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Involvement of Multiple Chromosome 17p Loci in Medulloblastoma Tumorigenesis

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Summary

Loss of heterozygosity for sequences located on chromosome 17p in several tumor types is often associated with mutations in the tumor suppressor gene *p53*. We previously showed consistent deletion of chromosome 17p12-13.1 in medulloblastoma, a common childhood brain tumor. Using denaturing gradient gel electrophoresis and direct sequencing, we have detected *p53* mutations in only two of 20 medulloblastoma specimens. Moreover, additional RFLP studies of these 20 specimens showed loss of heterozygosity at a more distal and distinct site, 17p13.3. Deletion of 17p almost invariably signified a negative prognosis. Our results suggest that *p53* mutations may contribute to the pathogenesis of medulloblastoma in relatively few cases. The consistent deletion of other discrete loci on 17p suggests that additional or alternative tumor suppressor genes may contribute to the tumor's phenotype.

Introduction

Initial evidence for an active tumor suppressor gene on chromosome 17p was the allelic deletion of DNA sequences including the *p53* locus in neoplasms of the colon (Baker et al. 1989), breast (Coles et al. 1990), lung (Yokota et al. 1987), and brain (James et al. 1988). Detection of somatic *p53* mutations in those neoplasms strongly supported *p53* as the principal operant tumor suppressor (Nigro et al. 1989). Recently, investigators have documented germ-line *p53* mutations, notably in families with a history of Li-Fraumeni syndrome (Malkin et al. 1990; Srivastava et al. 1990). Deletion and mutation of *p53* appear to be common events in the pathogenesis of human cancer (Levine et al. 1991). We report a series of 20 medulloblastomas in which deletion of multiple chromosome 17p loci other than *p53* fosters tumorigenesis.

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Material and Methods

RFLP analysis of medulloblastoma and peripheral blood (control) specimens was performed using Southern blot hybridization techniques that have been described elsewhere (Feder et al. 1985; Cogen et al. 1990). Blood and tumor specimens from 20 patients were studied, including 12 patients whose results were reported elsewhere (Cogen et al. 1990). The same specimens were analyzed for *p53* mutations using GC-clamped denaturing gradient gel electrophoresis (DGGE) followed by direct sequencing (Meyers et al. 1987; Sheffield et al. 1989). The highly conserved region of *p53* that contains all mutations reported previously (Lamb and Crawford 1986) was divided into four fragments, each defined by a set of PCR primers (fig. 1). The sequences for each primer set were, for exon 4, (AOG 9) 5′-ATC CTC ACC CAT CTA CAG TCC CCC TT-3′ (sense), and (AOG 10) 5′-GCC GGA ATT CCT CAG GGC AAC TGA CCG TGCA-3′ (antisense); for exon 5, (AOG 1) 5′-ATC CTT CCT CTT CCT GCA GTA CTC-3′ (sense), and (AOS 1) 5′-GCC CCA GCT GCT CAC CAT-3′ (antisense); for exon 6, (AOS 2) 5′-ACC ATG AGC GCT GCT CAG ATA GCG ATG GTG-3′ (sense), and (AOG 2)

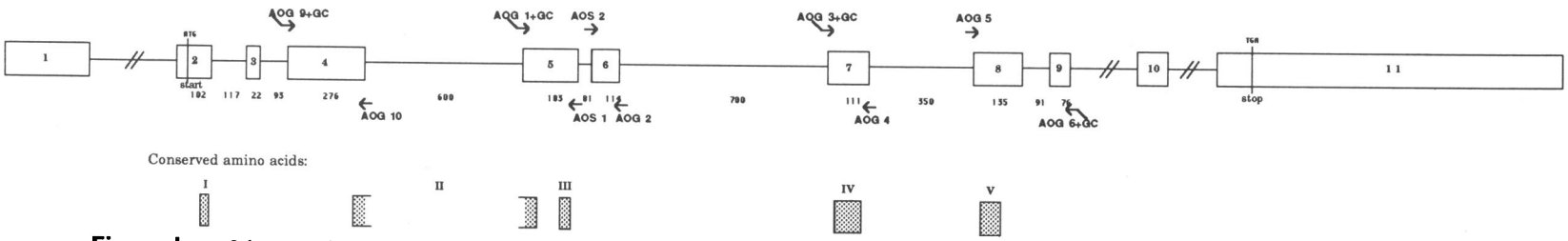

Figure 1 Schematic diagram of *p53* gene showing location of primer sets used for PCR-DGGE analysis. Primer start sites are indicated by the arrows.

