2q, 3q, 4q, 5p, 5q, 6p, 6q, 7p, 7q, 8q, 11q, 12p, 12q, 16q, 17q, 19q, 20q, and loss regions were on 1p, 3p, 3q, 4p, 4q, 5q, 6q, 7q, 8p, 8q, 9p, 9q, 10p, 10q, 11p, 13q, 14q, 16p, 17p, 18p, 18q, 19p, 19q, 21q, 22q, Xp, Xq, respectively. The frequent regional gain and loss loci (over 4 of 7 cell lines) are summarized in Table I. One gain region at 6p21.32 and six losses at 3p22.3, 3p14.2, 3p14.3, 4q13.1, 22q11.21 and 22q11.23 were detected in each of the seven GBC cell lines, respectively. Several known cancer-related genes (*PBX2*,

Table I. Chromosomal regions demonstrating DNA copy number abnormalities in seven GBC cell lines.

Gain	Loss
1p21.3, 1q21.1, *1q21.2-1q21.3*, 1q23.3, 1q24.1, 1q31.1	1p36.32-p36.33, 1p36.21, 1p34.3, 1p33-p34.2
2p24.1, 2p23.2-p23.3, 2p22.1-p23.1,	3p26.3, 3p25.1, [a]3p22.3, 3p21.31-p22.1,
2p16.1, 2p12, 2p11.2, 2q22.1, 2q23.3,	3p21.31, *3p21.2*, [a]3p14.3, [a]3p14.2,
2q32.2, 2q33.1, 2q34	3p14.1, 3p13-p14., 3p12.3, 3q22.2, 3q23
3q13.11, *3q26.2*, 3q27.3, 3q28	4p15.33-p16.1, [a]4q13.1, 4q13.3, 4q23,
4q28.1	4q25, 4q31.3, 4q35.1-q35.2
5p15.33, 5p15.1-p15.2, 5p13.2, 5p13.1,	5q13.1, 5q31.1, 5q33.3-q34
5p12, 5q14.2, 5q33.1, 5q33.3	6q15, 6q27
[a]*6p21.32*, 6q11.1	7q36.3
7p21.1-p21.2, *7p15.3*, 7p14.3, 7p13-p14.1,	8p23.2-p23.3, 8p21.3, 8q24.22
7p13, *7p11.2*, 7q21.2, 7q21.3	9p22.2, 9p21.3, 9q21.13, 9q34.3
8q21.13, 8q23.1-q23.3, 8q24.21, 8q24.3	10p15.3, 10p13, 10q11.21-q11.23,
11q13.3, 11q22.3	10q23.2, 10q24.33, 10q25.3, 10q26.13
12p13.33, 12p13.32, 12p12.1, 12q15,	11p15.5, 11p15.4, 11p15.2-p15.3, 11p13, 11p11.2
12q21.1, 12q21.32	13q12.3, 13q13.1-q13.2, 13q21.32
16q22.1	14q11.2, 14q22.3, 14q32.33
17q11.2, 17q21.31, 17q23.2	16p13.11, 16p12.1
19q13.32	17p12, 17p11.2
20q12, 20q13.2, 20q13.32, 20q13.33	18p11.21, 18q12.1, 18q12.2-q21.1,
	18q21.2-q21.31, 18q21.33, 18q21.33-q22.1, 18q23
	19p13.3, 19q13.42
	21q21.1, 21q21.3, 21q22.11, 21q22.11-q22.12,
	21q22.12, 21q22.13, 21q22-q22.3, 21q22.3
	[a]22q11.21, [a]22q11.23, 22q12.1, 22q12.2,
	22q12.3, 22q13.1, 22q13.31-q13.33
	Xp22.13, Xp11.3, Xp11.23, Xq22.2, Xq26.3, Xq28

[a]Indicates chromosomal abnormality regions detected in each of the seven GBC cell lines. Underlining represents regions that showed DNA copy number abnormalities in more than half of the number of cell lines (both GBC and BDC).

