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Advances in Brief

Deletion within the *D17S34* Locus in a Primitive Neuroectodermal Tumor[1]

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Abstract

Loss of heterozygosity on chromosome 17p13.3 is frequently observed in solid tumors, and the presence of a tumor suppressor gene has been predicted in this region of chromosome 17. We have analyzed a primitive neuroectodermal tumor sample exhibiting loss of heterozygosity at the *D17S34* locus, a commonly used telomeric marker on the short arm of chromosome 17. The remaining allele showed a rearrangement. Cosmids spanning the *D17S34* locus and probes from that region were used to demonstrate a 9-kb deletion within the *D17S34* locus and were found to contain evolutionary, conserved sequences. Genetic alterations in this region may also affect expression of immediately adjacent genes, such as *ABR*, and could be a common mechanism in the causation of primitive neuroectodermal tumors.

Introduction

PNETs[3] and medulloblastomas are the most common malignant tumors of the central nervous system in childhood (1). Most frequently, cytogenetic abnormalities have been found on chromosome 17. These include an isochromosome 17q and LOH on the short arm of chromosome 17 (2–8). LOH occurs when a chromosome or segments of it are lost in tumor tissue. When the remaining allele undergoes a mutation or a deletion at, for example, a tumor suppressor gene, this may lead to tumorigenesis.

A deletion of 17p13.3 has been reported to occur in 37–44% of medulloblastomas studied (6, 7). Loss of the most telomeric marker p144D6 (*D17S34*) has been demonstrated in all tumors displaying LOH on 17p, and allelic loss only at the *D17S34* locus has been described (4, 6). Moreover, a variety of other solid tumors have LOH on 17p13.3, such as breast cancer, ovarian cancer, astrocytoma, and hepatocellular carcinoma (9–16). These studies have indicated that a locus on distal chromosome 17p is involved in the development of tumors, which is distinct from the tumor suppressor gene *p53*. In the present study, we analyzed in detail the DNA of a PNET tumor, which had shown LOH for *D17S34* and for which Southern blot analysis suggested a chromosomal rearrangement in the other allele. Using cosmid cloning and mapping, we demonstrate that the other *D17S34* allele has undergone a microdeletion and have determined the boundaries of this deletion.

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[3] The abbreviations used are: PNET, primitive neuroectodermal tumor; LOH, loss of heterozygosity.

Materials and Methods

Tumor DNA. Matched tumor and normal DNAs from patient 14 were isolated as described previously (2). This patient had a PNET of the posterior fossa (medulloblastoma; Ref. 3). The tumor specimen showed allelic loss on 17p and a rearrangement with probe p144D6 (3, 5).

Cosmid DNA. Overlapping cosmids c68A3 and c48B2 were isolated using a *D17S34* probe and span a region of approximately 56 kb. Neither these cosmids nor additional ones isolated by walking from the ends contain sequences hybridizing with *ABR* cDNA. Previous experiments using fluorescent *in situ* hybridization showed *ABR* and *D17S34* to be located within 250 kb of each other (6, 17). Plasmid p144D6 (locus *D17S34*) was obtained from the American Type Culture Collection (Rockville, MD). This plasmid contains an insert of approximately 5.5 kb and was originally isolated by digestion of cosmid DNA with *Mbo*I (18). However, digestion of the 5.5-kb insert of p144D6 with *Mbo*I showed the presence of an internal *Mbo*I site. Hybridization to genomic and cosmid DNA showed that the p144D6 probe consists of two fragments of 5 and 0.5 kb ligated together, which in reality are located approximately 25 kb apart in the genomic DNA. On a genomic blot with total human DNA, the hybridization signal of the 0.5-kb fragment relative to the 5-kb fragment is extremely weak and is apparent only as faint background bands. Cosmid DNAs were mapped with different enzymes, and location of the p144D6 probe was determined by hybridization.

To isolate probe *b* (1.4 kb *Sst*I), an 8-kb *Eco*RI-*Bam*HI fragment from cosmid c68A3 was first subcloned into pSK (Stratagene). This plasmid was mapped, digested with different restriction enzymes, and hybridized with total human DNA to identify fragments that lack repetitive sequences. Probe *b* was identified in this manner as a repeat-free probe.

Results and Discussion

RFLP studies of PNET and medulloblastoma patients have demonstrated frequent LOH at the *D17S34* locus on chromosome 17p13.3. One such patient (patient 14) had PNET of the posterior fossa (medulloblastoma), and tumor DNA showed LOH with every informative marker on 17p between 17p11.2 and 17pter (2, 3). Of 11 different probes from 17p used to examine this DNA, one, the p144D6 probe, detected an abnormal-sized band in place of the remaining allele using *Rsa*I and *Pst*I digestions (5). We used the p144D6 probe to further analyze this rearrangement at the distal 17p locus.

