A copy number gain of the 6p arm is linked with advanced hepatocellular carcinoma: an array-based comparative genomic hybridization study

Yasuyo Chochi, Shigeto Kawauchi, Motonao Nakao, Tomoko Furuya, Kiichiro Hashimoto, Atunori Oga, Masaaki Oka and Kohsuke Sasaki

1Department of Pathology, Yamaguchi University Graduate School of Medicine, Ube 755-8505, Japan
2Department of Surgery, Yamaguchi University Graduate School of Medicine, Ube 755-8505, Japan

Abstract

In accordance with cancer progression, genomic aberrations accumulate in cancer cells in a stepwise fashion. However, whether there are genomic changes linked with tumour progression remains unclarified. The purpose of this study is to elucidate the relationship between genomic alterations and clinical stages in hepatocellular carcinoma (HCC). A technology of array-based CGH using DNA chips spotted with 1440 BAC clones was applied to 42 surgically removed HCCs to examine the DNA copy number aberrations. A frequent copy number gain was detected on chromosomal regions 1q, 8q and Xq. In particular, gains of 1q42.12, 1q43 and 8q24.3 were detected in more than 65% of tumours. A frequent copy number loss was detected on chromosomal regions 1p, 4q, 6q, 8p and 17p. Losses of 8p21 and 17p13 were detected in more than 55% of HCCs. However, the DNA copy number gains of clones on 6p and 8q24.12 were more frequent in stage III/IV tumours than in stage I/II tumours (p < 0.001). In particular, the gain of the whole 6p was virtually limited to advanced-staged HCCs. The gain of the whole 6p is suggested to be a genomic marker for the late stages in HCCs. These observations therefore support the concept of genomic staging in HCC.

Keywords: hepatocellular carcinoma; array-based comparative genomic hybridization; pathological stage; genomic stage; DNA copy number aberration

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world and approximately 600,000 patients die of HCC every year [1]. Now the incidence of HCC is rising, even in Western countries [2]. The strong association of HCC with chronic liver diseases such as viral hepatitis B and C is well recognized. An accurate diagnosis and staging are critical issues in not only estimating the prognosis for each HCC patients but also determining an optimal treatment.

HCCs and other solid tumours develop and progress as a consequence of the stepwise accumulation of genetic alterations and subsequent clonal selection. It is also thought that the genetic aberrations accompanied by hepatocarcinogenesis occur in a specific order [3]. This means that we can depict a genetic pathway to HCC development and progression. Many investigators have made attempts to identify the genetic alterations underlying hepatocyte carcinogenesis and furthermore to draft the genetic pathway to HCC [4]. However, the elucidation of a genetic pathway to HCC development and progression is not a simple matter, and information on the genetic pathway to hepatocarcinogenesis has been as yet limited [5]. In order to acquire clues to the demonstration of the genetic pathway to HCC progression, a comprehensive analysis of the genomic alterations for each tumour with the known clinicopathological features is necessary. Although we applied the chromosomal comparative genomic hybridization (cCGH) to HCCs in order to have a sketchy description of the genomic detailed changes during the process of hepatocarcinogenesis [6,7], more detailed information regarding the genomic changes is necessary to identify the genomic alterations linked with HCC progression. Array-based CGH (aCGH) has the potential to draft a genetic pathway to HCC development and progression. However, the application of aCGH to human HCCs is still limited and the relationship between the genomic changes and the stage of HCC remains to be clarified [8–11].
In this study, we identified late genomic events in HCC by aCGH. Advanced HCCs were characterized by DNA copy number gains of the whole 6p. The present study also supported the concept of a genomic stage as well as that of a pathological stage in HCC.