5′-GCC GGA ATT CAG TTG CAA ACC AGA CCT CAG-3′ (antisense); for exon 7, (AOG 3) 5′-ATC CGT GTT GTC TCC TAG GTT GGCT-3′ (sense), and (AOG 4) 5′-GCC GGA ATT CCA AGT GGC TCC TGA CCT GGA-3′ (antisense); for exon 8, (AOG 5) 5′-GCC GGG ATC CCC TAT CCT GAG TAG TGG TAA TC-3′ (sense), and (AOG 6) 5′-GCC GGA ATT CGT CCT GCT TGC TTA CCT CGC T-3′ (antisense). The G-C clamp sequence used to modify the sense primers for DGGE was 5′-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC GGG-3′. PCR products were processed, run on a DGGE apparatus, and scored for base-pair mutations by the presence of novel bands (Meyers et al 1987; Sheffield et al. 1989). Fifteen tumor specimens with known *p53* mutations were first run in blinded fashion on the DGGE apparatus to verify the sensitivity of the technique for screening samples. Fourteen of 15 *p53* mutations were identified; the undetected mutation deleted a PCR primer site. Genomic DNA from the medulloblastoma and blood specimens was extracted and used for *p53* amplification (Feder et al. 1985). All of the *p53* gene screened by DGGE was then sequenced to confirm the validity of the methodology while identifying and confirming mutations (Haltiner et al. 1985). The reaction products were sequenced using a modified T7 polymerase kit (Sequenase, U.S. Biologicals, Cleveland) (Haltiner et al. 1985).

The patients were classified as good risks or poor risks according to conventional clinical and radiological criteria (Kopelson et al. 1983).

Results

RFLP analysis showed deletion of 17p DNA in the tumor specimens from 10 (50%) of 20 patients studied (fig. 2). Six of these 10 patients (Med-17 to Med-26) have not been reported previously. In seven of the 10, a previously reported loss of heterozygosity for sequences located on chromosome 17p12-13.1 in medulloblastoma (Cogen et al. 1990) was confirmed. Two of these 10 patients showed deletion of the *p53* gene.

