Short Communication

Microsatellite Instability Is Infrequent in Neuroblastoma

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Abstract

Neuroblastoma (NB) is a childhood cancer of the autonomic nervous system. The molecular pathology of NB is not yet well understood. Both amplification of the proto-oncogene N-myc and loss of heterozygosity of several chromosomal loci occur in NB, representing genetic instability. In this study, we examined another type of genetic instability, microsatellite instability. Five chromosomal loci known to exhibit this alteration in colon, gastric, and pancreatic cancers were used in a PCR-based assay to examine 30 matched normal and tumor DNAs, which included all stages of tumor progression. Among these 30, only 2 (7%) manifested microsatellite instability. There was no correlation between the occurrence of microsatellite instability and the amplification of the N-myc gene. These data show that microsatellite instability is infrequent in neuroblastoma tumors.

Introduction

NB is a relatively common solid tumor of the autonomic nervous system of children, affecting 1 in 7000 children before the age of 5 years (1). It is unusual in that, for unknown reasons, it has a high rate of spontaneous regression. The proto-oncogene N-myc is amplified in 22% of NB patients and is associated with metastasis and fatal outcome (2). Loss of heterozygosity has been observed at several chromosomal loci, although no genes have yet been identified at these loci (3-5). Recently we have shown allelic imbalance at the APC locus in 38% of NB tumor DNAs (6). These data show that genetic instability, manifested as amplification of the N-myc gene, loss of heterozygosity at several loci, and allelic imbalance, occurs in a relatively small proportion of neuroblastoma patients.

Microsatellites, which are short nucleotide sequence repeats occurring approximately 100,000 times throughout the human genome, tend to be polymorphic among different individuals but stable within one individual (7, 8). Instability in microsatellite DNA, manifested as differences in the number of repeats between normal and tumor DNA from the same patient, has been observed at high frequencies in hereditary nonpolyposis colon cancer (79%), pancreatic cancer (67%), bladder cancer (41%), and gastric cancer (31-39%), among other tumor types (9-16). The frequency of microsatellite instability is not uniformly high in all types of cancer, however. It is exhibited in only 13-22% of sporadic colon, uterine, esophageal and ovarian cancers, and occurs in 0-4% of cancers of the breast, liver, lung, and testis (10, 12, 14, 15, 17). One cause of microsatellite instability appears to be mutation in mismatch repair genes. Human genes identified thus far include hMSH2, hMLH1, hPMS1, and hPMS2 (18-23). Because little is known about the molecular biology of NB, we examined 30 neuroblastomas and matched normal tissues for microsatellite instability.

Materials and Methods

Patient Samples. Purified DNA or tissue from 30 paired normal and tumor samples was obtained from the Pediatric Oncology Group neuroblastoma tissue bank. DNA was extracted by using standard methods (24). Matching data including patient age, site of primary tumor, stage, N-myc amplification, DNA index, survival, and follow-up time were available for each patient.

Analysis of Microsatellite Instability. Normal and tumor DNAs were PCR-amplified at microsatellite repeat loci D2S123, D2S147, D2S119, D10S197, and D11S904, which show relatively high instability rates in colorectal and gastric tumors (10, 13). We used multiplex PCR, in which more than one locus is amplified simultaneously in the same reaction tube. Loci D2S123 and D10S197 were amplified, as were loci D2S147, D2S119, and D11S904. PCR conditions consisted of 33 cycles at 95°C X 50 s, 58°C X 90 s, and 72°C X 90 s. PCR was performed by using 0.2 μCi of [33P]dCTP incorporated into a 10-μl reaction volume. After denaturation in 95% formamide, PCR products were electrophoresed on denaturing polyacrylamide gels and visualized by autoradiography. Instability was manifested as alteration in DNA fragment lengths in tumor relative to normal.

Results

Of 30 matched samples analyzed at each of the 5 loci, 2 (7%) exhibited microsatellite instability (Table 1). The DNA pattern of one of the positives, patient 747, is shown in Fig. 1. In the tumor DNA there was a novel band at locus D2S123, a shorter band containing a decreased number of repeats at D2S147, and a longer band suggesting an increased number of repeats at D2S119 and D11S904. The normal and tumor DNAs were derived from the same patient, as evidenced by the absence of band changes at locus D10S197, which was amplified in the same tube as D2S123 (see “Materials and Methods”). Furthermore, there were no differences between normal and tumor DNAs from this patient revealed by PCR primers from a region.