ITGA9, *Wnt-5a*, *BCR* and *RUTBC2*) were located on the following chromosomal locations: 6p21.32, 3p22.3, 3p14.3, 22q11.21 and 22q11.23, respectively (Tables III and IV). In terms of the frequent gain regions, the known cancer-related genes are *PDZK1* (1q21.1), *ARNT* (1q21.2-q21.3), *CD48* (1q23.3), *Tpr* (1q31.1), *KIAA0549* (2q33.1), *FHF1* (3q28), *RAD1*, *GDNF* (5p13.2), *SPARC* (5q33.1), *PBX2* (6p21.32), *IL6* (7p15.3), *GABS* (7p11.2), *IL7* (8q21.13), *BCL1/CCND1* (1q13.3), *krag* (12p12.1), *p53-associated* (12q15), *NF1* (17q11.2), *BRCA1* (17q21.31), *TBX2* (17q23.2), and *TOP1* (20q12). In terms of the frequent loss regions, the known cancer-related genes are *TGM4*, *IFRD2*/ *RASSF1*, *HYAL1* (3p21.31), *ACY1* (3p21.1), *FHI1*, *PTPRG* (3p14.2), *Wnt5a* (3p14.3), *AFP* (4q13.3), *RGS* (4q25), *APC* (5q33.3-q34), *PDCD2* (6q27), *NDRG1* (8q24.32), *RSU1* (10p13), *GFRA1* (10q25.3), *ILK*, *STS*, *MRV11* (11p15.4), *WT1*, *LMO2*, *EHF* (11p13), *DDB2* (11p11.2), *FLT1* (13q12.3), *BRCA2* (13q13.1-q13.2), *NRL* (14q1.2), *MRP* (16p13.11), *MBD*, *PCM1*, *DCC* (18q12.2-q21.1), *BCL2* (18q21.33), *TIAMI* (21q22.11), *ISK/KCNE2* (21q22.11-q22.12), *ETS2/E2* (21q22.1-q22.12), *BCR* (22q11.21), *BAM22* (22q12.2), *PDGFB* (22q13.1), *NDP* (Xp1.3), *PLP* (Xq22.2), *TRAG3*, and *X linked ALD* (Xq28). The largest, frequent gain was on chromosome 2q32.2 (~5.1 Mb in length); the largest loss was on 6q15 (~6 Mb in length).

Bile duct cancer cell lines. Different profiles of genomic copy-number abnormalities are demonstrated on various chromosomal regions in each of the BDC lines (Fig. 1B). All the chromosomes in each of the five BDC cell lines had DNA copy number abnormalities. Gain regions were on 1p, 1q, 2p, 2q, 3p, 3q, 4q, 5p, 5q, 6p, 6q, 7p, 7q, 8q, 9q, 10q, 11q, 12q, 13q, 14q, 17q, 20q, 22q, Xq, Yp and loss regions were on 1p, 3p, 3q, 4q, 6p, 6q, 8q, 0p, 9q, 11p, 11q, 14q, 15q, 16p, 17p, 18q, 19p, 19q, 21q, 22q, Xp, Xq, Yq, respectively. Frequent regional gain and loss loci (loci involved in over 3 of 5 cell lines) are summarized in Table II. Four gain regions at 7p21.1, 7p21.2, 17q23.3, 20q13.2 and five loss regions at 1p36.21, 4q25, 6q16.1, 18q21.31, and 18q21.33 were detected in each BDC cell line, respectively. Several known cancer-related genes as *HDAC9*, *Gax*, *TBX2*, *DOK5*, *NEDD4L* and *BCL2* were located on the following

Table II. Chromosomal regions demonstrating DNA copy number abnormalities in five BDC cell lines.

