Frequent Increase of DNA Copy Number in the 2q24 Chromosomal Region and Its Association with a Poor Clinical Outcome in Hepatoblastoma: Cytogenetic and Comparative Genomic Hybridization Analysis

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In a cytogenetic and comparative genomic hybridization (CGH) study of 38 hepatoblastomas, we found gain of 1q in 17 tumors (44.7%), that of 2/2q in 14 (36.8%), that of 20/20q in 9 (23.7%) and that of 8/8q in 8 (21.0%), loss of 4q in 4 (10.5%) and no DNA copy changes with normal karyotype or no mitotic cells in 11 (28.9%). Eleven tumors with 2/2q gain detected by CGH had a total chromosome 2 gain, a partial 2q gain, or a total chromosome 2 gain with an augmented partial 2q region; the common region for DNA copy gain was 2q24. Two-color fluorescence in situ hybridization (FISH) analyses using probes covering the centromere of chromosome 2 or HOXD13 (2q31) confirmed the CGH findings, and showed that the common region for gain in 2q was centromeric to HOXD13. Event-free survival (EFS)±±±±standard error (SE) at 5 years was lowest in patients with 2q gain [37±±±±15%], highest in those with no DNA copy changes [82±±±±12%], and intermediate in those with DNA copy changes other than 2q gain [74±±±±13%] (P==0.0549). Multivariate analysis showed that 2q gain was an independent factor predicting a poor outcome. These findings suggest the presence of a growth-promoting gene or an oncogene in the 2q24 chromosome band, and a tumor suppressor gene in terminal 4q, which have important roles in the development and progression of hepatoblastoma.

Key words: Hepatoblastoma — CGH — FISH — Chromosome abnormalities — 2q gain

Hepatoblastoma is a malignant hepatic tumor in children, which occurs mostly in the first 3 years of life.

Due to the rare incidence, cytogenetic and molecular-genetic studies in hepatoblastoma have been limited compared with those of other childhood tumors. Common cytogenetic abnormalities reported included trisomies of chromosome 2, 5, 8 and/or 20, and 1q trisomy, which is occasionally associated with 4q deletion; namely, der(4)t(1;4)(q12;q34).2-5 Recently, comparative genomic hybridization (CGH) analysis has been performed in 3 series of hepatoblastomas, which confirmed cytogenetic findings reported previously.6-8

Although most hepatoblastomas are sporadic, they also occur in association with Beckwith-Wiedemann (B-W) syndrome and familial adenomatous polyposis (FAP).9,10 APC mutation and/or loss of heterozygosity in the APC locus were reported in some children with both sporadic and the FAP-associated hepatoblastomas.11,12 More recently, mutation of the β-catenin gene was reported in approximately half of the sporadic hepatoblastomas examined.13 Although previous cytogenetic and CGH analyses reported common chromosomal regions of gain or loss, only one study attempted to determine the prognostic implication of these genetic changes,8 because of the small number of hepatoblastomas included in each series. We performed cytogenetic and CGH analysis in 38 children with hepatoblastoma, and identified frequent DNA copy changes in distinct chromosomal regions including 1q and 2q. Moreover, fluorescence in situ hybridization (FISH) and chromosome analyses narrowed down the region of 2q gain, and revealed a cytogenetic mechanism of DNA copy gain in 2q in one tumor. We also found that 2q gain was associated with poor outcome of hepatoblastoma patients.