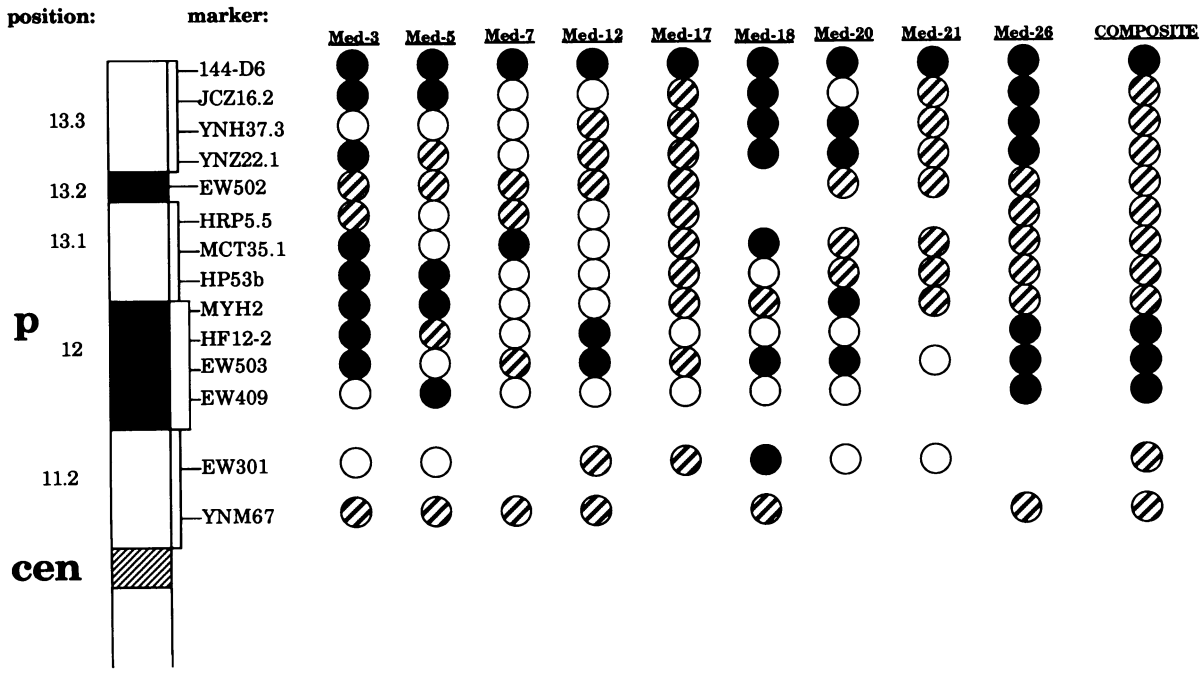

Figure 2 Current deletion map of chromosome 17 for medulloblastoma. The positions of the markers on the map are the consensus locations established at the most recent meeting of the Chromosome 17 Human Gene Mapping Committee (Wright et al. 1990; Fain et al. 1991). RFLP procedures have been reported (Feder et al. 1985; Cogen et al. 1990). Med-5, Med-7, Med-20, and Med-26 were obtained from patients clinically designated as poor risks at the time of their presentation. The remaining specimens were from patients judged clinically to be good-risk patients. (Adapted from Baker et al. [1989] with permission.) ● = Lost; ○ = noninformative; and ⊘ = preserved.

In only two of the 20 patients' samples were *p53* mutations identified, both of them medulloblastomas recurring after radiation therapy (fig. 3). The mutations were found in exon 7 at codons 242 (Cys→Tyr) (Med 15) and 248 (Arg→Gln) (Med 17), respectively, codons located in a region of *p53* where clustering of somatic and germ-line mutations is reported for other tumor types (Nigro et al. 1989; Malkin et al. 1990; Metzger et al. 1991). In both of these tumors, RFLP analysis showed that the *p53* locus was preserved. All mutations were detected by GC-clamped DGGE, validating this technique as a screening procedure. Confirmation sequencing of the PCR fragments from tumor DNA detected only the *p53* mutations detected by DGGE. No germ-line *p53* mutations were found in any control specimen.

In the 10 patients showing deletion of 17p DNA, RFLP studies showed consistent deletion of markers within 17p13.3 (fig. 2). In two (Med-17, Med-21), loss of DNA was confined to probes that mapped to this location. In the other eight, loss of heterozygosity was shown both at proximal and distal 17p sites separated by two or more preserved markers, a pattern also observed in breast cancer (Coles et al. 1990). The specificity of these 17p deletions was tested by hybridization of these same blots to probes mapped to all of the other chromosomes except 21 and the sex chromosomes. Loss of heterozygosity was found for chromosome 6 in one tumor, for chromosome 10 in three, and for chromosome 13 in three others. These results support the specificity of chromosome 17 deletion in medulloblastoma.

For the eight patients clinically judged to be poor risks, the 17p deletion data had no bearing on clinical outcome. Among the 12 good-risk patients, however, five of six showing loss of heterozygosity for 17p DNA had rapid regrowth of their original tumor, a result significantly different from the conventional prognosis (Kopelson et al. 1983; Packer and Finlay 1989). Four of these five tumors showed both proximal and distal losses of chromosome 17p DNA; in the fifth (Med-17), the distal loss of chromosome 17p DNA was associated with mutation of the more proximally located *p53* gene. The one good-risk patient in whom a loss of chromosome 17p DNA was associated with a good outcome had a deletion of the distal marker 144D-6 only, with preservation of all of the informative probes more proximally located. The six good-risk patients with no 17p DNA deletions are alive, with no evidence of disease.