Gain	Loss
1p21.3, 1q24.1, 1q25.2, 1q31.1	1p36.33, 1p36.32, [a]1p36.21, 1p36.12,
2p16.1, 2p12, 2q23.3, 2q33.1, 2q33.3	1p36.11-1p35.3, 1p33
3p21.1, 3q13.11	3p26.3, 3p25.3, 3p25.1, 3p22.3, 3p21.33-p22.1,
4q12	3p21.31, 3p21.1, 3p14.3, 3p14.2,
5p15.33, 5p15.2, 5p14.1-p15.1, 5p13.2,	3p14.1, 3p13-p14.1, 3q28
5p12-p13.1, 5q14.2	4q21.23, 4q22.1, 4q23, [a]4q25, 4q25-q26
6p21.32, 6q11.1	6p25.2-p25.3, 6p21.33, 6q15-q16.1,
[a]7p21.1-p21.2, 7p14.3, 7p13-p14.1, 7p13,	*6q16.1 6q23.2, 6q23.3, 6q24.1, 6q25.1, 6q26-q27
7p11.2, 7q21.12, 7q21.2, 7q21.3, 7q31.2,	8p23.3
7q32.1, 7q32.3, 7q33, 7q34, 7q35	9q12
8q21.3, 8q23.1-q23.3	11p15.5, 11p15.4, 11p11.2, 11q23.1-11q23.2
9q21.11, 9q33.1	14q32.33
10q26.13	15q25.2
11q14.2	16p13.11
12q21.1	17p13.3, 17p13.2, 17p13.1, 17p11.2-p13.1
13q13.1, 13q13.2-q14.11, 13q14.13, 13q14.3,	18q12.2-q21.1, 18q21.1, [a]18q21.31,
13q14.3-q21.32, 13q21.33, 13q22.2-q22.3,	18q21.31-q21.33, [a]18q21.33, 18q23
13q32.1, 13q33.1-q34	19p13.3, 19p13.2, 19q13.42
14q31.1	21q22.11-q22.12, 21q22.13, 21q22.2-q22.3,
17q11.2, 17q12, 17q21.2, 17q22, [a]17q23.2	21q22.3, 21q22.3, 21q22.3
20p12.3, 20p12.1, [a]20q13.2	22q11.21, 22q11.22-q11.23, 22q11.23, 22q12.1, 22q12.1-q12.2,
22q11.21	22q12.2, 22q12.3, 22q13.1, 22q13.1, 22q13.31, 22q13.33
Xq25	Xp11.23, Xp11.21, Xq28
Yp11.31	Yq11.223, Yq11.23

[a]Indicates common chromosomal abnormality regions detected in each of the 5 BDC cell lines. Underlining represents regions that showed DNA copy number abnormalities in more than half of the cell lines (both GBC and BDC).

chromosomal regions: 7p21.1, 7p21.2, 17q23.3, 20q13.2, 18q21.31, and 18q21.33, respectively (Tables III and IV). In terms of the frequent gain regions, known cancer-related genes are *Tpr* (1q31.1), *KIAA0549* (2q33.1), *II17BR* (3p21.1), *KDR* (4q12), *GDNF* (5p13.2), *PBX2* (6p21.32), *MET* (7q31.2), *DACH* (13q21.33), *EDNRB* (13q22.2-q22.3), *ERCC5* (13q33.1-q34), *TSHR* (14q31.1), *NF1* (17q11.2), *HER2*, *ERBB2* (17q12), *HLF* (17q22), and *TBX2* (17q23.3). On the frequent loss regions, known cancer-related genes are *CDC2L1* (1p36.33), *p73* (1p36.32), *FGR* (1p36.11-p35.3), *LMCD1* (3p25.3), *LFRD2/RSSF1*, *HYAL1* (3p21.31), *BAP1* (3p21.1), *Wnt5a* (3p14.3), *LPP* (3q28), *RGS* (4q25-q26), *IRF4* (6p25.2-p25.3), *HLA-B* (6p21.33), *MYB* (6q23.3), *PDCD2* (6q26-q27), *DDB2* (11p15.4), *MRP* (16p13.1), *LIS1* (17p13.3), *MBD*, *PCM1*, *DCC* (18q21.1), *BCL2* (18q21.31-q21.33), *ICAM1* (19p13.2), *ISK/KCNE2* (21q22.11-q22.12), *ETS2/E2* (21q22.2-q22.3), *BCR* (22q11.21), *BCR-ABL*, *RAB36* (22q11.22-q11.23), *EVS*, *BAM2* (22q12.2), *PDGFB* (22q13.1), *ECGF1* (22q13.33), *YRRM2* (Yq11.223), and *CDY1* (Yq11.23).