MATERIALS AND METHODS

Patients and samples (Table I) Tumors were obtained from 38 Japanese children with hepatoblastoma who underwent biopsy or surgery between March 1989 and September 1999. Thirteen and 25 tumors were obtained...
before and after chemotherapy, respectively. There were 21 males and 17 females, ranging in age from 3 months to 8 years with a median age of 1 year. Patients were staged according to the system proposed by the Children’s Cancer Study Group.¹⁴ The majority of patients were treated according to the protocols of the Japan Hepatoblastoma Study Group.¹⁵ The pathological diagnosis was made based on routine hematoxylin/eosin-stained slides by

### Table I. Clinical and Pathological Characteristics of 38 Patients with Hepatoblastoma

| Case No. | Age/sex | Histologic type | AFP⁶ (ng/ml) | Stage | Chemotherapy before surgery | Survival⁸ (months) | Present⁹ status | Comment¹⁰ |
|----------|---------|----------------|--------------|-------|-----------------------------|-------------------|----------------|-----------|
| N patients (n=11): normal CGH pattern and normal karyotype or no mitotic cells |
| 356 | 6m/M | Unclassifiable | Negative | 1 | – | 134+ | NED |
| 669 | 1/F | Embryonal | 330 000 | 4 | – | 107+ | NED |
| 834 | 6m/F | Embryonal | 2 600 000 | 4 | + | 94+ | NED |
| 893 | 5m/F | Fetal | 2 070 000 | 2 | + | 92+ | NED |
| 1303 | 3/F | Fetal | 398 106 | 1 | – | 52+ | NED |
| 1384 | 8/M | Macrotabecular | Negative | 4 | + | 8 | DOD |
| 1471 | 2/F | Embryonal | 525 800 | 4 | + | 48+ | NED |
| 1891 | 1/M | Embryonal | 2 1845 | 1 | – | 24+ | NED |
| 1918 | 4m/M | Fetal | 378 500 | 2 | + | 27+ | NED |
| 2116 | 11m/M | Fetal | 905 160 | 2 | + | 13+ | AWD |
| 2201 | 5/F | Embryonal | 600 000 | 4 | + | 10 | DOD |
| A1 patients (n=14): 2q gain detected by CGH and/or cytogenetic analysis |
| 843 | 8/F | Embryonal | 1 110 000 | 4 | + | 8 | DOD |
| 972 | 1/F | Fetal | 1 400 000 | 2 | + | 16 | DOD |
| 991 | 7m/M | Embryonal | 776 | 4 | + | 80+ | NED |
| 1067 | 1/M | Mixed (E & M) | 300 000 | 2 | + | 89+ | NED |
| 1103 | 2/M | Embryonal | 16 348 | 4 | + | 22 | DOD |
| 1117 | 1/F | Fetal | 414 | 1 | – | 83+ | NED | CHD |
| 1134 | 2/M | Embryonal | 2 110 000 | 3 | – | 5 | DOD | CHD, epilepsy, MR |
| 1408 | 1/F | Embryonal | 611 596 | 4 | + | 15 | DOD |
| 1694 | 1/M | Embryonal | 252 250 | 2 | + | 24+ | NED | CHD, microcephalus |
| 1975 | 2/F | Embryonal | 64 000 | 2 | + | 21+ | NED |
| 2093 | 1/M | Embryonal | 87 411 | 3 | + | 14+ | AWD |
| 2150 | 3/F | Unclassifiable | 846 214 | 4 | + | 11+ | AWD |
| 2198 | 1/F | Embryonal | 177 190 | 1 | – | 10+ | NED |
| 2230 | 1/F | Fetal | 53 350 | 4 | + | 10+ | AWD |
| A2 patients (n=13): CGH and/or cytogenetic changes other than 2q gain |
| 692 | 3m/F | Fetal | 329 287 | 1 | – | 105+ | NED | B-W syndrome |
| 769 | 1/F | Embryonal | 390 000 | 1 | – | 105+ | NED |
| 990 | 1/F | Fetal | 920 | 1 | – | 82+ | NED |
| 1057 | 8m/M | Unclassifiable | 5 480 | 2 | + | 85+ | NED |
| 1107 | 2/M | Unclassifiable | 45 830 | 3 | + | 22 | DOD | Prematurely born |
| 1113 | 4/M | Unclassifiable | 875 | 4 | + | 116+ | NED |
| 1148 | 6m/M | Fetal | 290 000 | 2 | + | 69+ | NED |
| 1194 | 8m/M | Fetal | 420 000 | 2 | – | 65+ | NED |
| 1358 | 7/M | Small cell | Negative | 2 | – | 54+ | NED | Li-Fraumeni syndrome |
| 1416 | 3/M | Fetal | 41 987 | 3 | – | 17 | DOD |
| 1495 | 4m/M | Fetal | 1 405 020 | 2 | + | 39+ | NED |
| 1748 | 4m/M | Embryonal | 500 000 | 2 | + | 24+ | NED | B-W syndrome |
| 1905 | 1m/F | Fetal | 5 723 300 | 2 | + | 15+ | NED |