Materials and methods

Tumour tissue specimens and DNA extraction

We examined 42 surgically removed HCCs in this study (Table 1). The patients consisted of 31 men and 11 women with an average age of 64 (range, 39–78) years. The patients were positive for the anti-HBV and/or anti-HCV antibody (six HBV-positive, 34 HCV-positive and two HBV- and HCV-positive). The pathological staging of these tumours was made pursuant to the tumour-node-metastasis (TNM) classification of the International Union against Cancer, and this series included 14 stage I, 18 stage II, eight stage III and two stage IV HCCs. In this study, we divided these tumours into three groups for convenience, stages I, II and III/IV. Histological differentiation was done in line with The General Rules for the Clinical and Pathological Study of Primary Liver Cancer [12]. The tumour tissue specimens were stored frozen at −80°C until use. The study protocol was approved by the Institutional Review Board for Human Use, Yamaguchi University Graduate School of Medicine, and informed consent for this study was obtained from all patients. High-molecular weight DNA was extracted from each tumour specimen using a DNA extraction kit (SepaGene, Sankojyunyaku Co., Tokyo, Japan) according to the manufacturer’s instructions, as previously described [6–9]. Control DNA was obtained from Promega (Madison, WI, USA) and was used for reference.

Array CGH

The BAC DNA array used in this study consisted of 1440 human bacterial artificial chromosome (BAC) clones, including 356 cancer-related genes, which are spaced approximately 2.3 Mb across the whole genome (Macrogen Inc., Seoul, Korea). The BAC chip information, together with information of end-sequenced BAC clones, is provided through the following website: http://www.macrogen.co.kr/eng/biochip/karyo_summary.jsp. The experiments were performed as previously described [13–15]. Briefly, tumour DNA and gender-matched reference DNA (Promega) were labelled with Cy5 and Cy3-dCTP (Perkin-Elmer Life Science), respectively, with a random primer labelling kit (BioPrime® DNA Labelling System, Invitrogen®). For hybridization, labelled DNA was mixed with Cot-1 DNA (50 mg, Gibco BRL, Gaithersburg, MD, USA) and ethanol precipitated. The precipitated DNA was dissolved in 40 µl hybridization mix. The probe mixture was denatured at 75°C for 5 min and incubated at 37°C for 60 min for blocking of repetitive sequences. The arrays were prehybridized with salmon sperm DNA to reduce non-specific background staining. The probe mixture was applied to the array. The arrays were placed in a moist chamber at 37°C for 72 h for hybridization. The array slides were washed twice in 2× standard saline citrate (2× SSC), 50% formamide, pH 7.0, at 45°C. Then the array slides were washed well in phosphate buffer with 0.1% NP-40, pH 8.0, in 2× SSC at room temperature.

Imaging and analysis

After hybridization, the slides were scanned on a GenePix 4000A scanner (Axon Instruments, Union City, CA, USA) and the 16-bit TIFF images were captured using GenePix Pro 5.0 software. The fluorescence images were analysed using MAC Viewer™ software (Macrogen Inc.) optimized for analysis of

| Table 1. Clinicopathological features on 42 hepatocellular carcinomas |
|---------------------------------------------------------------|
| **TNM stage**                                               |
| **All** | **I** | **II** | **III–IV** |
|----------|-------|--------|-----------|
| Gender   |       |        |           |
| Male     | 31    | 10     | 13        | 8         |
| Female   | 11    | 4      | 5         | 2         |
| Age (years) |       |        |           |
| Average (range) | 64 (39–78) | 65.5 (55–74) | 61.5 (49–75) | 63.5 (39–78) |
| Viral infection |       |        |           |
| HBV-positive | 6 (14.3%) | 0 (0%) | 3 (16.7%) | 3 (30%) |
| HCV-positive | 34 (81.0%) | 12 (85.7%) | 15 (83.3%) | 7 (70%) |
| HBV- and HCV-positive | 2 (4.7%) | 2 (14.3%) | 0 (0%) | 0 (0%) |
| Tumour size |       |        |           |
| Average diameter (cm) | 4.62 | 3.0 | 4.4 | 8.47 |
| Grade of differentiation |       |        |           |
| Well | 6 (14.3%) | 4 (28.6%) | 2 (11.1%) | 0 (0%) |
| Moderately | 29 (69.0%) | 10 (71.4%) | 13 (72.2%) | 6 (60%) |
| Poorly | 7 (16.7%) | 0 (0%) | 3 (16.7%) | 4 (40%) |

