High-Resolution Deletion Mapping of Chromosome Arm 17p in Childhood Primitive Neuroectodermal Tumors Reveals a Common Chromosomal Disruption Within the Smith-Magenis Region, an Unstable Region in Chromosome Band 17p11.2

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Loss of heterozygosity (LOH) on chromosome arm 17p is the most common genetic aberration in childhood primitive neuroectodermal tumors (PNETs). To determine the frequency and extent of 17p deletions, 29 loci on 17p were investigated in 24 tumors by using restriction fragment length polymorphism (RFLP) and microsatellite analysis. LOH on 17p was found in 9 of 24 tumors. In all tumors with LOH, a continuous stretch from the telomere to chromosome band 17p11.2 was completely deleted, and no interstitial or terminal small-scale deletions were detected in the remaining 15 tumors. In four tumors with LOH on 17p, the chromosomal breakpoint was located between D17S953 and D17S805. To identify this deletion breakpoint on the cytogenetic map of chromosome 17 and to exclude uniparental disomy, we verified our data by using fluorescence in situ hybridization (FISH) analyses. By using two yeast artificial chromosome (YAC) clones that were positive for D17S689 and D17S953, the same breakpoint was confirmed in two specimens of cerebrospinal fluid (CSF) metastases by using FISH on interphase preparations. We demonstrate that, in most childhood PNETs with LOH on 17p, the breakpoint is close to, but not within, the centromere. It varies, and it occurs predominantly between the two markers D17S689 and D17S953, which is an unstable chromosomal region that is deleted or duplicated in the Smith-Magenis syndrome. Because LOH of 17p is associated with the formation of isochromosome 17q in the majority of PNETs, this study provides entry points to determine the molecular nature of this phenomenon.

INTRODUCTION

Primitive neuroectodermal tumors (PNETs) are the most frequent malignant childhood brain tumors, with a cumulative incidence of 16/100,000 children below 16 years of age in Germany (Haaft et al., 1993). Medulloblastomas are the most frequent PNETs and arise in the posterior fossa within the cerebellar vermis or hemispheres. The supratentorial PNET (stPNET) displays very similar histological features; therefore, both tumors have been proposed to represent a distinct biological entity (Rorke et al., 1985; World Health Organization, 1994). Medulloblastomas are the most frequent PNETs and arise in the posterior fossa within the cerebellar vermis or hemispheres. The supratentorial PNET (stPNET) displays very similar histological features; therefore, both tumors have been proposed to represent a distinct biological entity (Rorke et al., 1985; World Health Organization, 1994). 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Saylors et al., 1991; Batra et al., 1995). Due to the rare occurrence of mutations within the TP53 and loss of heterozygosity (LOH) studies showing restricted deletions of chromosome band 17p13.3, it has been suggested that there may be a second tumor suppressor gene in 17p13.3 that is involved in the pathogenesis of PNETs (Biegel et al., 1992; Cogen et al., 1992; McDonald et al., 1994).

Even though most LOH studies on 17p in PNETs have been performed by using restriction fragment length polymorphism (RFLP) and microsatellite analyses with only a limited repertoire of polymorphic markers, they have revealed several deleted regions along the short arm of chromosome 17 (McDonald et al., 1994; Batra et al., 1995). In contrast, we used a previously ordered set of 29 polymorphic markers (Wilgenbus et al., 1996) from 17p to determine the frequency and the accurate extent of chromosomal loss of the short arm of chromosome 17 in childhood PNET. The density of the markers was especially high in the telomeric region (subband 17p13.3), because a putative tumor suppressor gene deleted in PNET has been located to this region (Biegel et al., 1992; Cogen et al., 1992). A high-resolution analysis was also performed for the chromosomal region between loci D17S689 and D17S953, because this region turned out to encompass a common chromosomal breakpoint in tumors. We reasoned that this high-resolution deletion mapping of chromosome arm 17p in childhood PNET might aid in circumscribing a locus that harbors a tumor suppressor gene relevant for PNET development.

