Role for the Wilms tumor gene in genital development?

(kidney/gonad/cancer/pulsed-field gel electrophoresis/deletion on human chromosome 11)

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ABSTRACT Detailed molecular definition of the WAGR region at chromosome 11p13 has been achieved by chromosome breakpoint analysis and long-range restriction mapping. Here we describe the molecular detection of a cytogenetically invisible 1-megabase deletion in an individual with aniridia, cryptorchidism, and hypospadias but no Wilms tumor (WT). The region of overlap between deletion and one associated with WT and similar genital anomalies but no aniridia covers a region of 350–400 kilobases, which is coincident with the extent of homozygous deletion detected in tumor tissue from a sporadic WT. A candidate WT gene located within this region has recently been isolated, suggesting nonpenetration for tumor expression in the first individual. The inclusion within the overlap region of a gene for WT predisposition and a gene for the best-documented WT-associated genitourinary malformations leads us to suggest that both of these anomalies result from a loss-of-function mutation at the same locus. This in turn implies that the WT gene exerts pleiotropic effect on both kidney and genitourinary development, a possibility supported by the observed expression pattern of the WT candidate gene in developing kidney and gonads.

Wilms tumor (WT) is a kidney malignancy—the most frequently seen solid tumor of childhood. Most cases are sporadic and unilateral, with no associated anomalies. Bilateral and familial tumors, comprising approximately 7% and 1% of the incidence, respectively (1), have an earlier age of onset than unilateral cases. On the basis of such statistics, the two-hit hypothesis formulated originally for retinoblastoma was extended to WT (2). This model suggests that two mutational steps are required for the development of these childhood tumors. In the hereditary (bilateral and familial) cases, the first predisposing mutation is postulated to be present already in the germ-line and therefore present constitutively in all cells of the target tissue—hence, the earlier age of onset. In sporadic cases, two mutations have to accumulate postzygotically within the same target cell. Ninety-five percent of all WT cases have been postulated by the age of 10 years (1), suggesting that the target-cell population disappears as development to mature form is completed.

Apart from rare familial incidence, the most clear-cut evidence for the presence of a predisposing DNA-level change has come from the unexpectedly frequent association (1 in 100) of WT with aniridia (absence of iris) (3). After this observation, the diagnosis of sporadic aniridia in newborn infants, especially when accompanied by other anomalies, became a prognostic indicator for the possible development of WT. In many such cases, additional genitourinary abnor-

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development, at least in males. This idea is supported by the elevated frequency of genitourinary anomalies accompanying WT and by the common embryological origins of the genital system and the metanephric kidney. The argument in favor of this dual role for the WT gene in the development of both the kidney and genital system is further strengthened by the pattern of expression of the recently isolated candidate gene for the WT locus.

MATERIALS AND METHODS

Clinical Summary and Cytogenetic Analysis. The patient, PAZO, presented initially with sporadic aniridia. Subsequently cryptorchidism (undescended testes) and hypospadias (misplaced penile external urinary orifice) were noted. Mild mental retardation and developmental delay were observed. No WT has been found in this boy, now age 12. Karyotype analysis of chromosome preparations from peripheral blood and later from the Epstein-Barr virus-transformed lymphoblastoid cell line (12) revealed no abnormalities. In particular no deletion was detected on the chromosome 11 short arm.

The second patient, DAR, has been described in detail (13). He presented with WT at age 21 months. There was no sign of aniridia or cataract. He required surgery to correct abnormal external genitalia: chordee (abnormally curved penis) and bilateral cryptorchidism. Karyotype analysis of chromosomes from peripheral blood cultures revealed deletion of most of band 11p13. Catalase activity was reduced, suggesting deletion of the locus CALC encoding this enzyme.

Somatic Cell Hybrids. Fusions were carried out between Epstein-Barr virus-transformed lymphoblastoid cells from the patients and mouse myeloma cell lines as described (6, 12). Stable cell lines were selected by fluorescence-activated cell sorting and subcloning of live cells with high-level expression of appropriate human chromosome 11-encoded cell surface markers (6, 14). The newly produced hybrids from the PAZO cell line were designated PAX and were analyzed for heterozygous marker alleles to ensure the presence of each chromosome 11 homolog in independent hybrids. DAR 15.4 has been described (6).