J Pathol 2009; 217: 677–684 DOI: 10.1002/path
Copyright © 2008 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.
the array, as previously reported [13–15]. The fluorescence spots were defined using the automatic grid feature and adjusted manually. Then the ratio of the red : green channel of each clone was calculated and all CGH ratios were converted to log base 2 (log2 ratios). The clones with log2 ratios that exceeded ±0.25 were considered gain and loss of the copy number. We defined a log2 ratio > 1.0 as an amplification. In order to define chromosomal regions with DCNAs, the adaptive weights smoothing (AWS) procedure with the gain and loss analysis of DNA (GLAD) algorithm [16] was applied to the ratio data, as previously described [17].

Statistical analysis
The differences in the prevalence of common gains and losses between the groups were determined using the χ² test. Differences in the total number of changes and frequency were tested by Student’s t-test, Welch’s t-test or non-parametric Mann–Whitney U test. A difference was considered significant at p < 0.05.

Results
Stage I tumours consisted of well- and moderately differentiated HCCs, and well-differentiated HCCs were not detected in stage III/IV tumours, as shown in Table 1. In contrast, stage III/IV HCCs consisted of moderately and poorly differentiated tumours, and poorly differentiated HCCs were not detected in stage I tumours. However, the correlation coefficient between disease stage and histological differentiation was as low as 0.39.

The gain of 19q13.41 and loss of 4q21.22 were linked to histological differentiation of HCC (p < 0.01) (Table 2). These aberrations were frequently detected in poorly differentiated HCCs but were not detected in well-differentiated tumours (Table 2). However, the number of copy number gains and losses was not statistically affected by histological differentiation of HCC; 111 ± 53.2 and 127.5 ± 46.8 for well-differentiated tumours, 140.8 ± 63.4 and 128.2 ± 73.6 for moderately differentiated tumours, and 88.0 ± 69.9 for poorly differentiated tumours, respectively.

Table 2. Chromosomal regions with copy number aberrations of which frequency is different between HCCs with different histological differentiation

| Chromosome region | Gene | Histological differentiation | p Value |
|-------------------|------|-----------------------------|---------|
| 1q21.22           |      | Well Moderate Poor          |         |
| 1q23.3            | 71   |       |      | 0.000622595 |
| 8q24.3            | 71   |       |      | 0.008315262 |
| 1q25.2            | 70   |       |      | 0.001106195 |
| 4q21.2           | 68   |       |      | 0.003414791 |

This table contains only data of p < 0.01.

Table 3. Chromosomal regions with highly recurrent DNA copy number aberrations in HCC

| Chromosomal region | Frequency (%) | Candidate gene |
|--------------------|---------------|----------------|
| DNA copy number gain |                |               |
| 1q23.1             | 52             | Taz1           |
| 1q23.2             | 52             | Taz1           |
| 1q42.12            | 55             | A0099l7        |
| 1q42.13            | 57             | MAGF           |
| 1q44               | 57             | TAZ-1          |
| 1q25.3             | 57             | MAFG           |
| 8q24.3             | 56             | —              |
| 1q23.1             | 52             | RPI-1-T1C1     |
| 8q24.3             | 52             | EXTL3          |
| 1q24.3             | 52             | CD48           |
| 1q24.11-8q24.12    | 52             | KIAA1389       |
| 8q24.23-8q24.3     | 52             | (8q terminal)  |
| 1q23.3             | 50             | D5S1108        |
| 1q22.2             | 50             | KIAA1389       |
| 1q15.3             | 50             | (5p terminal)  |
| 8q23.1             | 50             | WI-13991       |
| 8q24.22            | 50             | D5S1108        |