**MATERIALS AND METHODS**

**Tumors and Patients**

Tumor biopsies of 25 patients (see Table 1) were snap frozen in liquid nitrogen immediately after removal. Histologic diagnosis was made according to the WHO classification of brain tumors (World Health Organization, 1994). Informed consent was obtained from the children’s parents and from the local ethics committee to withdraw blood when venipuncture was necessary for routine care. Tumor cells of cerebrospinal metastases that were obtained

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*All patients except for FV and NF were treated by surgery, chemotherapy, and radiotherapy according to a standard protocol that has been published elsewhere (Kuhl et al., 1993). PNET, primitive neuroectodermal tumor; stPNET, supratentorial PNET; LOH, loss of heterozygosity; n.d., not done.
during routine diagnosis of two patients were used for fluorescence in situ hybridization (FISH) analysis.

**DNA Samples**

Whole genomic high-molecular-weight DNA was isolated from 24 tumor biopsies and from the patients' peripheral blood cells (PBL) by using a salting-out method (Miller et al., 1988). In ambiguous cases, comparison of the patients' microsatellite patterns with their parents' DNA patterns was helpful to differentiate between homozygous uninformative allelic configuration, preservation of both alleles with similar repeat numbers (see, e.g., Fig. 2, tumors MW and SG, locus D17S805), and amplification artifacts.

**Microsatellite and RFLP analysis**

Microsatellite analysis was carried out by using 28 short tandem repeat polymorphisms (STRP) that were located previously to the short arm of chromosome 17 (Gyapay et al., 1994; Gerken et al., 1995). Several of the annealing temperatures for polymerase chain reaction (PCR) amplification were determined empirically and are indicated when they are different from published data. The microsatellites analyzed in our study were as follows: D17S643 (60°C), D17S654 (65°C), D17S731, D17S755, D17S606 (60°C), D17S675, D17S678 (58°C), D17S689 (65°C), D17S695, D17S900 (60°C), D17S906 (60°C), D17S513 (60°C), D17S620 (60°C), D17S926 (58°C), D17S938 (58°C), D17S796 (58°C), D17S786 (60°C), D17S805 (60°C), D17S841 (65°C), D17S945 (65°C), D17S947 (58°C), D17S849 (52°C), D17S922 (55°C), D17S953 (55°C), D17S691 (58°C), D17S793 (56°C), D17S1794 (60°C), D17S71 (65°C), D6S311 (60°C), D9S277 (54°C), and D16S752 (58°C). One PCR reaction typically contained 150 ng DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 200 µM of each deoxynucleotide triphosphate, and 1.5 U Taq Polymerase in a final volume of 30 µl. For radioactive labeling of PCR products, 5 µCi [α³²P]-dCTP (specific activity, 3,000 Ci/mmol) was added to the reaction. Denaturation occurred at 94°C for 1 minute, and extension occurred at 72°C for 1 minute. Aliquots of PCR products were heat denatured and separated on denaturing 6% polyacrylamide gels. The gels were subsequently dried and exposed to x-ray films for 1–7 days. LOH of marker D17S34 was performed by RFLP studies on Southern blots of MspI-digested cellular DNA using clone p144D6 (American Tissue Culture Collection) as hybridization probe.

**Figure 1.** Results of loss of heterozygosity (LOH) and fluorescence in situ hybridization (FISH) using polymorphic markers that were separated according to ordered bins along the short arm of human chromosome 17 (Wilgenbus et al., 1996). Relative positions of yeast artificial chromosome (YAC) clones B38A4 and 907E8 along chromosome arm 17p are indicated by circles. Squares indicate microsatellite data, and circles indicate FISH data. Solid squares, LOH; half solid squares, not informative; open squares, both alleles preserved; n.e., not evaluable.
RESULTS

LOH Studies

Twenty-nine loci on chromosome arm 17p were analyzed in 24 tumors (Table 1) by microsatellite and RFLP analyses. LOH was observed in nine cases, with deletion of a continuous stretch from the telomere to band 17p11.2 in all nine tumors (Fig. 1). No difference in extent of LOH was observed between one stPNET (tumor TG) and infratentorial medulloblastomas. No circumscribed small-scale deletions were detected in any of the remaining 15 tumors. No significant background of low-intensity bands was observed in the tumor probes with apparent LOH on chromosome arm 17p. This was also true for six of the 15 remaining tumor probes without LOH of chromosome arm 17p, in which LOH of chromosomes 6, 9, and 16 was detected by using the same experimental approach (Table 1). These data suggested a homogeneous tumor cell population in the PNET probes analyzed in our study without significant contamination of normal tissue (e.g., infiltrating lymphocytes, blood vessels, etc.). The determination of the chromosomal breakpoint between two adjacent microsatellite markers was possible in five tumors: tumor MW (D17S71 and D17S805), tumor PR (D17S689 and D17S691), tumor SG (D17S71 and D17S805), tumor SA (D17S1794 and D17S689), and tumor MF (D17S71 and D17S805; see also Fig. 1). In four other tumors, there was at least one interspersed marker that was not informative. In tumor MA, the breakpoint could be located between D17S689 and D17S953; in tumor AM, it was between D17S841 and D17S689; in tumor TP, it was between D17S805 and D17S953; and, in tumor TG, it was between D17S841/D17S691 and D17S805 (Fig. 1; data on AM not shown). Karyotype analysis of tumor AM showed an i(17q), suggesting breakage of this chromosome very close to or within the centromere (data not shown). In summary, the LOH studies demonstrated that disruption breakpoints of PNETs on 17p are clustered...
between the two polymorphic markers D17S805 and D17S953.