a) Mixed (E & M), mixed epithelial and mesenchymal; small cell, small cell undifferentiated.

b) AFP, α-fetoprotein.

c) + after survival indicates that the patient is alive.

d) NED, no evidence of disease; AWD, alive with disease; DOD, died of disease.

e) B-W, Beckwith-Wiedemann; CHD, congenital heart disease; MR, mental retardation.
pathologists at each institution according to the classification proposed by Haas et al.18) Of the 38 hepatoblastomas, 15 were classified as fetal histologic type, 15 as embryonal histologic type, 1 as mixed epithelial and mesenchymal histologic type, 1 as macrotrabecular histologic type, 1 as small cell undifferentiated histologic type, and 5 as unclassifiable because of necrosis due to preoperative chemotherapy. Hepatoblastomas occurred in 2 patients with B-W syndrome (Nos. 692 and 1748), in 3 patients with congenital heart disease with or without other malformations (Nos. 1117, 1134 and 1694), in 1 patient with Li-Fraumeni syndrome (No. 1358), and in 1 patient (No. 1107) who was born prematurely.

**Cytogenetic studies** Chromosomes were studied as described previously.19) Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) 1995.18) The BAC probe was located in 2q31.19) The BAC probe was labeled with biotin (bio)-16-dUTP (Boehringer, Mannheim, Germany) by nick translation. Interphase and metaphase cells were stained with DAPI. Two-color FISH analysis using probes covering the centromere of chromosome 2 (CEP 2, Spectrum Orange) (VYSIS, Downers Grove, IL), and a BAC probe (RBP1) covering HOXD13 (Fig. 1), 12 had trisomy, tetrasomy, or hexasomy 1q, 6 had trisomy 8, and 4 had trisomy 20. Two tumors had an unbalanced 1;4 translocation resulting in a deletion of 4q34–4qter and 1q trisomy. Combined findings of cytogenetic and CGH analyses

**RESULTS**

**Cytogenetic findings** Of 38 hepatoblastomas in which cytogenetic analysis was performed, 9 (23.7%) had no mitotic cells, 13 (34.2%) had only normal mitotic cells, and 16 (42.1%) had clonal abnormal cells (Table II). Twelve tumors had trisomy 2 or trisomy or pentasomy 2q (Fig. 1), 12 had trisomy, tetrasomy, or hexasomy 1q, 6 had trisomy 8, and 4 had trisomy 20. Two tumors had an unbalanced 1;4 translocation resulting in a deletion of 4q34–4qter and 1q trisomy.

**CGH findings** Findings from CGH analysis of 38 hepatoblastomas are presented in Table II and Fig. 2. Of 38 tumors, 24 (65.8%) had DNA copy number changes in at least one chromosomal region. Fourteen tumors had gain of 1q, 11 had gain of 2/2q, 5 had gain of 20/20q and 3 had gain of 8q. Eight tumors had loss of chromosome arm 4q. Of the 11 tumors with 2/2q gain, 4 (Nos. 1003, 1117, 1134 and 1975) had a total chromosome 2 gain, 3 (Nos. 1694, 2198 and 2230) had a partial 2q gain, 3 (Nos. 843, 972 and 991) had a total chromosome 2 gain with an augmented partial 2q region (Fig. 1), and one (No. 1408) had gains of 2pter–2q24 and 2q32–qter. The most common region for increased DNA copy number was 2q24.

