The Association of Chromosome 8p Deletion and Tumor Metastasis in Human Hepatocellular Carcinoma

Lun-Xiu Qin, Zhao-You Tang, Jonathan S. T. Sham, Zeng-Chen Ma, Sheng-Long Ye, Xin-Da Zhou, Zhi-Quan Wu, Jeffrey M. Trent, and Xin-Yuan Guan

Liver Cancer Institute, Zhongshan Hospital, Shanghai Medical University, Shanghai, Peoples Republic of China [L-X. Q., Z-Y. T., Z-C. M., S-L. Y., X-D. Z., Z-Q. W.]; Department of Clinical Oncology, Queen Mary Hospital, University of Hong Kong, Hong Kong [J. S. T. S., X-Y. G.]; and Cancer Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, Maryland [J. M. T.]

Abstract

To understand the genetic mechanisms underlying the progression of hepatocellular carcinoma (HCC) metastasis, differences of genomic alterations between 10 pairs of primary HCC tumors and their matched metastatic lesions were analyzed by comparative genomic hybridization. Several chromosomal alterations including loss of 8p, 4q, 17p, and 19p, gain of 5p and high-level amplification of 1q12-q22 were detected in two or more cases. The most significant finding is the loss of 8p which was detected in 8 metastatic tumors but only in 3 corresponding primary tumors (P = 0.03). This result suggests that the deletion of chromosome 8p might contribute to the development of HCC metastasis.

Introduction

HCC is one of the most frequently occurring human cancers worldwide and has been ranked as the second cancer killer in China since the 1990s (1, 2). Although encouraging long-term survivals of HCC patients have been obtained in some clinical centers, HCC carries a very poor prognosis. One of the major reasons of the poor prognosis is the recurrence and metastasis after surgery. It has been reported that the 5-year recurrence rate of HCC after resection is higher than 50% with most of the recurrences due to invasion-related spreading (1–3). Thus, understanding the metastatic mechanisms of HCC becomes an imperative. Many efforts have been made to explore the mechanisms involved in the recurrence and metastasis of HCC, however, the genetic events involved in the HCC metastasis is still unclear (1–3).

Recently, the genomic alterations in HCC have been studied by CGH, and several recurrent chromosome changes have been reported (4, 5). The gain of 1q, 8q, and 20q and the loss of 16q, 4q, 17p, 1p, and 8p were commonly detected in HCC. The relationship between these recurrent alterations and the clinical phenotypes is still unknown. A considerable amount of genetic alterations on solid tumors has been documented during the last seven years since the CGH technique was developed (6), however, only a few of these genetic alterations have been associated with the metastatic phenotype (7–9). It has been shown that the progression from the primary tumor to metastasis is due either to the acquisition of additional genetic changes or to the alteration of regulatory processes without DNA changes (7–9).

We hypothesized that the progression from the primary tumor to metastasis in HCC may also be due to the acquisition of further genetic changes that inactivate the tumor suppressor gene(s) or activate the expression of oncogene(s) related to the tumor metastasis. To detect the specific chromosome alterations related to HCC metastasis, the differences of genomic changes between 10 primary HCC tumors and their corresponding metastatic lesions were analyzed by CGH.

Materials and Methods

Tumor Specimens and DNA Extraction. Ten pairs of untreated primary HCC tumors and their corresponding metastatic lesions (thrombosis in portal vein or bile duct or intrahepatic spreading nodules) were collected at the time of surgical resections from the Liver Cancer Institute, Zhongshan Hospital, Shanghai Medical University (Shanghai, China). Among the 10 samples of metastases, 5 cases were tumor thromboses in the portal vein (which is one of the most common places of HCC spreading), 2 thromboses in the bile duct, 2 intrahepatic spreading nodules, and 1 spreading nodule in the abdomen cavity. All of the primary tumors and their metastases were histologically proven to be HCC. The clinical and pathological characteristics of the patients and the tumors are summarized in Table 1. The specimens were frozen in liquid nitrogen within 30 minutes after surgical resection and stored at –80°C until DNA extraction.