DNA Markers. The derivation and characterization of the DNA markers used in these analyses have been described in detail as follows: probes specific for follicle-stimulating hormone β gene (FSHβ), for the calcitonin gene (CALC), for CAT, and for monoclonal antibody markers for MIC11 and MICC4 (14); D11S16 (15); L65-6/74 (D11S12), C65-6/6 (D11S102), L65-6/22B (D11S107), and P9RH (16); 582 (D11S317), 495 (D11S310), M20 (D11S377) (7); p5 (D11S323) (17); S1 (D11S87) (9); NBC12 (D11S294) (8); LP2G4 (D11S48), LP4F11 (D11S382), LP11F9 (D11S49) (18); and D11S39 (19).

Repeat-free probes used in this study have been described as follows: probes specific for piscine-stimulating hormone gene (FSHβ), for the calcitonin gene (CALC), for CAT, and for monoclonal antibody markers for MIC11 and MICC4 (14); D11S16 (15); L65-6/74 (D11S12), C65-6/6 (D11S102), L65-6/22B (D11S107), and P9RH (16); 582 (D11S317), 495 (D11S310), M20 (D11S377) (7); p5 (D11S323) (17); S1 (D11S87) (9); NBC12 (D11S294) (8); LP2G4 (D11S48), LP4F11 (D11S382), LP11F9 (D11S49) (18); and D11S39 (19).

Repeat-free probes used in this study have been described (8). Additionally, JM44 is a 4.4-kilobase (kb) BamHI fragment from L65-6/74 DNA marker subcloned into pBlueBscript.

PFGE. High molecular weight DNA was prepared, and PFGE was performed as described (8). Size markers were oligomers of phage λ DNA and chromosomess of Saccharomyces cerevisiae, strain YPH 148.

RESULTS

A series of PAX hybrids were produced from the PAZO lymphoblastoid cell line known to be heterozygous for the polymorphic marker AN2. Analysis of these hybrids revealed that one allele was present in PAX 1 and the alternate allele in PAX 17, thus demonstrating the separation of the two chromosome 11 homologs (Fig. 1a).

FIG. 1. Deletion mapping by DNA blot analysis in somatic cell hybrids. Digested DNA was loaded for hybrids (10 μg) and species controls (5 μg). "11-only" denotes a control somatic cell hybrid with chromosome 11 as its sole human component. Thermus aquaticus Tag 1 digests were probed for CALC polymorphism (a), and BamHI digests were probed for DNA markers NBC12 (b), L65-6/74 (c), and S1 (d).

Initial hybridization with available markers for the 11p13 region showed no evidence of deletion, even with DNA markers such as NBC12 and L65-6/74, which were located close to the WT and AN2 loci (8) (Fig. 1 b and c). As further markers became available the hybrids were examined for their presence. In PAX 1, but not PAX 17, the markers S1 (Fig. 1d) and 495 were deleted as well as the intervening marker p5 (data not shown). Results are summarized in Fig. 2, which illustrates the extent of deletions in the two patients described here.

The physical map of the WAGR region, summarized in Fig. 3, suggested that the DNA markers NBC12 and L65-6/74 should closely flank the deletion in PAZO DNA (8). Characterization of PAZO DNA by PFGE demonstrated that the proximal deletion breakpoint disrupts the 900-kbNorI fragment hybridizing to NBC12 (Fig. 4B), although the proximal 90-kb region of this fragment was unaltered (data not shown). In a similar manner, the distal deletion breakpoint interrupts the 1.4-megabase (Mb) BszHII fragment [these CpG island sites are coincident with NorI sites (8, 20)] hybridizing to L65-6/74 (Fig. 4A). The presence of an unaltered Sac II fragment restricts the position of this breakpoint to the centromeric 400-kb of the BszHII (NorI) fragment (20). This fragment directly abuts the AN2 translocation, which has been studied in detail (21).

The distal deletion breakpoint in the DAR parent lymphoblastoid line cannot be localized precisely. No altered NorI fragment could be detected with probe p5, which therefore places the deletion breakpoint proximal to the 325-kb fragment on which p5 resides (17).