DNA copy number loss

| Chromosomal region | Frequency (%) | Candidate gene |
|--------------------|---------------|----------------|
| 17p13.2            | 60             | —              |
| 17p13.3            | 57             | ABR            |
| 17p13.1            | 57             | SCDI           |
| 17p12              | 57             | —              |
| 8p21.1             | 57             | EXTL3          |
| 17p13.1            | 56             | GAS7           |
| 4q21.22            | 55             | —              |
| 15q25.2            | 55             | Wi-22674       |
| 8p21.3             | 55             | —              |
| 17p12              | 50             | —              |
| 17p13.1            | 50             | ALOX12         |
| 8p12               | 50             | V5             |
| 22q11.21           | 50             | BCR            |

DNA copy number gain

| Chromosomal region | Frequency (%) | Candidate gene |
|--------------------|---------------|----------------|
| 1q23.3             | 71             | —              |
| 8q24.3             | 71             | REC Q4         |
| 1q25.2             | 70             | GST            |
| 4q21.22            | 68             | LBR            |
| 1q43               | 67             | RYR2           |
| 1q43               | 67             | DYS204         |
| 1q23.3             | 64             | PBX1           |
| 1q42.12            | 64             | ITPKB          |
| 1q25.3             | 63             | —              |
| 1q44               | 62             | (1q terminal)  |
| 1q23.1             | 60             | PRCC           |
| 1q44               | 60             | Akt3, DKFZp434N0250 |
| 1q32.1             | 57             | —              |
| 1q32.2             | 57             | M6N199         |
| 1q22.3             | 57             | SC20A1, NEK2   |
| 1q41               | 57             | TGFR2          |
| 1q42.13            | 57             | M-ABC2         |
| 1q44               | 57             | TAZ-1          |
| 1q25.3             | 57             | MAGF           |
| 8q24.3             | 56             | —              |
| 1q42.12            | 55             | A0099l7        |
| Xq23               | 54             | TED            |
| 1q31.1             | 52             | RPI-1-T1C1     |
| 8q24.11-8q24.12    | 52             | EXTL3          |
| 8q24.23-8q24.3     | 52             | (8q terminal)  |
| 1q23.3             | 50             | CD48           |
| 1q24.11-8q24.12    | 52             | EXTL3          |
| 1q24.23-8q24.3     | 52             | (8q terminal)  |
| 1q24.3             | 52             | —              |
| 1q24.11-8q24.12    | 52             | EXTL3          |
| 1q24.23-8q24.3     | 52             | (8q terminal)  |
| 1q23.3             | 50             | D5S1108        |
| 1q24.22            | 50             | D5S1108        |

DNA copy number loss

| Chromosomal region | Frequency (%) | Candidate gene |
|--------------------|---------------|----------------|
| 17p13.2            | 60             | —              |
| 17p13.3            | 57             | ABR            |
| 17p13.1            | 57             | SCDI           |
| 17p12              | 57             | —              |
| 8p21.1             | 57             | EXTL3          |
| 17p13.1            | 56             | GAS7           |
| 4q21.22            | 55             | —              |
| 15q25.2            | 55             | Wi-22674       |
| 8p21.3             | 55             | —              |
| 17p12              | 50             | —              |
| 17p13.1            | 50             | ALOX12         |
| 8p12               | 50             | V5             |
| 22q11.21           | 50             | BCR            |
Figure 1. (a) Overall frequency of DCNAs for each clone/gene in 42 hepatocellular carcinomas. A DNA copy number gain is frequent for clones on chromosomal regions 1q, 6p and 8q and, in particular, gains of 1q42.12, 1q43 and 8q24.3 were detected in >65% of tumours. A DNA copy number loss is frequent for clones/genes from 1p, 4q, 6p, 8p, 13q, 16q, 17p and Y chromosome, and losses of 8p21.3, 17p13.1, 17p13.3 and 22q11.21 were detected in >55% of tumours. The upper part of the figure indicates the frequency of clones with relative increase in DNA copy number and the lower part of the figure indicates the frequency of clones with relative decrease in DNA copy number. The numbers on the horizontal axis denote chromosome number (corresponding to BAC clones spotted). The numbers on the vertical axis denote the frequency of DNA copy number aberration for each clone; minus (−) indicates the frequency of DNA copy number loss. (b) An aCGH profile of stage II HCC from a 63 year-old male. A DNA copy number gain is observed at 10p, 13 and 14q and a loss is observed at 14q, 4, 10q, 11, 12p, 13q, 17q and 22. It is obvious that DCNAs extend to the whole chromosomal arm.