Genomic DNA was extracted from the tumor samples with a proteinase K/SIDS digestion and phenol/chloroform/isoamylalcohol extraction. Normal reference DNA was prepared from peripheral blood lymphocytes of healthy donors.

CGH. Metaphases were prepared following standard procedures from peripheral blood lymphocytes of a healthy male donor. The slides with the metaphase spreads were stored at room temperature until use. CGH was performed essentially as described by Kallioniemi et al. (10). Briefly, 1 μg of genomic DNA from a HCC tumor sample and a sex-matched normal reference was labeled directly with Spectrum green-dUTP and Spectrum red-dUTP (Vysis, Downers Grove, IL), respectively, by nick translation. Three hundred to 500 ng of labeled tumor DNA and normal DNA probes were used in a 10-μl hybridization mixture containing 55% formamide, 2× SSC, and 10 μg of human Cot I DNA), which was denatured at 75°C for 5 min. The slide containing normal metaphase spreads was treated with Nuclease (100 U/ml) at 37°C for 1 h and then denatured at 75°C in 70% formamide and 2× SSC for 5 min. Hybridization with probes was then carried out at 37°C in a moist chamber for 48 h. The slide was then washed in 0.4× SSC/0.3% NP40 at 75°C for 2 min and then in 2× SSC/0.1% NP40 at room temperature for 2 min. After the washing, the slide was counterstained with 1 μg/ml DAPI in an antifade solution.

Digital Image Analysis. The hybridized metaphase chromosomes were analyzed using a digital image analysis system containing a Zeiss Axioshot microscope equipped with a Metachrome II cooled-charged device camera (Zeiss, Oberkochen, Germany). Three images of each metaphase were captured using filter wheel-mounted, single-band excitation Rhodamine, FITC, and DAPI filters. The image analyses were carried out using Quips CGH program (Vysis, Downers Grove, IL). Five metaphases were analyzed to generate fluorescent ratio profiles in each case. Interpretation of the profiles was performed according to the program guidelines.

Results

CGH analysis was carried out on 10 primary HCC tumors and their corresponding metastatic lesions. The copy-number changes for the entire genome detected in these 10 primary HCC and their...
Compared to their corresponding primary tumors, the copy number metastatic tumors. High copy-number amplification of 1q with a tumors), 8q (6 of 10 in primary tumors and 7 of 10 in metastatic most frequently detected sites of chromosome gain were 1q (10 of corresponding metastatic lesions are summarized in Table 2. The (differentiation of tumor cells).

All of the CGH results mentioned above were derived from com-}

**Discussion**

The importance of chromosomal abnormalities in tumor development and progression has been known since pioneering cytogenetic studies successfully defined recurrent chromosome changes in specific types of tumors (11). In the course of carcinogenesis, cells experience several genetic alterations that are associated with the transition from a preneoplastic lesion to an invasive tumor and finally to the metastatic state. Many studies on the cytogenetic abnormalities of HCC have been performed using CGH and LOH analysis. Several recurrent chromosome alterations have been detected, including the gain of 1q, 8q, and 20q, and the loss of 4q, 16q, 1p, 17p, and 8p (4, 5). However, little is known about

Table 2 Summary of genomic alterations in 10 pairs of primary and metastatic HCC detected by CGH