Received 3/9/95; revised 5/31/95; accepted 6/2/95.

1 This work was supported in part by American Cancer Society Grant 93-01 and NIH/Pediatric Oncology Group Grant CA-30969 (P.E.B.) and American Cancer Society Grant PDT-419, NIH Grant RO1 DK77167-01, and a grant from the Department of Veterans Affairs (S.M.).

2 To whom requests for reprints should be addressed, at University of Maryland School of Medicine, Department of Pediatrics, 655 West Baltimore Street, Room 10-031, Baltimore, MD 21201.

3 The abbreviation used is: NB, neuroblastoma.
Table 1  Microsatellite instability in NB: patients showing genetic instability

<table>
<thead>
<tr>
<th>Patient</th>
<th>POG no.</th>
<th>D2S2119</th>
<th>D2S123</th>
<th>D2S147</th>
<th>D10S197</th>
<th>N-myc</th>
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<tbody>
<tr>
<td>34</td>
<td>106003</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>200</td>
</tr>
<tr>
<td>254</td>
<td>109444</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>326</td>
<td>110590</td>
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<td></td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>747</td>
<td>114792</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* POG, Pediatric Oncology Group.
* The copy number of the N-myc gene is given only if amplified.

Table 2  Microsatellite instability in NB: clinical data of patients showing microsatellite instability

<table>
<thead>
<tr>
<th>Patient</th>
<th>N-myc copy no.</th>
<th>DNA index</th>
<th>Age Dx (month)</th>
<th>Clinical stage</th>
<th>Site of primary</th>
<th>Follow-up time (month)</th>
<th>Outcome</th>
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<tr>
<td>254</td>
<td>1</td>
<td>ND</td>
<td>19</td>
<td>B</td>
<td>A/P/RP</td>
<td>51</td>
<td>Alive</td>
</tr>
<tr>
<td>747</td>
<td>75</td>
<td>1.0</td>
<td>14</td>
<td>D</td>
<td>Adrenal</td>
<td>25</td>
<td>Alive*</td>
</tr>
</tbody>
</table>

* Age Dx, age at diagnosis.
* Staging according to POG: B, incomplete gross resection of primary tumor with negative lymph nodes and liver; D, metastases beyond intracavity nodes.
* A, abdomen; P, peritoneum; RP, retroperitoneum.
* ND, not determined.
* Alive but relapsed.

containing a variable number of tandem repeats within the p53 gene.

Patient 254 exhibited instability at locus D10S197, consisting of an abnormally long band suggesting a gain of repeats (data not shown).

Clinical and molecular data for the two patients manifesting microsatellite instability are displayed in Table 2. Among the 30 patients analyzed in this study, 6 (20%) showed amplification of the N-myc gene (Tables 1 and 2), which is representative of the frequencies reported in previous studies of NB patients (2). Patient 747, who exhibited microsatellite instability, showed amplified N-myc and a DNA index of 1.0, both indicators of a poor prognosis. This patient was stage D and is alive but has relapsed. In contrast, patient 254 exhibited microsatellite instability, but only a single copy of N-myc, was stage B, and the patient is alive after 51 months.

Discussion

We analyzed 30 matched normal/tumor DNAs at 5 loci shown previously to exhibit microsatellite instability (10) and detected two patients (7%) with altered numbers of repeats. This rate lies between the 0-4% prevalences reported for cancers of the lung, breast, liver, and testis and the 13-22% reported for sporadic colon, uterine, esophageal, and ovarian cancers (9, 11, 13, 14). These data suggest that microsatellite instability is an infrequent occurrence in neuroblastoma tumors.

The genetic instability often manifested by tumor cells can consist of DNA amplification, loss of heterozygosity, and aneuploidy (25), all of which are known to occur in NB. Recent studies show that microsatellite instability may constitute another type of genetic instability (9-17). The interrelationships among these various types of genetic instability are not yet known. Of the six NB tumors known to exhibit an amplified N-myc gene in this study, only one manifested microsatellite instability; conversely, only one of the two tumors exhibiting microsatellite instability showed N-myc amplification. These

preliminary data suggest there is no apparent correlation between microsatellite instability and N-myc amplification in these tumors.

Acknowledgments
We wish to thank Jonathan Bell for performing some of the key assays in this study.

References
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