>65% of tumours (Figure 1a). A frequent copy number loss was detected on chromosomal regions 1p, 4q, 6q, 8p and 17p. Losses of 8p21.3, 17p13 and 22q11.21 were detected in >55% of HCCs (Figure 1a). The number of clones with DNA copy number gains was 128.4 ± 48.6 for stage I tumours, 128.9 ± 67.3 for stage II tumours, and 157.1 ± 75.2 for stage III/IV tumours. The number of clones with DNA copy number losses was 139.4 ± 67.2 for stage I tumours, 126.7 ± 71.3 for stage II tumours and 120 ± 59.1 for stage III/IV tumours (Figure 2a). The disease progression was accompanied by a slight increase in the number of clones with DCNA, but no significant difference in the number of DCNAs was found between tumours with different stages (Figure 2a). In addition, concerning the number of recurrent DCNAs, no correlation was observed between the pathological stage and the average number of clones with DCNAs detected in >50% of tumours (Figure 2b). DCNAs extending to the whole chromosomal arm were not infrequent in HCC, as shown in Figure 1b.

Gains of 1q, 6p, 8q, and Xq, and losses of 6q, and 8p were frequent in stage III/IV HCCs. The comparisons of the aCGH profiles with the pathological stage of the HCCs revealed that gains of almost all the clones on 6p and 8q24.12 were much more frequent in stage III/IV tumours than in stage I/II tumours (p < 0.001). For example, the gain of 6p23 (clone ID 814) was detected in 0/10 (0%), 1/16 (6.3%) and 7/9 (77.8%) for stage I, II and III/IV tumours, respectively (Table 4). The frequency of the 6p23 gain was significantly different between the stage I and stage III/IV tumours (p = 0.00045), the stage II and III/IV tumours (p = 0.00023) and the stage I/II and III/IV tumours (p = 0.0000053) (Table 3). In particular, the gain of the whole 6p arm was virtually limited to the advanced HCCs. The AWS procedure with the GLAD algorithm highlighted this feature (Figure 3). Although the frequency of a gain of the entire length of chromosome 2 was not high, the chromosome 2 gain was exclusive for stage III/IV HCCs (Figure 3). The results in this study were not affected by any difference in the hepatitis virus type. The gain of BAC clones on 6p was infrequently associated with histological differentiation.

DNA amplification was a rare event in HCC; however, it was exceptionally detected at Xq23 (clone ID, 326) with a frequency of 4/14 (28.6%) for stage I, 7/18 (38.9%) for stage II and 4/10 (40.0%) for stage III/IV tumours. Detailed information of aCGH for 42 HCCs was available through a supplementary data file.

Discussion

Many genes including cancer-related genes are involved in HCC as well as in other solid tumours. The genetic alterations depend on the organ and type of cancer, and the same goes for the chromosomal regions with DCNA [18,19]. In this study, the 1.4 K BAC DNA array, which may be a low-density array,
allowed the drawing of a sketch of DCNAs on chromosomes, and the AWS procedure with the GLAD algorithm for CGH data provided interesting information on chromosomal regions with genomic aberrations. The particular genomic alterations that occur in early cancers may be responsible for tumour development, and the genomic alterations that are detected exclusively in advanced cancers are connected to the tumour progression part from the matter of a cause or a consequence [20,21]. Non-random genomic aberrations that occur at the early stage of carcinogenesis usually persist even at an advanced stage of tumours [22,23].