<table>
<thead>
<tr>
<th>Case</th>
<th>Gain</th>
<th>Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>1P</td>
<td>1q12-q25, 2q22-q32, 5q, 8q, 8q22.1-qter, 13, 17q</td>
<td>1pter-p32, 4q, 11, 12, 14, 16q, 17p. (3) b</td>
</tr>
<tr>
<td>1M</td>
<td>1q12-q25, 2q22-q32, 5q, 8q, 8q22.1-qter, 13, 17q (6)</td>
<td>1pter-p32, 4q, 11, 12, 14, 16q, 17p. (8, 19)</td>
</tr>
<tr>
<td>2P</td>
<td>4q, 6q</td>
<td>4p, 9p, 10q, 12q22-qter</td>
</tr>
<tr>
<td>2M</td>
<td>1q, 6p, 5q11-q13, 12q15-q21, 20p</td>
<td>9p, 9p, 10p, 12q22-qter, (4q, 8p, 17p)</td>
</tr>
<tr>
<td>3P</td>
<td>1q, 6p, 7q, 20q, (2q12-q22, 13q22-q23)</td>
<td>4q, 17p, 18q, 19p</td>
</tr>
<tr>
<td>3M</td>
<td>4p, 1q12-q21, 2q, 6p, (3p)</td>
<td>4q, 17p, 18q, 19p, (14, 16q, 21)</td>
</tr>
<tr>
<td>4M</td>
<td>1q12-q21, 2q, 6p</td>
<td>1pter-p34, 4, 9, 17pter-q21, 19p</td>
</tr>
<tr>
<td>5P</td>
<td>1q, 5q, 7q11-q34, 18pter-q12</td>
<td>1pter-p35, 3p, 4q, 6q, 9q, 17p, 20p, 21, (12p)</td>
</tr>
<tr>
<td>5M</td>
<td>1q, 5q, 7q11-q34, 18pter-q12</td>
<td>1pter-p35, 3p, 4q, 6q, 9q, 17p, 20p, 21, (8p, 19p)</td>
</tr>
<tr>
<td>6P</td>
<td>1q, 8q, 8q13-qter, (12p)</td>
<td>1pter-p35, 3p, 4q, 6q, 9q, 17p, 20p, 21, (16p, 15q)</td>
</tr>
<tr>
<td>6M</td>
<td>1q, 5q, 8q13-qter</td>
<td>1pter-p35, 3p, 4q, 6q, 9q, 17p, 20p, 21, (2p, 17p)</td>
</tr>
<tr>
<td>7P</td>
<td>1q12-q22, 5q, 6q23-qter, 7pter-p21, 8q21-qter, 10q, 19q13.1-q13.2</td>
<td>1q12-q22, 5q, 6q23-qter, 7pter-p21, 8q21-qter, 10p, 19q13.1-q13.2</td>
</tr>
<tr>
<td>7M</td>
<td>1q12-q22, 5q, 6q23-qter, 7pter-p21, 8q21-qter, 10p, 19q13.1-q13.2</td>
<td>1q12-q22, 5q, 6q23-qter, 7pter-p21, 8q21-qter, 10p, 19q13.1-q13.2</td>
</tr>
<tr>
<td>8P</td>
<td>1q, 3q, 8q, 10p, 19q12-q13.3</td>
<td>4q, 5q, 8q, 9q, 10q, 11q22-q21, 13q12-q13, 16q, 18q, 19p</td>
</tr>
<tr>
<td>8M</td>
<td>1q, 3q, 8q, 10p, 19q12-q13.3</td>
<td>4q, 5q, 8q, 9q, 10q, 11q22-q21, 13q12-q13, 16q, 18q, 19p</td>
</tr>
<tr>
<td>9P</td>
<td>1q12-q24, 1q12-q24, 1p12-q12, 1p12-q12, 1p12-q12, 1p12-q12</td>
<td>1p12-q34, 4q, 5q11-qter, 8q, 14, 16q, 17p</td>
</tr>
<tr>
<td>9M</td>
<td>1q, 2q24-q32, 11p, (5p)</td>
<td>1p, 5p</td>
</tr>
<tr>
<td>10P</td>
<td>1q12-q22, 8q</td>
<td>1p, 5p</td>
</tr>
<tr>
<td>10M</td>
<td>1q12-q22, 8q21-qter, 10q</td>
<td>(8pter-q13, 14, 17p, 18p)</td>
</tr>
</tbody>
</table>