The largest, frequent gain was on chromosome 13q14.3-q21.32 (~11 Mb in length); the largest loss was on 18q12.2-q21.1 (~15 Mb in length). Chromosome numbers 12, 13 and 21 displayed DNA copy number abnormalities in each chromosomal region (data not shown).

Unique candidate genes related to biliary tract cancer cells. We tried to determine the genes that were related to malignancy uniquely in GBC and BDC by comparing the incidence of DNA copy number abnormalities in each chromosomal region. In the BDC cell lines, the candidate genes, *NA4B* on the locus 7q32.3 and *SEC8L1* on the locus 7q33, were extracted by their significant DNA copy number gain. In the GBC cell lines, *SAMD10* and *SOX18* on the locus 20q13.33 were nominated as unique cancer-related genes based upon gains in the DNA copy number, whereas, *ROBO1* on 3p12.3 and *ITK* and *CYFIP2* on 5q33.3 were unique candidate genes because they displayed significant losses of DNA copy number. Although the 4q13.1 locus showed a significant DNA copy number loss in the GBC cell lines, no cancer-related gene was identified (Table V).

Discussion

By our high resolution BAC array CGH, loss regions were more precisely detected in this study than previous studies by

Table III. The incidence of cell lines showing DNA copy number loss and the candidate cancer-related genes on individual chromosomal regions of GBC and BDC cell lines.

No.	Chromosomal regions	Rate of abnormal DNA copy number in each cell line		Candidate cancer-related gene
		GBC (n=7)	BDC (n=5)	
1	1p36.21	85.7% (6/7)	100% (5/5)	
2	1p34.1	57.1% (4/7)	0%	*PTCH2*
3	3p12.3	85.7% (6/7)	0%	
4	3p14.2	100% (7/7)	80% (4/5)	*FHIT*, *PTPRG*
5	3p14.3	100% (7/7)	60% (3/5)	*Wnt5a*
6	3p22.3	100% (7/7)	60% (3/5)	*ITGA9*
7	4q13.1	100% (7/7)	40% (2/5)	
8	4q25	57.1% (4/7)	100% (5/5)	
9	5q31.1	57.1% (4/7)	0%	
10	5q33.3	57.1% (4/7)	0%	
11	6p21.33	0%	80% (4/5)	*HLA-B*
12	6q16.1	42.9 % (3/7)	100% (5/5)	
13	6q23.2	0%	60% (3/5)	
14	7q36.3	57.1% (4/7)	0%	
15	9p21.3	57.1% (4/7)	0%	
16	9q21.13	57.1% (4/7)	0%	
17	16p12.1	57.1% (4/7)	0%	
18	18q21.31	71.4% (5/7)	100% (5/5)	
19	18q21.33	85.7% (6/7)	100% (5/5)	*BCL2*
20	22q11.21	100% (7/7)	80% (4/5)	*BCR*
21	22q11.23	100% (7/7)	80% (4/5)	*RUTBC2*

Table IV. The incidence of cell lines showing DNA copy number gain and the candidate cancer-related genes on the individual chromosomal regions of GBC and BDC cell lines.