**Combined findings of cytogenetic and CGH analyses** Of 29 tumors from which mitotic cells were obtained, 6 tumors had normal karyotypes and normal CGH patterns, 7 had normal karyotypes and abnormal CGH patterns, 3 had abnormal karyotypes and normal CGH patterns, and 13 had abnormal karyotypes and abnormal CGH patterns. Of the other 9 tumors from which no mitotic cells were obtained, 5 and 4 had normal and abnormal CGH patterns, respectively.

Combined findings of cytogenetic and CGH analyses showed gain of 1q in 17 tumors (44.7%), that of 2/2q in 14 (36.8%), that of 20/20q in 9 (23.7%) and that of 8q in 8 (21.0%), and loss of 4q in 4 (10.5%). The most common regions for gain were 1q32–1qter and 2q24, and the most common region for loss was 4q34–4qter.

**Interphase and metaphase FISH findings** Two-color FISH analysis using probes covering the centromere of chromosome 2 or HOXD13 (2q31) was performed in 9 of
Table II. Cytogenetic, CGH and FISH Findings in 38 Hepatoblastomas

| Case No. | Karyotype                                      | CGH findings       | HOXD /CEP2 | Gains of 1q | 2q |
|----------|-----------------------------------------------|--------------------|------------|-------------|----|
|          |                                               | Gain               | Loss       |             |    |
| A1 patients (n=14): 2q gain detected by CGH and/or cytogenetic analysis |                   |                     |            |             |    |
| 843      | 53.X−,X_dup(2)(q23q37),+dup(2)(q23q37),+5,+12, +der(16)(1;16)(q12;q24),+20,+3mar | 1q21−qter, 2qter−qter(2q23−q36), 6,10q24−q26, 20'  | Xp         | 4/3         | +  |
| 972      | 47.X−,X,+2,der(4)(1;4)(q21;q34),+mar,ace      | 1q21−qter, 2qter−qter(2q23−q31)' | QXq26−qter  |             |    |
| 991      | 48.XY,+i(1)(q10),dup(2)(q24q34),+dup(2)(q24q34) | 1q12−qter, 2qter−qter(2q24−q34), 9, 16, 17, 22 | Q433−qter   | 3/3         | +  |
| 1067     | 51.XY,+2,der(4)(1;4)(q12q34),+5,+8,+8,+20    |                     |             |             |    |
| 1103     | 52.XY,+2,+3,+7,+15,+20                        | 1q12−qter, 2, 8q'  |             |             |    |
| 1117     | 52.XY,+2,+3,+7,+15,+20                        |                     |             |             |    |
| 1134     | 47.XY,add(2)(q37),+add(2)(q37), other changes | 1q31−qter, 2, 17q22−qter |             |             |    |
| 1408     | 48.XY,+20,+der(1)(?)/(q31;?),dimin            | 1q31−qter, 2ter−q22, 2q23−qter, 20 | Q434−qter   | 2/3         | +  |
| 1694     | 46.XY,dup(2)(q24q31),der(21)t(1;21)(q21;p11) /48,idem,+dup(2)(q22q31),+mar | 1q21−qter, 2q21−q31 |             |             |    |
| 1975     | 48.XY,+2,+7,der(22)t(1;22)(q12;q11)           | 1q12−qter, 2, 7    |             |             |    |
| 2093     | 47.XY,+2                                      |                     |             |             |    |
| 2150     | 50.XY,+2,+5,+8,der(20)t(1;20)(q12;q11), +der(20)(1;20)(q12;q11) | 1q23−qter, 19 |             |             |    |
| 2198     | 51.XY,+2,+5,+8,+der(1)(?)/(q21;?),+mar        | 1q24−qter, 2q24qter |             |             |    |
| 2230     | 46.XY,dup(2)(q23q37),der(17)t(1;17)(q21;q12) | 2q23−q37           |             |             |    |
|          |                                               |                     |            |             |    |
| A2 patients (n=13): CGH and/or cytogenetic changes other than 2q gain |                   |                     |            |             |    |
| 692      | 46.XY                                         | 1q31−qter, 5q31,'  |             |             | +  |
| 769      | 46.XY                                         | 1q24−q44           |             | Q421−qter   | +  |
| 990      | 46.XY                                         | 20q                |             |             |    |
| 1057     | 51.XY,+i(1)(q10),+i(1)(q10),+8,+20,+21       | 1q23−qter, 6q13−q23 |             |             |    |
| 1107     | NM                                            | 1q24−qter, 8q, 17,' 20q |             |             |    |
| 1131     | 61−91,i(1)(q10), other complex changes        | 1q23−qter, 1p34−qter |             |             |    |
| 1148     | 46.XY                                         | X'                 |             |             |    |
| 1194     | 46.XY                                         | 21q22−qter         |             |             |    |
| 1358     | 46.XY                                         | 3p24−qter, 5q33−q35,' |             |             |    |
| 1416     | NM                                            | 6p12−qter          |             |             |    |
| 1495     | NM                                            | Xq21−q28           |             |             |    |
| 1748     | 48.XY,+8,+20                                 | 6p12−p21           |             |             |    |
| 1905     | 46.XY                                         | 1p34−qter, 19, 20  |             |             |    |