* High copy-number gain is shown in bold.

b The differences of genomic alterations between primary and metastatic tumors are shown in parentheses.
the relationship between these recurrent chromosome changes and the metastasis phenotypes of HCC. In this study, we analyzed 10 pairs of primary HCC tumors and their corresponding metastatic lesions by CGH to identify specific chromosome alterations related to the metastasis phenotype in HCC. The majority of the chromosomal aberrations detected in this study in both primary and metastatic HCC were consistent with those in previous reports.

The most interesting finding in this study is the deletion of 8p that was detected in eight metastatic tumors but in only three corresponding primary tumors. In the present study, the difference in 8p deletion between primary and metastatic lesions of HCC is significant (30% versus 80%; \( P = 0.03 \)), compared with the other differences of genomic alterations between primary tumor and its corresponding metastatic tumor. This suggests that 8p may harbor a putative tumor suppressor gene that plays an important role in the HCC progression especially in the tumor metastasis. LOH studies have demonstrated that the loss of 8p is frequently detected in many other tumors including breast, prostate, and ovarian cancers (12–14). In colorectal cancer, deletion of 8p was detected as a late event in the multistep model of colorectal carcinogenesis and was significantly correlated with the invasion of lymphatics, vessels, or perineurium (15). Another study has shown that the loss of 8p is associated with tumor metastasis in renal cell carcinoma (16). Several very small overlapping deleted regions on 8p have been detected in a variety of cancers including those of prostate, breast, ovarian, colon, renal cell, and liver (12–17). Most of those small overlapping deleted regions on 8p were localized on 8p12-p22. Several candidate tumor suppressor genes have been mapped to 8p including the \textit{DLC-1} (8p21.3-p22; Ref. 18) and \textit{FEZ1} (8p22; Ref. 19).

Another interesting finding in this study is the detection of the minimum high-level amplification region on 1q12-q22. The gain of 1q is one of the most frequently chromosomal changes in HCC detected by CGH (4, 5). In most cases, the gain of 1q involved the whole long arm. In the present study, high copy-number amplification on 1q was detected in four primary tumors and six metastatic tumors, with a minimum overlapping amplified region at 1q12-q22 (Table 2). Most interestingly, in two cases (Cases 8 and 10), the high-level amplification of 1q12-q22 was detected only in metastatic tumors. This implies that the overexpression of an oncogene(s) at 1q12-q22 confers a selective advantage in HCC. The gain of 1q may be one of the early genetic changes in HCC because it was one of the most commonly detected alterations in HCC. However, high copy-number amplification of 1q12-q22 may occur only in the later stage of HCC and provide more advantage of growth selection. The amplification of 1q was also commonly observed in breast cancer, colorectal cancer, bladder cancer, and lipoma-like liposarcoma (20–23). In one study, high copy-number amplification at 1q21-q24 was detected in 3 of 26 cases of bladder cancer (22). In another study, a minimum amplified region at 1q21-q23 was observed in four of eight cases with lipoma-like lipo-
The gain of 8q were detected in both the primary tumor (left labeled with Spectrum red, dUTP tumor (right). This study provides a practicable model to detect specific genetic changes in a candidate region for gene cloning. Further study of the 8p deletion may contribute to the metastasis of HCC. This suggests that 8p may harbor a novel tumor suppressor gene that plays an important role in HCC development.

Acknowledgments

We thank Drs. J. Fan, Q. H. Ye, H. C. Sun, Y. Yu, J. Zhou, Q. Niu, and L. H. Zhang for their help in collecting tumor specimens.

References

The Association of Chromosome 8p Deletion and Tumor Metastasis in Human Hepatocellular Carcinoma


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/22/5662

Cited articles
This article cites 23 articles, 4 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/22/5662.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/59/22/5662.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.