Loss of Maternal Alleles on Chromosome Arm 11p in Hepatoblastoma

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Abstract

Hepatoblastoma is the most common primary malignant liver tumor in children, yet little is known about molecular genetic changes in these tumors. Previous studies report loss of heterozygosity on chromosome arm 11p in some hepatoblastomas. We used the polymerase chain reaction to amplify multiple microsatellites on chromosome arm 11p to assess loss of heterozygosity in 18 hepatoblastomas. Loss of heterozygosity on 11p was found in six of them. The common region of overlap was restricted to the telomeric portion of 11p (11p15.5) and therefore excluded the W7-T1 tumor suppressor gene at 11p13. Parental origin of the lost allele could be determined in all six cases and was exclusively maternal. These results indicate that a tumor suppressor gene at 11p15.5 is involved in the pathogenesis of hepatoblastoma and also suggest that this chromosomal region is imprinted.

Introduction

HB is the most common primary malignant hepatic tumor of childhood, making up approximately 50—60% of such tumors (1). However, HB is still a very rare tumor with an incidence of 0.7—1 new cases/million children 15 years of age or younger (2). Little is known about the etiology of HB. While most cases are sporadic, some are associated with BWS (3) or with familial adenomatous polyposis coli (4). Previous studies of molecular genetic changes in HB have shown LOH on chromosome arm 11p in some cases (3, 5, 6). Therefore, we decided to study a series of 18 HBs to assess the frequency and extent of LOH on chromosome arm 11p in these tumors. Since there is predominantly maternal allelic loss in some other so-called embryonal tumors of childhood (7), we also decided to determine the parental origin of the lost 11p alleles in HB.

Materials and Methods

Patient Population. HBs from a total of 18 patients were studied. These children had all received operations in the Department of Pediatric Surgery at the Hannover Medical School (Hannover, Germany). There were 11 boys and 7 girls, ranging in age from 3 to 48 months (mean, 17.9 months). All tumors were primary lesions, except for case D166, which was a recurrent lesion. In three cases (D162, D166, and D22), the tumors were multifocal, i.e., they grew as several discrete, noncontiguous nodules. Histologically, HBs are predominantly maternal allelic loss in some other so-called embryonal tumors of childhood (7), we also decided to determine the parental origin of the lost 11p alleles in HB.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: HB, hepatoblastoma; BWS, Beckwith-Wiedemann syndrome; LOH, loss of heterozygosity; PBL, peripheral blood leukocytes; PCR, polymerase chain reaction; IGF2, insulin-like growth factor II; RFLP, restriction fragment length polymorphism.
Table I Summary of clinical data

<table>
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<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (mo)</th>
<th>Chemo</th>
<th>Type</th>
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<td>Mixed</td>
</tr>
<tr>
<td>D34</td>
<td>F</td>
<td>48</td>
<td>Yes</td>
<td>Epithelial</td>
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<tr>
<td>D92</td>
<td>M</td>
<td>12</td>
<td>No</td>
<td>Epithelial</td>
</tr>
<tr>
<td>D104</td>
<td>F</td>
<td>24</td>
<td>No</td>
<td>Epithelial</td>
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<tr>
<td>D116</td>
<td>F</td>
<td>9</td>
<td>Yes</td>
<td>Mixed</td>
</tr>
<tr>
<td>D123</td>
<td>M</td>
<td>3</td>
<td>Yes</td>
<td>Mixed</td>
</tr>
<tr>
<td>D158</td>
<td>F</td>
<td>8</td>
<td>No</td>
<td>Mixed</td>
</tr>
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<td>M</td>
<td>11</td>
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<tr>
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<td>11</td>
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<td>M</td>
<td>16</td>
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<td>M</td>
<td>13</td>
<td>No</td>
<td>Epithelial</td>
</tr>
</tbody>
</table>

a Chemo, chemotherapy.
b Patient with Beckwith-Wiedemann syndrome.
c Recurrent tumor.
d Sister died of HB.
e Primary tumor and cell line studied.
f Brothers.

(MBI Fermentas, Vilnius, Lithuania), an isoenzyme of MvnI. The digested PCR products were run on 6% denaturing polyacrylamide gels on an automated sequencing apparatus (DNA sequencer 373A; Applied Biosystems), and the results were analyzed using the Genescan 672 software package (Applied Biosystems).