NM, no mitotic cells; * high-level DNA copy gain; HOXD/CEP2, see the legend to Fig. 2.
11 tumors with gain of 2/2q detected by CGH (Fig. 2, Table II). The 2 probes were chosen because \textit{HOXD13} was located close to the common region for 2q gain, and the centromere probe was needed as a control to show the number of chromosome 2. Two tumors (Nos. 1134 and 1975) with a total chromosome 2 gain had 3 centromeric and 3 \textit{HOXD13} signals as expected. The signal number of \textit{HOXD13} was greater than that of the centromere in 5 of the 6 tumors with a partial 2q gain (Nos. 1694, 2198 and 2230) or a total chromosome 2 gain with an augmented partial 2q region (Nos. 843 and 991), and the same signal number of \textit{HOXD13} and the centromere was seen in the other (No. 972). One tumor (No. 1408) with gains of 2pter–2q24 and 2q32–qter had 3 centromeric and 2 \textit{HOXD13} signals, and the finding confirmed the normal DNA copy of the 2q31 region. Thus, the interphase FISH findings were consistent with the CGH findings, and indicated that \textit{HOXD13} was included in the common region for DNA copy gain of 5 out of 6 tumors, and that the common region was centromeric to the \textit{HOXD13} (2q31) region.

FISH revealed metaphase cells in one tumor (No. 991) (Fig. 3). Normal chromosome 2 had 1 centromeric and 1
HOXD13 signals as expected, and 2 dup(2) chromosomes had 1 centromeric and 2 HOXD13 signals, which were apart. Cytogenetic, CGH and FISH findings indicated that the tumor cells had 5 copies of the 2q24–q34 region as a result of duplication of the 2q24–q34 region, and the subsequent duplication of the dup(2) chromosome.

**Statistical findings** Patients were classified into 3 groups on the basis of combined cytogenetic and CGH findings; namely, 11 N patients with normal CGH and normal or no cytogenetic findings, and 27 A patients with abnormal CGH and/or abnormal cytogenetic findings. The 27 A patients were further classified as 14 A1 patients with 2q gain and 13 A2 patients with abnormalities other than 2q gain (cytogenetic and CGH classification 1), or 17 A3 patients with 1q gain and 10 A4 patients with abnormalities other than 1q gain (cytogenetic and CGH classification 2). Event-free survival (EFS)±standard error (SE) at 5 years was lowest in A1 patients [37±15%], highest in N patients [82±12%] and intermediate in A2 patients [74±13%] (P=0.0549) (Fig. 4). EFS±SE at 5 years was lower in A3 patients [40±14%] than in N patients [82±12%] or in A4 patients [80±13%] (P=0.1062). Overall survival (OS)±SE at 5 years was lower in A1 patients [48±17%] than in N patients [81±12%] or in A2 patients [82±12%] (P=0.1397) (Fig. 5). OS±SE at 5 years was lower in A3 patients [54±14%] than in N patients [82±12%] or in A4 patients [88±12%] (P=0.2286).

