Genomic Aberrations in Human Hepatoblastomas Detected by 2-Dimensional Gel Analysis

Hiyoshi Nagai, Hiroaki Tsumura, Mathurose Ponglikitmongkol, Yong-Sung Kim, and Kenichi Matsubara

Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan [H. N., M. P., Y-S. K., K. M.]; First Department of Surgery, Hiroshima University Medical School, 1-2-3, Kasumi-cho, Hiroshima 720, Japan [H. T.]; Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI road, Bangkok 10400, Thailand [M. P.]; and Department of Molecular Biology, Genetic Engineering Research Institute, KIST, P.O. Box 115, Yusong, Taejon, 305-600, Korea [Y-S. K.]

Abstract

Hepatoblastoma is a frequent embryonic tumor found in children. The mechanism of its formation and development remain unknown. We analyzed genomic DNA aberrations associated with three hepatoblastomas by means of restriction landmark genomic scanning (I. Hatada et al., Proc. Natl. Acad. Sci. USA, 88:9523—9527, 1991). About 2000 NotI restriction landmarks were monitored in each of the hepatoblastomas, which revealed that the intensity of eight spots decreased in the tumor genome, suggesting allelic loss. Three of these spots were identical to those that decreased in human hepatocellular carcinomas. These results suggested that common, early events are involved in the genesis of the two types of liver tumor.

Introduction

Embryonic tumors in children, including hepatoblastoma, Wilms' tumor, retinoblastoma, and rhabdomyosarcoma, represent a group of inappropriately proliferating tissue frequently associated with congenital malformations (1). In hepatoblastomas, aberrations in a region of chromosome 11p have been suggested in association with the Beckwith-Wiedmann syndrome (2), but the causative aberration has not been mapped, and other changes have not been discovered. Analyzing human genomes by means of RLGS3 (3) allows wide genomic regions from different samples to be compared. About 2000 landmark sites in genomic DNAs can be surveyed on a single two-dimensional gel (4, 5) without using polymorphic DNA markers (6). Genomic DNAs were extracted from the tumor tissues, as well as from their normal counterparts. The genomic DNA was analyzed by densitometry on a PDQUEST (PDI Inc. USA).

Materials and Methods

Three hepatoblastomas and their normal counterparts were surgically resected at Hiroshima University Medical School. Records of the three hepatoblastomas are shown in Table 1. None of the three patients had Beckwith-Wiedmann syndrome or other congenital malformations. The hepatoblastoma samples were quickly frozen in liquid nitrogen and stored until use. Tissue samples were then sliced using a cryostat; then appropriate pieces were stained with hematoxylin-eosin to monitor the shape of the tumor (5). The tumor was separated from normal portions of the dissected tissues using a scalpel (7). The adjacent nonneoplastic parts of the same patient were used as normal counterparts. The genomic DNA was extracted from each sample, and 1 μg of the DNA was analyzed with RLGs as described (5). The electropherograms were analyzed by densitometry. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1 Records of the three hepatoblastomas and the intensity of seven spots in RLGS profiles of the genomic DNA from hepatoblastomas relative to the corresponding spot intensities from the DNA of normal counterparts

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: RLGS, restriction landmark genomic scanning; HCC, hepatocellular carcinoma.

Table 1

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<td>Well</td>
<td>Mod.</td>
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<table>
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</tr>
</tbody>
</table>

4 M, male; F, female.
5 Well, well differentiated; Mod., moderately differentiated. The intensity of seven spots in the DNA of hepatoblastoma relative to that of the DNA in normal counterpart is represented as follows: 1 (unchanged), 1/2 (decreased to one-half: 0.44 ± 0.12). For details, see Fig. 1.
6 Spot decrease in HCC indicates whether the same spot has undergone an intensity decrease in more than two HCC cases in our previous analyses of 16 HCCs (5).
Fig. 1. RLGS profiles of genomic DNAs from hepatoblastoma (C) no. 1 and its normal counterpart (N). The DNA samples were radiolabeled at NotI sites as landmarks. For sample preparation and analysis, see text and Nagai et al. (5). Unlike HCCs (5), there were no intensified spots in the hepatoblastomas. The intensity of spots 5, H1, H2, and H3, (arrowheads) decreased in this hepatoblastoma. Among these, spot 5 was also decreased in HCC (5), whereas H1, H2, and H3 were unique to hepatoblastomas.

Fig. 2. RLGS profiles of genomic DNAs from hepatoblastoma nos. 2 (A) and 3 (B). Genomic DNA samples from the hepatoblastomas were analyzed as described in the legend to Fig. 1. Only regions of interest in the electropherograms are displayed. In addition to the four spots that decreased in intensity in sample no. 1, an additional spot (no. 4) decreased in intensity in sample no. 2, and the intensity of spots 4, 5, 6, and H4 decreased in sample no. 3. The profiles of the normal counterparts were omitted for clarity.
Only the RLGS profiles of these hepatoblastoma samples are shown for clarity.

Nagai et al. (5) have similarly analyzed genomic DNAs from 16 HCCs. They reported over 100 spots that decreased in intensity, among which 60 occurred among several samples, whereas others were sporadic. There were fewer changed spots in hepatoblastomas than in HCC samples. Furthermore, there were no intensified spots in the hepatoblastoma samples, whereas such changes occurred in the HCC (14 of 16 samples examined). Although we analyzed only three hepatoblastoma samples, these differences between hepatoblastomas and HCCs seem to be significant.

A comparison of the changed spots in hepatoblastomas with those in HCCs showed that spots 4, 5, and 6 in the former were identical to those that decreased in intensity in HCC (4, 5, and 6 among the 60 dwindling spots). We reported that the frequency of the decrease of spots 4, 5, and 6 was 56, 60, and 50%, respectively, among 16 HCCs examined (5). H1 through H5 were unique to hepatoblastomas, however. These changes in spot intensity are summarized in Table 1.

The spot intensity in RLGS reflects the ploidy of the DNA in the genome. Thus, the intensity of spots in diploid DNA is twice as intensive as those of haploid DNAs (3). The spots in which the intensity was halved are likely to represent loss of one allele, which has been verified by cloning the DNA in the spot.4 The reduced spot intensity in the hepatoblastomas, especially the recurrent decrease in spots 5, H1, H2, and H3 in the three hepatoblastoma samples, as well as spot 4 in samples 2 and 3, must have an important connection with the onset/development of this malignant tissue. Spots 4, 5, and 6 also dwindle in more than 50% of HCC samples (5). These observations suggest that hepatoblastomas that arise from hepatoblasts and HCC that arise from hepatocytes share the same mechanism, at least in part.

A loss of heterozygosity of some gene loci or of chromosomal regions has been reported in hepatoblastoma and HCC (3, 8–13). The common changes reported here should be characterized, and we are currently cloning these spots. The spot amplification in HCCs was not evident in the three hepatoblastomas we analyzed. In addition, about 60 spots that decreased in HCCs have not been detected in hepatoblastomas. Although analyses of more hepatoblastomas are needed, the differences in genomic aberrations between hepatoblastomas and HCCs should provide clues to the mechanism of initiation of these two tumors, as well as in understanding the course of development of these tumors.

References


4 H. Nagai and M. Ponglikitmongkol. Cloning of NotI-cleaved genomic DNA fragments appearing as spots on 2D gel electrophoresis, submitted for publication.
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