It follows that early genomic events are frequently found in both early and advanced tumours, and that late genomic events are detected preferentially in advanced tumours. Accordingly, it is legitimate to assume that the recurrent DCNAs independent of disease stage are implicated in HCC development, and that the recurrent events preferentially detected in advanced tumours are responsible for HCC progression. In this context, it is likely that gains of 1q,

Table 4. Chromosomal regions where the frequency of copy number gain is statistically different between disease stages (p < 0.001)

| Region   | Genes* | Stage I | Stage II | Stage I/II | Stage III/IV | p Value** |
|----------|--------|---------|----------|------------|--------------|-----------|
| 6p23     | RNF182 | 0/10    | 1/16     | 7/9        | 0.000449377  |
|          |        |         | 0/10     | 0.000233166 |
|          |        |         | 0.005302446 |
| 6p12.1   | DST    | 0/14    | 6/10     | 16.8 ± 9.6  | 26.7 ± 9.6   | 0.000817973 |
| 6p11.2   | —      | 0/14    | 6/10     | 0.000817973  |
|          |        |         | 2.53258E-05 |
| 6p24.3   | BMP6   | 0/18    | 6/10     | 16.8 ± 9.6  | 26.7 ± 9.6   | 0.000209327 |
| 6p22.3   | ATXN1  | 0/18    | 6/10     | 0.000209327  |
|          |        |         | 4.421945E-05 |
| 6p21.2   | —      | 2/18    | 8/10     | 0.000267124  |
| 6p24.3   | —      | 0/17    | 6/10     | 0.000335232  |
|          |        |         | 0.000293053 |
| 6p22.2   | TRIM38 | 4/32    | 7/10     | 0.000306363  |
| 6p21.1   | PTK7   | 4/32    | 7/10     | 0.000306363  |
| 6p21.1   | SAMD12 | 8/29    | 9/10     | 0.000596845  |
| 6p21.1   | PPARD  | 7/32    | 8/10     | 0.000812847  |
| 6p21.1   | —      | 7/32    | 8/10     | 0.000812847  |

* Candidate genes are not identified in the relevant BAC clone.
** p Values between Stage I, II or I and II and Stage III and IV tumours.
Figure 3. The AWS procedure using the GLAD algorithm is applied to aCGH data. The procedure makes clear chromosomal regions with DCNA. (a) The frequency of DCNAs for stage I HCCs. In 14 stage I HCCs, gains of 1q and 8q and losses 4q and 17p are frequent, and in particular the 1q gain and 17p loss are detected in approximately 70% and 60% of stage I HCCs, respectively. (b) The profile of aCGH in 18 stage II HCCs is similar to that in stage I, although the number of chromosomal regions with DCNA appears less in stage II than in stage I tumours. (c) The frequency of DCNAs for 32 HCCs at stage I or II. The profile is similar to those from HCCs in stage I or II. (d) The frequency of DCNAs for 10 HCCs in stage III/IV. The profile is considerably different from those of HCCs in stage I or II. Gains of 1q, 6p and 8q and losses of 6q and 8p were distinct. In particular, a DNA copy number gain covering the entire length of 6p is a characteristic features in stage III/IV HCCs (solid arrow). Although the frequency of a gain of the whole chromosome 2 is low, the gain of the chromosome is also a characteristic change in stage III/IV HCCs (open arrow).

8q and Xq and losses of 1p, 4q, 6q, 8p and 17p correspond to genomic changes associated with HCC development. The gains of clones on 6p and 8q preferentially detected in advanced HCC seem to be linked with progression of HCC. In particular, the gain of the whole 6p was virtually limited to advanced-staged HCCs; in other words, advanced HCCs were characterized by DNA copy number gains of the entire 6p arm. DCNAs extending to broad chromosomal regions are not unusual for HCC [6,7,9,24]. The AWS procedure with the GLAD algorithm clearly defined the chromosomal regions involved. There have so far only been a few reports concerning the relationship between genomic alterations and tumour progression in HCC and, in addition, the controversy as to the relationship exists between these studies [3,6,22,25]. The chromosomal regions involved in advanced HCCs are different from our data. The cause of the difference is unexplained, although the concept that DNA copy number gains on some particular chromosomal regions
contribute to the progression of HCC is common to these studies. The high-level gain of 6p21 harbouring VEGFA, of which activation stimulates hepatocyte proliferation, was detected in 14/210 HCCs [24], although a BAC clone harbouring VEGFA was not spotted on the array used in the present study.