Seven of the 11 N patients were treated with chemotherapy before surgery. To avoid the possible influence of chemotherapy, the 7 patients were excluded from the next analysis. The P values for EFS and OS were 0.0378 and 0.0503, respectively, among 4 N, 14 A1 and 13 A2 patients, and 0.0758 and 0.1126, respectively, among 4 N, 17 A3 and 10 A4 patients. The findings indicated that the differences in EFS and OS became more significant in the analysis excluding the 7 N patients than in the analysis including them.

*Fig. 3.* A. Hybridization of Spectrum Orange-labeled CEP2 to a cell from tumor 991. An arrow and arrowheads show normal chromosome 2 and dup(2) chromosomes, respectively. B. Hybridization of a biotin-labeled BAC clone covering HOXD13 to the same cell as in A. Normal chromosome 2 had 1 HOXD13 signal, and dup(2) chromosomes had 2 HOXD13 signals.

*Fig. 4.* Event-free survival curves for 3 groups of patients classified by CGH and cytogenetic findings (log-rank, P=0.0549). N patients had no DNA copy changes and normal karyotypes or no mitotic cells. A1 patients had 2q gain, and A2 patients had DNA copy and/or cytogenetic changes other than 2q. ---- N (11), ---- A2 (13), ---- A1 (14).
Survival analyses on 3 groups of patients classified only by chromosome findings showed no differences among 13 N, 12 A1 and 4 A2 patients; 9 patients, whose tumors had no mitotic cells, were excluded from the analysis. The analysis on 3 groups of patients classified only by CGH findings showed a difference in OS ($P = 0.0356$), but no difference in EFS ($P = 0.1724$) among 14 N, 11 A1 and 13 A2 patients. Thus, the CGH analysis is more useful than the chromosome analysis to detect prognostic subgroups among hepatoblastoma patients.

Patients were grouped on the basis of various factors, including age ($<5$ years vs. $\geq 5$ years), stage of the disease (I+II vs. III+IV), histological type of tumor (fetal histologic type vs. other types) and presence or absence of 2q gain (A1 patients vs. A2 patients; A1 patients vs. N patients). Cytogenetic and CGH classification 1 (N, A1, and A2), which showed a more significant $P$ value on survival analysis than cytogenetic and CGH classification 2 (N, A3, and A4), was chosen for multivariate analysis on prognosis. The findings are summarized in Table III. Since no differences in EFS and OS were found between patients with and without fetal histologic type (data not shown), the histologic type was excluded from multivariate analysis. The patient’s age and the groups classified by the cytogenetic and CGH classification had a similar contribution to the EFS. The patient’s age had the largest contribution to the OS, followed by the cytogenetic and CGH classification and the stage of the disease. The findings show that the cytogenetic and CGH classification is an independent prognostic factor.

**DISCUSSION**

We examined 38 hepatoblastomas by both cytogenetic and CGH methods. CGH analysis detected the presence or absence of DNA copy changes in all 38 tumors including 9 that showed no mitotic cells by cytogenetic analysis. Moreover, CGH analysis detected DNA copy changes in 7 of 13 tumors that showed only normal mitotic cells; malignant cells may not have been in the mitotic phase, or were overlooked in the 7 tumors by cytogenetic analysis. However, cytogenetic analysis detected cells with chromosome abnormalities in 3 tumors that showed normal CGH pattern; the coexistence of tumor cells and an overwhelming number of non-tumor cells, which may have obscured the DNA copy changes, may explain the discrepancy. Thus, although CGH analysis gives more information on DNA copy changes than cytogenetic analysis, cytogenetic analysis sometimes complements CGH analysis.