No.	Chromosomal regions	Rate of abnormal DNA copy number in each cell line		Candidate cancer-related gene
		GBC (n=7)	BDC (n=5)	
1	1Q21.1	57.1% (4/7)	0%	*PDZK1*
2	1Q21.2-Q21.3	57.1% (4/7)	0%	*ARNT*
3	2Q33.3	0%	60% (3/5)	
4	3Q28	57.1% (4/7)	0%	
5	3Q28	57.1% (4/7)	0%	
6	3Q28	57.1% (4/7)	0%	
7	3Q28	57.1% (4/7)	0%	*FHF-1*
8	4Q12	0%	60% (3/5)	*KDR*
9	4Q28.1	57.1% (4/7)	0%	
10	6P21.32	100% (7/7)	60% (3/5)	*PBX2*
11	7P21.1	57.1% (4/7)	100% (5/5)	*HDAC9*
12	7P21.2	85.7% (6/7)	100% (5/5)	*Gax*
13	7P21.2	0%	60% (3/5)	*ETV1*
14	7Q32.3	0%	80% (4/5)	
15	7Q33	0%	80% (4/5)	
16	7Q34	0%	60% (3/5)	*TCRB*
17	12P12.1	57.1% (4/7)	0%	*krag*
18	13Q14.13	0%	60% (3/5)	
19	13Q21.32	0%	60% (3/5)	
20	17Q23.3	85.7% (6/7)	100% (5/5)	*TBX2*
21	20Q13.2	71.4% (5/7)	100% (5/5)	*DOK5*
22	20Q13.33	57.1% (4/7)	0%	

Table V. Candidate genes related to malignancies unique in GBC/BDC.

No.	Region	Abnormality	Incidence		P-value	Candidate gene (s)
			GBC	BDC		
1	7q32.3	Gain	0	4	0.0101	*PLXNA4B*
2	7q33		0	4	0.0101	*SEC8L1*
3	20q13.33		5	0	0.0278	*SAMD10*, *SOX18*
4	3p12.3	Loss	6	0	0.0152	*ROBO1*
5	4q13.1		7	2	0.0455	
6	5q33.3		5	0	0.0278	*ITK*, *CYFIP2*

The p-value was obtained by Fisher's exact test.

conventional CGH (5,6). In 21 chromosomal regions of either BDC or GBC cell lines, the DNA copy number loss was exhibited frequently (Table III). Regions 3p12.3 and 6p21.33 should be noted in terms of unique DNA copy number loss. At region 3p12.3, six of the seven GBC cell lines showed a DNA copy number loss, although each of the 5 BDC cell lines did not show a DNA copy number loss. On the contrary, at region 6p21.33, the 7 GBC cell lines did not show a DNA copy number loss, although four of the five BDC cell lines showed a DNA copy number loss (Table III).

In addition, 22 chromosomal regions demonstrated DNA copy number gains in either the BDC or GBC cell lines (Table IV). However, no cancer-related gene has been identified on 7q32.3 or 7q33 regions. Further studies on these gain regions might be significant for distinguishing GBC and BDC (Table IV).

Seven well-known cancer-related genes (*PBX2*, *FHIT*, *PTPRG*, *Wnt5a*, *ITGA9*, *BCR* and *RUTBC2*) were detected in the seven gallbladder cell lines (Tables III and IV). The *PBX2* gene is located on 6p21.32 and encodes a homeodomain protein, which is a member of the three-amino-acid loop extension (TALE) family. PBX proteins interact with a number of Hox proteins. *PBX* genes, including *PBX2*, have an essential role in embryonic development (10). *FHIT*, *PTPRG* and *Wnt5a* genes are located on 3p14.2-p14.3. These regions have already been reported to display a loss of heterozygosity (LOH) in lung, esophageal, stomach and gallbladder cancers (11-14). On the 3p22.3 region, the *ITGA9* gene encodes alpha integrin. We first found that this region was deleted commonly in gallbladder cancers, but we could not determine whether the deletion was homozygous or heterozygous as in renal, lung and breast cancers (15). The *BCR* gene is located on the 22q11.21 region and this particular region is the site of translocation t(9:22) in chronic myelogenous leukemia (16). The *RUTBC2* gene (one of the human proteome genes) (17) is located on the 22q11.23 region. This locus often displays LOH or homozygous deletions in some pediatric rhabdoid tumors (18,19), but the loss of this region in gallbladder cancers has not been reported.