Tumor and normal DNA of PNET patient 14 and control DNAs from four unrelated individuals were digested with different restriction enzymes and hybridized with the p144D6 probe (Fig. 1). A polymorphic hybridization pattern was detected in digestions with *Bgl*II, *Eco*RI, and *Hin*dIII. The normal DNA of patient 14 was informative at *D17S34*, and the tumor DNA exhibited LOH (Fig. 1*A*, *Lanes N* and *T*). However, the remaining allele in the tumor DNA was different in size from that in the corresponding normal DNA of patient 14; in all control DNAs, the probe detected polymorphic *Bgl*II fragments varying in size from 4.5 to 6.5 kb, whereas an aberrant 14-kb *Bgl*II fragment was detected in the tumor DNA (Fig. 1*A*, *Lane T*). The control DNA samples, including the nontumor control of patient 14, contained two *Hin*dIII fragments larger than 20 kb, but the tumor showed one fragment of 16 kb (Fig. 1*B*, *Lane T*). The patient's normal

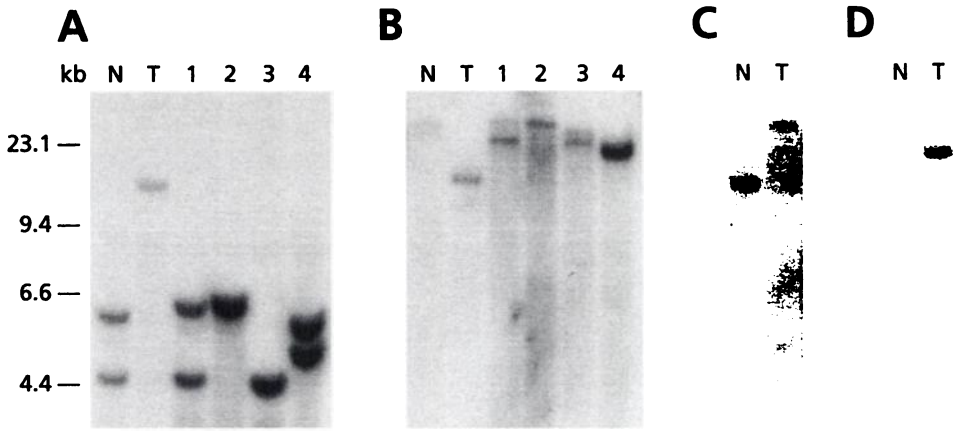

Fig. 1. Rearrangement at *D17S34* in the PNET. DNAs include normal (*N*) and tumor (*T*) DNAs from the patient, and four unrelated DNAs (labeled *1–4*). DNAs were digested with *Bgl*II (*A*), *Hin*dIII (*B*), *Eco*RI (*C*), and *Bam*HI (*D*). The blot was hybridized to the p144D6 probe. The sizes of molecular weight markers are indicated to the *left*. Fragments above 20 kb do not transfer well; as a consequence, their hybridization signal is relatively weak.

DNA digested with *Eco*RI yielded two fragments of 15 and 30 kb, whereas in tumor DNA only a single fragment of 30 kb was detected (Fig. 1*C*, *Lane T*). Finally, instead of the expected *Bam*HI wild-type fragment of approximately 30 kb seen in the nontumor control of patient 14, a single fragment of 20 kb was detected in tumor DNA (Fig. 1*D*, *Lane T*). Note that the differences in intensities among different bands (*e.g.*, in the *Eco*RI digest; Fig. 1*C*, *Lane N*, compare *upper* and *lower* band) are caused by the large sizes of some of the fragments; fragments in excess of 20 kb will be underrepresented, will transfer poorly, and thus will yield a relatively weak hybridization signal. Additional digestions with other enzymes consistently showed a single abnormal-sized band in the tumor DNA (data not shown).

To define the type of rearrangement in the tumor sample on a genomic level, we mapped the region containing *D17S34* using cosmids. The probe p144D6 (Fig. 2, probe *a*) detected a 6-kb *Bgl*II, an 18-kb *Eco*RI, a 16-kb *Hin*dIII, and a very large *Bam*HI fragment in cosmid DNA (Fig. 2). This region, which is detected by the probe p144D6, contains a variable number tandem repeat (Fig. 1, *A* and *B*, *Lanes 1–4*). Because the *Bam*HI fragment in patient 14 tumor DNA was significantly smaller than in the patient's normal DNA (Fig. 1*D*, *Lanes N* and *T*), we investigated the possibility that a deletion had occurred in the tumor sample. A repeat-free probe (Fig. 2, probe *b*) was prepared 15 kb distant from the probe p144D6. If there had been a deletion, both probes could detect the same *Bgl*II, *Eco*RI, *Bam*HI, and/or *Hin*dIII fragments in the tumor DNA, depending on the location and extent of the deletion. In the cosmids, probe *b* hybridized to different restriction enzyme fragments than probe *a*, including 18-kb *Bgl*II, 19-kb *Eco*RI, and 9-kb *Hin*dIII fragments (Fig. 2). The same, very large *Bam*HI fragment was detected by probes *a* and *b*.