Discussion

Loss of heterozygosity for markers at 17p12-13.1 in other tumors is frequently associated with mutations in *p53*. In the two medulloblastomas showing loss of heterozygosity for *p53* in our study, no mutations were found in the remaining allele. Our specimens showed a consistent loss of markers located at 17p12-13.1 and/or 17p13.3; but, as only two of the

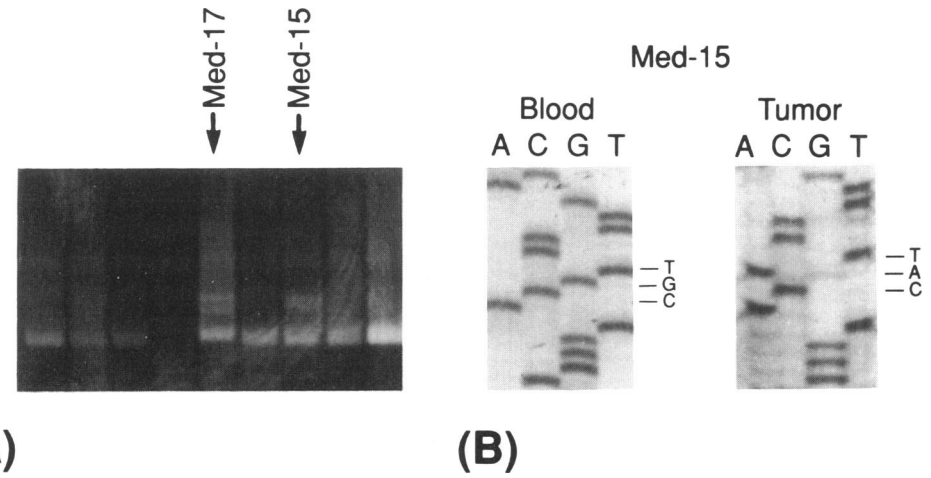

Figure 3 Results of PCR-DGGE study of *p53* gene mutations in medulloblastoma. *A*, Denaturing gradient gel showing new bands observed in the medulloblastoma specimens from two patients (Med-15, Med-17) in whose tumors these mutations were detected. *B*, Sequence data for one of two *p53* mutations found in medulloblastoma tumor specimens (Med-15). The wild-type sequence is shown for comparison. A = adenine; C = cytosine; G = guanine; T = thymine.

20 medulloblastomas showed *p53* mutations, such mutations may contribute to the pathogenesis of medulloblastoma in only a small proportion of cases. That both of the *p53* mutations were detected following radiation therapy of the tumors suggests that the mutations may have been acquired, leading to a more aggressive population of cells and so resulting in recurrence. Although we did not scrutinize the entire gene, we systematically examined the entire conserved region in which all of the numerous *p53* mutations were located in other tumors (Nigro et al. 1989; Malkin et al. 1990; Metzger et al. 1991). Similar results have been reported from another *p53* mutational analysis of medulloblastoma (Saylors et al. 1991). Moreover, several medulloblastomas showed concurrent loss of markers at both 17p12-13.1 and 17p13.3 with preservation of intervening markers, suggesting that additional tumor suppressor genes or other loci on chromosome 17p may be involved in the tumor's etiology.

A direct correlation has been shown between allelic deletions of chromosome 1p36 and amplification of the *N-myc* oncogene with a poor outcome in neuroblastoma (Seeger 1985; Fong et al. 1989), a childhood tumor bearing strong histologic similarity to medulloblastoma (Rorke 1983). By analogy, our findings in medulloblastoma suggest that further molecular genetic investigations may permit a distinction between tumors that otherwise appear identical.

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