**YAC Mapping and FISH Analysis**

To define and verify the deletion breakpoint between D17S953 and D17S805, we isolated and characterized YAC clones flanking the breakpoint region. YAC clones 907E8, 838E4, and 818H9 were selected for retention of STSs D17S689/D17S805 and D17S953/D17S122. The size of these clones was verified by using PFGE, and whole yeast DNA from yeast colonies was also tested for retention of ST Ss D17S689/D17S805 and D17S953/D17S122. The size of these clones was verified by using PFGE, and whole yeast DNA from yeast colonies was also tested for retention of ST Ss. The order of these STSs was determined previously on a radiation hybrid map panel of 17p (Wilgenbus et al., 1996). The respective sizes of YACs and mapping results are summarized in Figure 3A. Subsequently, IRS-L R PCR products from YAC clones 907E8 and 838E4 were hybridized to human metaphase chromosomes, and both probes revealed strong signals only on chromosome segment 17p11-12 (907E8; see Fig. 3B). IRS-L R PCR products derived from YACs 907E8 and 838E4 were also used for interphase FISH analysis on whole CSF cytospin preparations of metastasizing PNETs (tumors MA, TM, and SK). The hybridization probe derived from YAC clone 907E8 revealed preservation of both alleles in three CSF metastasis specimens with LOH on chromosome arm 17p (Fig. 4; tumors MA, TM, and SK). In contrast, hybridization with a complex probe derived from YAC 838E4 revealed only single signals on interphase preparations from CSF cytospin preparations of metastasizing PNETs (Fig. 4B; tumors MA and TM). Thus, we were able to demonstrate clearly that the disruption breakpoint leading to a monoallelic deletion of chromosome segment 17p11.2-17pter is located between these two YAC clones. In two metastases (tumors T M and SK), loss of chromosome arm 17p has also been demonstrated by using FISH analysis of tumor cells with YAC 677H10, which maps to 17p13.1 (Wilgenbus et al., 1996).

**DISCUSSION**

The aim of our study was to identify the frequency and extent of allelic loss on chromosome arm 17p in PNETs. We reasoned that this particular chromosome arm would harbor an important tumor suppressor gene that is critical for maintenance of a benign phenotype of the primitive neuronal stem cell.

In contrast to previously published reports, we used significantly more polymorphic markers obtained from a set of ordered ST RPs that were assigned previously to a somatic and radiation hybrid panel (Wilgenbus et al., 1996). The exact chromosomal position of each marker was assessed before. Considering a size of 28 Mb of the entire short arm of human chromosome 17 (Morton et al., 1991) and the fact that, in total, we used 29 markers located over the entire region, we had an average spacing of markers of less than 1 Mb. Thus, we were not able to detect smaller hemizygous or homozygous deletions.

The numbers of PNETs investigated in our study (n = 24) were comparable to those of Thomas and Raffel (1991; n = 23) but lower than those of McDonald et al. (1994; n = 32), Biegele et al. (1995; n = 34), and Batra et al. (1995; n = 28). The frequency of PNETs with monoallelic deletions on chromosome arm 17p in these studies was determined by RFLP analysis and/or FISH and was 26, 44, 50, and 29%, respectively, which is comparable with our results. However, due to our experimental approach, we were not able to reveal hemizygous deletions of chromosome arm 17p in PNETs with hyperdiploid karyotypes, as it has been described...
by cytogenetic or FISH analysis (Biegel et al., 1995). One would expect minor intensity bands after PCR-based microsatellite analysis in these tumors compared with control tissue. Because PCR is not a quantitative assay, we reasoned that this approach would generate ambiguous results and is not suitable for mapping the exact extension of chromosomal deletions. Therefore, the relative number of tumors with hemizygous deletions may be lower in series using LOH techniques compared with studies using FISH (Biegel et al., 1995).