By combined cytogenetic and CGH analysis we confirmed the findings previously reported that the most frequent chromosomal regions for DNA copy gain were 1q and 2q.\(^2,8\) Frequent gain of 1q has been reported in various embryonal tumors and adult carcinomas by both cytogenetic and CGH analyses.\(^21,22\) In contrast, frequent gain of 2q has been reported only in ovarian carcinoma by CGH analysis.\(^23\) Recently, three groups of investigators reported frequent gain of 2/2q in hepatoblastomas by

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**Table III. Results of Multivariate Analysis of 38 Patients with Hepatoblastoma Evaluated for Event-free and Overall Survival**

| Prognostic factors | Event-free survival | Overall survival |
|--------------------|---------------------|-----------------|
|                    | Relative risk (95% CI) | P  | Relative risk (95% CI) | P  |
| Patient’s age      |                      |     |                          |     |
| $\geq 5$ years vs. $\leq 4$ years | 3.90 (1.05–14.51) | 0.0423 | 27.28 (3.39–219.41) | 0.0018 |
| Stage of the disease |                      |     |                          |     |
| III, IV vs. I, II  | 2.28 (0.64–8.11)     | 0.2047 | 2.98 (0.55–15.99)     | 0.2036 |
| Cytogenetic and CGH classification |          |     |                          |     |
| A1 patients vs. A2 patients | 3.80 (0.96–14.96) | 0.0563 | 20.88 (1.99–218.84) | 0.0112 |
| A1 patients vs. N patients | 4.94 (1.02–23.84) | 0.0465 | 6.62 (1.05–41.68) | 0.0441 |
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CGH analysis. Two of them reported high-level gain in a restricted region at 2q24. The present study also showed a partial 2q gain in 6 tumors by CGH analysis, and identified 2q24 as the most common region for DNA copy gain (Fig. 2). Furthermore, FISH and cytogenetic analysis revealed that the clone covering HOXD13 (2q31) was located outside the common region for the gain, and that partial 2q gain detected by CGH was produced by a cytogenetic mechanism of duplication of the 2q24–q34 region followed by duplication of dup(2) chromosome in one tumor (No. 991). These findings suggest that a gene located at 2q24 may have amplified and increased its product, and given the hepatoblastoma cells the growth advantage needed for proliferation. Candidate genes at 2q24 included the activin receptor-like kinases (ALK) gene, the tumor necrosis factor receptor-associated factor (I-TRAF) gene and the FRZB-1 gene. The HOXD genes, which are located close to the common region, were excluded from candidacy by the present study.

In the present series of hepatoblastoma patients, EFS was lowest in A1 patients, highest in N patients and intermediate in A2 patients (Fig. 4). Seven (64%) of 11 N and 19 (70%) of 27 A patients have been reported with unbalanced 1;4 translocations resulting in the loss of terminal 4q detected by cytogenetic analysis, or loss of 4q21–qter or 4q33 or 34–qter detected by CGH analysis. Previous cytogenetic and CGH studies reported similar unbalanced 1;4 translocations in 5 hepatoblastomas, and loss of chromosome 4 in 6 hepatoblastomas, respectively. Taken together, the terminal 4q is the most frequent region for chromosome loss, and is the target site for the molecular cloning of a tumor suppressor gene responsible for the development of hepatoblastoma.

Patients with familial adenomatous polyposis coli, which is caused by germline mutation of the APC gene, have an increased incidence of hepatoblastoma, and somatic mutation of the APC gene was reported in some sporadic hepatoblastomas. However, the present cytogenetic and CGH study showed no loss of 5q and no gain of 3p, where APC and β-catenin genes are located, respectively.

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