Results

LOH. The LOH data are summarized in Fig. 1. From 15 to 18 tumors yielded interpretable results at a given locus. A typical result is shown in Fig. 2A. Six HBs had LOH on chromosome arm 11p. Two tumors (DZ25 and D104) had LOH at all 11p loci for which they were informative and also at D11S490, which is located at 11q23.3. This is consistent with loss of one whole chromosome 11 in these two tumors. In one case (D166), the tumor had LOH at all loci on 11p for which the patient was informative but maintained heterozygosity at D11S490, indicating loss of one entire short arm. In three cases (D162, DZ18, and D158), LOH involved only the telomeric portion of 11p. In the six tumors, the region of overlap was confined to 11p15.5. The highest rates of LOH were 50% at IGF2 (3') and HBB and 57% at IGF2 (5').

In the case where both the primary HB tumor and a cell line derived from that tumor were studied (DZ25), the primary tumor always had one allele that was noticeably reduced in intensity when compared to the patient's constitutional DNA. In the cell line, however, this allele was completely absent (Fig. 2B, Lanes N, T, and CL). Similarly, in case D104, LOH appeared as a difference in the staining intensities of the tumor alleles at informative loci. This could be most easily seen with the IGF2 polymorphism. Both alleles were of equal intensity in the patient's constitutional DNA, whereas one allele was reduced by 55–60% compared to the other allele in the tumor (Fig. 2C).

The tumors from the patient with BWS (case D123) and from the two brothers (cases DZ28 and DZ29) did not have LOH. However, case D123 was only informative at Wi'-!, DRD4, and D11S490.

Parental Origin of LOH. For all six HBs with LOH on 11p, parental genomic DNA was available. Parental origin of the lost allele was determined for between three to six loci with LOH in these tumors. In all instances, the lost allele was of maternal origin (Fig. 2B).

Discussion

HB is known to be one of four malignancies associated with BWS, the other three being Wilms' tumor (or nephroblastoma), rhabdomyosarcoma, and adrenocortical carcinoma (3, 21). Since Wilms' tumor and rhabdomyosarcoma both show LOH on chromosome arm 11p and BWS has been linked to 11p15.5 (3), previous studies of LOH in HB have also focused on chromosome arm 11p. Kiechle-Schwarz et al. (5) found LOH on 11p in one of four informative HBs; the tumor had LOH at all loci for which the patient was informative (HRAS1, INS, PTH, and CALCA). Koufos et al. (6) found LOH on 11p15 in two of
LOH IN HEPATOBLASTOMA

Fig. 2. A. PCR analysis of a dinucleotide repeat at the HBB locus (11p15.5) in three HBs. Arrowheads, both strands of each allele. The other faint bands represent shadow-bands, a common occurrence when dinucleotide repeats are amplified by PCR. Case D161, noninformative; case D175, informative without LOH; case D158, LOH. (N, patient's constitutional DNA; T, tumor DNA). B. PCR analysis of a tetranucleotide repeat at the TH locus (11p15.5) in case DZ25 to determine parental origin of the lost allele. M, P, N, maternal, paternal, and patient's constitutional DNA; T, tumor DNA; CL, DNA from tumor-derived cell line. The lost allele is of maternal origin. C. PCR analysis of a dinucleotide repeat at the IGF2 locus (11p15.5) in case D104 (electropherogram). The patient (N) is informative at both the 5' and 3' ends of the repeat. Note the equal height of the peaks. In the tumor (T), one allele is reduced by 55—60%.

three informative HBs. Byrne et al. (3) found LOH on 11p in four of six HBs, one of them from a patient with BWS. Two of their tumors had LOH at 11p15.5, and one had LOH at 11p15.5 and CALCA (11p15.1) but was noninformative at the intervening loci; one had LOH restricted to CALCA. Their findings in the first three tumors are comparable to ours. Since we did not examine CALCA, we cannot rule out that some tumors in our series could have had an isolated LOH at that locus. However, none of the tumors with LOH in our study had such a single-locus LOH. Significantly, the WT-1 tumor suppressor gene at 11p13 which is mutated and undergoes LOH in some Wilms' tumors (3) was not part of the common deletion in our series, indicating that it is unlikely to be involved in the pathogenesis of HB.