Since the disease stage largely affects the prognosis of a cancer patient, the treatment strategy usually depends on the disease stage in an individual patient. Therefore, it is of practical importance to differentiate between early cancers and advanced cancers prior to the start of treatment. To this end, attempts have been made to distinguish genomic alterations for HCC development from those for HCC [7,10,22,26]. The present study revealed that the CGH profiles were generally mirrored by the pathological stages. As mentioned above, some genomic changes, such as gains of 6p and 8q, are directly linked with the genomic stage and may be an alternative to the pathological stage. The genomic stage can be determined in biopsy specimens. This means that the disease stage can be estimated by an analysis of the genomic alterations on the particular chromosomal regions prior to the initiation of treatment.

It is known that histological differentiation and stage are roughly related in HCC, and this relation was apparent in the present study as well. However, the relationship between DCNAs and histological differentiation of HCC had less impact than that between DCNAs and disease stage. In addition, the gain of BAC clones on 6p was barely associated with histological differentiation of HCC in this study. Moinzadeh and colleagues [27], who made a CGH meta-analysis, also revealed no linkage between 6p gain and histological differentiation. In conclusion, advanced HCCs were characterized by DNA copy number gains of the entire 6p arm. The present data support the concept of a genomic stage in HCC.

Acknowledgements

We thank Ms Takae Okada for her technical assistance with array CGH analysis. We also acknowledge financial support from the Ministry of Education and Culture of Japan (Grant Nos 15639087, 16390107 and 19390102) and the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

Supporting information

The aCGH data for 42 HCCs are available as supporting information to this article. BAC chip information together with information of end-sequenced BAC clones is provided through the following website: http://www.macrogen.co.kr/eng/biochip/karyo_sum mary.jsp