In BDC, four well-known cancer-related genes (*HDAC9*, *Gax*, *TBX2*, and *DOK5*) were detected in all five BDC cell lines (Tables III and IV). The *HDAC9* gene is located on 7p21.1 and is a member of the histone deacetylase family. It is known that this gene has alternative splicing variants to generate multiple protein isoforms that may play distinct biological roles and that this gene is associated with human cancer (20). The *Gax* gene is located on 7p21.2 and is one of the homeobox genes that is closely related to angiogenesis (21). The *TBX2* gene is located on 17q23.3 and this region is also the site of gains in many cancers (22-25). The *TBX2* gene is a member of the T-Box transcription factors contributing to oncogenic transformation (26). The *DOK5* gene is located on 20q13.2 and is a member of a downstream of tyrosine kinase family related to myeloid homeostasis and leukemia (27). We discovered a loss region (18q21.33), which encodes a well-known *BCL2* gene and this gene plays an anti-apoptotic role (28). However, on other loss regions (1p36.21, 4q25 and 6q16.1) commonly detected in all five BDC lines, no cancer-related gene was found (Tables III and IV).

In the statistical analysis, the significant gain genes were *PLXNA4B* (7q32.3, p=0.0101) and *SEC8L1* (7q33, p=0.0101) in BDC. The *PLXNA4B* gene produces a protein product that is a member of the semaphorin family, and semaphorins are related to one of the axon guidance molecules (29). The *SEC8L1* gene is a member of the *sec8* gene that is related to the exocyst complex (30).

The significant gains in GBC were in the *SAMD10* and *SOX18* genes (20q13.33). The *SAMD10* gene produces a protein product that has a sterile alpha motif domain which not only exhibits diverse protein-protein interactions but also has the ability to bind RNA, defining a new type of post-transcriptional modification gene (31). The *SOX18* gene is one of the Sry-type high-mobility group box gene family, which encode transcription factors in diverse developmental processes, and the mutation of this gene is related to hypotrichosis-lymphedema-telangiectasia (32). On the contrary, the significant losses were only apparent in GBCL: *ROBO1* (3p12.3, p=0.0278) and *ITK* and *CYFIP2* (5q33.3, p=0.0278). The *ROBO1* gene, which encodes a member of the neural cell adhesion molecule family of receptors, was recently cloned from the lung cancer tumor suppressor gene region 2, and resides in a region that has been the site of overlapping homozygous deletions characterized in both

small cell lung cancer cell lines and in a breast cancer cell line (33). The *ITK* gene encodes a product that is a member of T cell-specific Tec family, plays a role in the maturation of thymocytes, is required for intracellular signaling following T cell receptor (TCR) crosslinking, and is involved in the generation of second messengers that mediate cytoskeletal reorganization (34). The *CYFIP2* gene encodes a protein that is a member of a highly conserved protein family and that is expressed mainly in brain tissue, white blood cells and the kidney. The *CYFIP2* is a *p53*-inducible protein, thus, possibly a pro-apoptotic gene (35). Although the 4q13.1 locus was also the site of significant loss (p=0.0455) in GBC, on this locus no cancer related gene has been identified.

Although DNA copy number abnormalities were observed in every chromosome, chromosomes 12, 13 and 21 demonstrated abnormalities of all the BAC clones in each chromosomal region. These observations suggest that chromosomes 12, 13 and 21, at least in part, might be linked to cancer developments and progression.

In conclusion, both GBC and BDC cell lines have DNA copy number abnormalities of gains and/or losses on every chromosome and we were able to determine the genetic differences between gallbladder and bile duct cancer cell lines using BAC array CGH. Therefore, BAC array CGH has potential application in the screening for DNA copy number abnormalities in cancer cell lines and tumors.

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