In contrast to reports published by other investigators, our high-resolution deletion mapping approach could not detect several independently deleted regions on chromosome arm 17p or any tumors showing loss of chromosomal material from 17p13.3 only (Cogan et al., 1992; McDonald et al., 1994; Batra et al., 1995). However, our results are in accordance with the data of Biegel et al. (1995), who compared conventional karyotyping with FISH studies. In all but one tumor, one complete 17p was deleted, as revealed by LOH and FISH analysis. In this analysis, the loss of 17p was assessed by hybridization with three probes (D17S34, TP53, and D17S122) to interphase nuclei. Because D17S122 (the most centromeric probe on chromosome arm 17p used in this study) is located telomeric to YAC838E4 (see Fig. 3A), these data are in accordance with our results. Our data suggest that a possible medulloblastoma candidate gene may be located anywhere telomeric of D17S953 and is not restricted to the telomeric subband 17p13.3, as suggested before (Biegel et al., 1992; Cogen et al., 1992). Molecular analysis of exons 5–8 of the TP53 gene in 10 of the 24 tumors (including tumors MA, AM, MW, and TP with LOH on 17p) using single-strand conformation polymorphism (SSCP) and direct sequencing techniques (data not shown) did not show any evidence for point mutations within this tumor suppressor gene, supporting previous findings that the TP53 gene is not a likely candidate gene (Ohgaki et al., 1991; Sayers et al., 1991; Batra et al., 1995).

Another explanation for different breakpoints on chromosome arm 17p in our series compared with previous reports (Biegel et al., 1992; Cogen et al., 1992) may be that all tumors investigated displayed an i(17q), especially because karyotype analysis demonstrated isolated LOH of chromosome arm 17p without formation of i(17q) only in a minority of PNETs (Bigner et al., 1988; Griffin et al., 1988; Arnoldus et al., 1992; Biegel et al., 1992, 1995; Jennings et al., 1992; Karnes et al., 1992; Vagner-Capodano et al., 1992; Neumann et al., 1993; Stuart et al., 1993; Fujii et al., 1994). Our deletion mapping experiments demonstrated that most of the observed chromosomal disruptions are located in chromosome band 17p11.2 within a region that has been described previously as the critical region for Smith-Magenis syndrome (Greenberg et al., 1991). Smith-Magenis syndrome encompasses a complex phenotype that includes developmental and growth delay, facial anomalies, and unusual behaviors. The complex phenotypic abnormalities have been proposed to be due to haploinsufficiency of several contiguous genes; however, so far, no increased risk of developing any malignancies has been reported in Smith-Magenis syndrome patients. The molecular defect underlying the Smith-Magenis syndrome is either an interstitial deletion or a duplication in chromosome band 17p11.2 (Brown et al., 1996). Even though there is no increased incidence of PNET in Smith-Magenis patients, the underlying mechanism leading to chromosomal disruption and recombination within this region might have the same molecular basis.

Considering the high frequency of i(17q) in childhood PNETs and the fact that all observed monoallelic deletions encompass nearly the complete short arm of chromosome 17, it is highly suggestive that the LOH observed in our patient samples is due to the formation of dicentric isochromosomes for 17q. The classical theory explains the generation of isochromosomes with a misdivision of the centromere during cell division (de la Chapelle, 1982). However, it has been shown that, in man, the majority of breakpoints resulting in isochromosome formation are not in the centromere, thus leading to dicentric isochromosomes (Testa and Cohen, 1986; Wolff et al., 1996). Even though it has been speculated that certain pericentromeric sequences that are prone to chromosomal breakage are responsible for the generation of isochromosomes, no such sequences have been described as yet.

The data presented here show evidence for the clustering of chromosomal deletion breakpoints of PNETs in the juxtacentromeric region of the short arm of chromosome 17 in a region that has been described previously as the Smith-Magenis region. Further studies will determine whether the observed phenomenon is due to the formation of dicentric isochromosomes. High-resolution analysis of this region is underway to characterize the molecular structure of this putative, highly unstable region on human chromosome 17 and a possible pathomechanism for isochromosome formation.
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