References

1. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907–1917.
2. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127(S suppl 1):S27–34.
3. Poon TC, Wong N, Lai PB, Rattray M, Johnson PJ, Sung JJ. A tumor progression model for hepatocellular carcinoma: bioinformatic analysis of genomic data. Gastroenterology 2006;131:1262–1270.
4. MA, Sun B, Satiroglu Tufan NL, Liu J, Pan J, Lian Z. Genetic mechanisms of hepatocarcinogenesis. Oncogene 2002;21:2593–2604.
5. Cha C, DeMatteo RP. Molecular mechanisms in hepatocellular carcinoma development. Best Pract Res Clin Gastroenterol 2005;19:25–37.
6. Kusano N, Shiraiishi K, Kuba K, Oga A, Okita K, Sasaki K. Genetic aberrations detected by comparative genomic hybridization in hepatocellular carcinomas: their relationship to clinicopathological features. Hepatology 1999;29:1858–1862.
7. Kusano N, Okita K, Shirahashi H, Harada T, Shiraiishi K, Oga A, et al. Chromosomal imbalances detected by comparative genomic hybridization are associated with outcome of patients with hepatocellular carcinoma. Cancer 2002;94:746–751.
8. Takeo S, Arai H, Kusano N, Harada T, Furuya T, Kawauchi S, et al. Examination of oncogene amplification by genomic DNA microarray in hepatocellular carcinomas: comparison with comparative genomic hybridization analysis. Cancer Genet Cytogenet 2001;130:127–132.
9. Hashimoto K, Mori N, Tamesa T, Okada T, Kawauchi S, Oga A, et al. Analysis of DNA copy number aberrations in hepatitis C virus-associated hepatocellular carcinomas by conventional CGH and array CGH. Mod Pathol 2004;17:617–622.
10. Patil MA, Gutgemann I, Zhang J, Ho C, Cheung ST, Ginzinger D, et al. Array-based comparative genomic hybridization reveals recurrent chromosomal aberrations and Jabl as a potential target for 8q gain in hepatocellular carcinoma. Carcinogenesis 2005;26:2050–2057.
11. Park SJ, Jeong SY, Kim HJ. Y chromosome loss and other genetic alterations in hepatocellular carcinoma cell lines analyzed by CGH and CGH array. Cancer Genet Cytogenet 2006;166:56–64.
12. Liver Cancer Study Group of Japan. The General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 5th edn. Kenehara-shuppan: Tokyo, 2008; 40–42.
13. Ohguri T, Hisaoka M, Kawauchi S, Sasaki K, Aoki T, Kane-mitsu S, et al. Cytogenetic analysis of myxoid liposarcoma and myxofibrosarcoma by array-based comparative genomic hybridization. J Clin Pathol 2006;59:978–983.
14. Cho YL, Bae S, Koo MS, Kim KM, Chun HJ, Kim CK, et al. Array comparative genomic hybridization analysis of uterine leiomyosarcoma. Gynecol Oncol 2005;99:545–551.
15. Yamamoto Y, Chochi Y, Matsuyama H, Eguchi S, Kawauchi S, Furuya T, et al. Gain of 5p15.33 is associated with progression of bladder cancer. Oncology 2007;72:132–138.
16. Hupé P, Stransky N, Thiery JP, Radvanyi F, Barillot E. Analysis of array CGH data: from signal ratio to gain and loss of DNA regions. Bioinformatics 2004;20:3413–3422.
17. Tanaka M, Okada T, Ikemoto K, Furuya T, Oga A, Kawauchi S, et al. The development of a novel method for the classification of the aCGH profiles based on genomic alterations. Bull Yamaguchi Med Sch 2006;63:37–43.
18. Beijani BA, Shaffer LG. Application of array-based comparative genomic hybridization to clinical diagnostics. J Mol Diagn 2006;8:528–533.
19. Oga A, Kawauchi S, Izumi H, Ping LX, Furuya T, Sasaki K. New perspectives for tumor pathology provided by comparative genomic hybridization. Int J Clin Oncol 2002;7:133–137.
20. Komarova NL, Lengauer C, Vogelstein B, Nowak MA. Dynamics of genetic instability in sporadic and familial colorectal cancer. Cancer Biol Ther 2002;1:685–692.
21. Wilkins L, Fleming P, Gebel M, Bleck J, Terkamp C, Win- gen L, et al. Induction of aneuploidy by increasing chromosomal instability during dedifferentiation of hepatocellular carcinoma. Proc Natl Acad Sci USA 2004;101:1309–1314.
22. Sy SM, Wong N, Lai PB, To KF, Johnson PJ. Regional over-representations on chromosomes 1q, 3q and 7q in the progression of hepatitis B virus-related hepatocellular carcinoma. *Mod Pathol* 2005;18:686–692.

23. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759–767.

24. Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, *et al*. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res* 2008;68:6779–6788.

25. Wong N, Lai P, Lee SW, Fan S, Pang E, Liew CT, *et al*. Assessment of genetic changes in hepatocellular carcinoma by comparative genomic hybridization analysis: relationship to disease stage, tumor size, and cirrhosis. *Am J Pathol* 1999;154:37–43.

26. Marchio A, Meddeb M, Pineau P, Danglot G, Tiollais P, Bernheim A, *et al*. Recurrent chromosomal abnormalities in hepatocellular carcinoma detected by comparative genomic hybridization. *Genes Chromosomes Cancer* 1997;18:59–65.

27. Moinzadeh P, Breuhahn K, Stützer H, Schirmacher P. Chromosome alterations in human hepatocellular carcinomas correlate with aetiology and histological grade — results of an explorative CGH meta-analysis. *Br J Cancer* 2005;92:935–941.