In two of our cases (D104 and DZ25), LOH did not appear as a complete loss of one allele but as a significant reduction in the intensity of one allele compared to the other. This apparent “partial” LOH could simply represent contamination of the tumor by constitutional DNA from nontumorous elements such as stroma or leukocytes. This is the most likely explanation for case DZ25, where one allele was quite faint in the original tumor and completely absent in the cell line. However, in case D104, one allele was only reduced by 55—60%. Review of the frozen section from the tumor fragment selected for DNA extraction showed only minimal amounts (<10%) of contaminating nontumorous tissue. Furthermore, this consisted only of poorly cellular connective tissue, which could not have contributed much to the DNA extracted from this fragment. This may represent LOH in an aneuploid tumor or LOH in a tumor composed of two or more clones, some with LOH on 11p and some without.

Interestingly, the three multifocal HBs included in our series all had LOH on 11p, and these are also the only tumors associated with a fatal outcome. A multifocal growth pattern is an indicator of poor prognosis in HB (22). While the numbers are too small to draw definite conclusions, these results suggest that LOH on 11p in HB may be associated with multifocal growth and more aggressive behavior.

Deletions at 11p15.5 occur not only in HB but in a wide variety of malignancies, including Wilms’ tumor, ovarian and testicular tumors, rhabdomyosarcoma, breast cancer, and lung cancer (23). More than one subregion of 11p15.5 may be involved (3). Since all the tumors in our series with LOH on 11p had LOH at all 11p15.5 loci for which they were informative, we could not determine whether different subregions are deleted in individual HBs. To answer this question will require further studies using additional polymorphisms. Nevertheless, these observations point to the existence of at least one tumor suppressor gene at 11p15.5.

If LOH occurred at random, one would expect the allelic loss to affect maternal and paternal alleles at the same frequency, i.e., 50%. However, as reviewed elsewhere (7), in retinoblastoma, osteosarcoma, embryonal rhabdomyosarcoma, neuroblastoma, and Wilms’ tumor, LOH involves predominantly or exclusively the maternal allele. Parental origin of LOH has not been examined previously in HB. As our results indicate, HB also belongs to this group of tumors. The probability of finding only maternal LOH in six tumors purely by chance is 1 of 64, and the same holds true for finding only paternal LOH. The probability (P) of finding uniparental LOH in six tumors by chance only is, therefore, 1 of 32 or 0.03125.

Uniparental LOH implies that there is imprinting in the region of the allelic loss. Imprinting refers to parental allele-specific expression of a gene such that only the maternal or the paternal allele is expressed. If a tumor suppressor gene is imprinted, the imprint itself would represent the first of the two hits postulated by Knudson (7) since it constitutionally inactivates one allele. The active (nonimprinted) allele would be removed in a second hit by deletion. This “inactivation model” (7) would explain uniparental LOH in tumors.

However, the telomeric region of chromosome arm 11p contains not only at least one putative tumor suppressor gene but also an important growth-promoting gene, i.e., IGF2. IGF2 is a major fetal growth factor and appears to act in an autocrine fashion (24). It is expressed at high levels in fetal liver and the same has been shown in a hepatoblastoma cell line (21). IGF2 is one of the few genes known to be imprinted in humans. Only the paternal allele is expressed in fetal liver (maternal imprint), but in the adult liver, imprinting is relaxed, and both alleles are expressed (25). Monoallelic expression of IGF2 in HB has been reported recently in three of three HBs that were informative for an intragenic RFLP in the IGF2 gene (21). Although
parental origin of the expressed allele was not determined, it seems reasonable to assume that it was indeed the paternal one. This could explain our observation of maternal LOH on 11p15.5 in HBs. Paternal LOH at 11p15.5 would cause loss of the active IGF2 allele, whereas maternal LOH would preserve it. At the same time, maternal LOH would also remove the active maternal allele of a paternally imprinted tumor suppressor gene. In this scenario, cells with maternal LOH would have no active tumor suppressor gene but still retain an active IGF2 allele. This may have a synergistic effect on cell growth.

In summary, our results indicate that LOH on 11p occurs in a significant fraction of HBs. The common deletion was 11p15.5 and did not include the WT-1 gene at 11p13. These results provide further evidence of at least one tumor suppressor gene lying in the telomeric region of chromosome arm 11p. As in several other embryonal tumors of childhood, the lost allele was of maternal origin. This is most likely due to maternal LOH on 11p15.5. LOH would preserve it. At the same time, maternal LOH would also remove the active maternal allele of a paternally imprinted tumor suppressor gene. In this scenario, cells with maternal LOH would have no active tumor suppressor gene but still retain an active IGF2 allele. This may have a synergistic effect on cell growth.

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References
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