In control genomic DNA, probe *b* detected polymorphic restriction enzyme fragments with *Bgl*II and *Eco*RI; probe *b* detects another variable number tandem repeat (Fig. 3*A*, *Lanes 1–4* and data not shown). The nontumor DNA sample of the patient showed two *Bgl*II fragments of 15 and 18 kb, which were clearly distinct from those detected by probe *a* (compare Figs. 1*A* and 3*A*, *Lanes N*). In contrast, using probe *b*, the tumor sample showed a single 14-kb *Bgl*II fragment

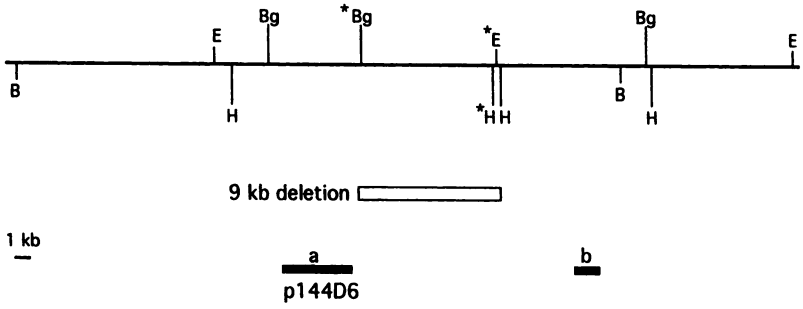

Fig. 2. Physical map of cosmids c68A3 and c48B2 containing the *D17S34* locus. The deletion observed in the PNET patient 14 is indicated by an *open box*. The p144D6 probe is indicated by the *black bar a*; the 1.4-kb *Sst*I probe is indicated by the *black bar b*. *B*, *Bam*HI; *E*, *Eco*RI; *H*, *Hin*dIII; *Bg*, *Bgl*II. Note that for clarity, only those restriction sites are indicated that correspond to fragments discussed in the text. *, restriction enzyme sites deleted in the tumor DNA of patient 14.

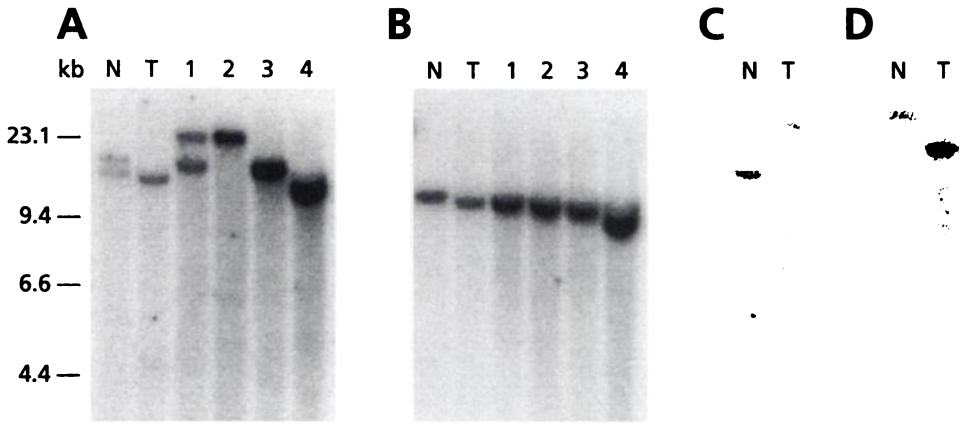

Fig. 3. Analysis of the deletion in the PNET sample. See Fig. 1 legend for a description of the DNA samples used. The blot shown in Fig. 1 was rehybridized to probe *b*.

of a size indistinguishable from that detected by probe *a* (Figs. 1*A* and 3*A*, *Lanes T*). Moreover, *Eco*RI fragments of 19 and 30 kb were detected in the patient's normal DNA, but a single identically sized fragment was detected in tumor DNA by both probes *a* and *b* (compare Figs. 1*C* and 3*C*, *Lanes T*). In addition, identically sized *Bam*HI fragments of 20 kb were detected with both probes *a* and *b* in tumor DNA (Figs. 1*D* and 3*D*, *Lanes T*), whereas in the nontumor control DNA of patient 14, one single large *Bam*HI fragment was detected by both probes (Figs. 1*D* and 3*D*, *Lanes N*). This provides strong evidence that the tumor DNA had undergone a deletion, resulting in the disappearance of *Bgl*II and *Eco*RI sites (Fig. 2, indicated with an *asterisk*). Probe *b* detected 9-kb *Hin*dIII fragments in all of the samples, and this allowed us to delineate the boundary of the deleted region (Fig. 3*B*). These data established that *Bgl*II, *Eco*RI, and *Hin*dIII restriction sites were lost because of an internal deletion of approximately 9 kb in the tumor DNA of patient 14.

The loss of a specific region of a chromosome in a malignant specimen suggests that the locus may contain sequences important to the development of that tumor. The most telomeric gene on chromosome 17p thus far identified is *ABR*, which is located within 250 kb from locus *D17S34* (6, 17). It is possible that the deleted region found in this PNET tumor sample contains regulatory elements of the *ABR* or of another currently unidentified gene located between *ABR* and *D17S34*. In this regard, it is of significance to note that in preliminary experiments we have found two DNA segments that are relatively well-conserved between man and rodents in this region (data not shown). Immediate future experiments are designed to isolate a putative cDNA corresponding to the *D17S34* locus, which is altered in at least one PNET.

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