DISCUSSION

Molecular analysis of 11p13 has allowed several groups to delineate a consensus map of the DNA in the WAGR region (7, 8, 17, 20). In this study we have demonstrated the presence of a 1-Mb deletion in a patient with aniridia, hypospadias, cryptorchidism, and mental retardation but no WT. It is worth noting that a karyotypically undetectable deletion can involve loss of such a large amount of DNA.

The smallest region that must contain the WT gene in the development of the patients and mouse myeloma cell lines (6, 12) has been studied in detail (21). In a similar manner, the distal deletion breakpoint interrupts the 1.4-megabase (Mb) BszHII fragment [these CpG island sites are coincident with NorI sites (8, 20)] hybridizing to L65-6/74 (Fig. 4A). The presence of an unaltered Sac II fragment restricts the position of this breakpoint to the centromeric 400-kb of the BszHII (NorI) fragment (20). This fragment directly abuts the AN2 translocation, which has been studied in detail (21).

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since the latter is considered to be a fully penetrant dominant mutation (7, 8, 22). The position of the AN2 gene has also been defined at the molecular level in aniridia-associated translocations (21, 22). 

FIG. 2. Map defining the smallest region of overlap between the DNA deletions in PAZO and DAR cells and the extent of the DNA deletion in WT13 tumor cells. The markers are described in the text. Horizontal divisions delineate the separate compartments, which have been defined by independent breakpoints. T-ALL, T-cell acute lymphocytic leukemia.

FIG. 3. The consensus long-range map of the chromosome 11p13 region. The size of the normal Not I fragments in kb is shown, with the positions of well-characterized translocation breakpoints marked by arrows. Asterisks denote the site of CpG islands associated with the ends of genes. The regions that must contain genes responsible for the observed phenotypes in the two deletions are shown as boxes. The coincidence between the homozygous WT13 tumor DNA deletion and the region of deletion overlap between PAZO and DAR DNAs is marked. The expected sizes of the altered PFGE fragments in PAZO DNA can be deduced from the position of the restriction sites shown. T-ALL, T-cell acute lymphocytic leukemia; GUD, genitourinary dysplasia.

FIG. 4. PFGE analysis of the PAZO DNA breakpoints. Position of the yeast size markers is indicated. (A) BssHII digest was probed with the single-copy subclone of L65-6/74: homozygosity for the normal 1.4-Mb fragment is seen in control DNA, while PAZO DNA is heterozygous for the normal fragment on one homolog and a new smaller fragment on the deletion chromosome. (B) Not I digest was probed with the single-copy subclone of NBC12: homozygosity for the normal 500-kb fragment is seen in control DNA, while PAZO DNA is heterozygous for the normal fragment and an ~1.5-kb altered fragment.

sporadic WT in a patient with no constitutional chromosome 11 deletion. However, the tumor itself was shown to be homozygously deleted for the 11p13 marker S1 (9). Subsequent analysis of the two chromosome 11 homologs revealed independent overlapping deletions (10). The region of homozygous deletion is presumed to include the WT locus, and its existence permitted the isolation of a strong candidate for the WT gene (11). Recently, homozygous deletions within the same small region were described in two further sporadic tumors and a second cloning of the same candidate WT gene was reported (23).

It is only in the light of the location of these homozygous tumor-associated deletions that we could conclude that the
patient PAZO, although nonessential for tumor expression, must lack the WT loci. Therefore, the smallest region of overlap between the proximal breakpoint in PAZO DNA and the distal one in DAR DNA includes the WT gene and covers almost exactly the same 350-kb region as the homozygous deletion in WT13 DNA (10). It is interesting to note that patients PAZO and DAR also share the hypospadias—cryptorchidism phenotype. This implies that a gene involved in aspects of male genital development also lies within the region of deletion overlap. Could it be that the gene involved in predisposition to WT also plays a role in genital development?

The candidate WT gene of Call et al. (11) was generously made available to us. We have been studying the expression pattern of this gene in normal development of early embryos. At the level of RNA blot analysis, expression is clearly seen in fetal testis and ovary in addition to developing kidney (11, 29). mRNA in situ hybridization in early embryos confirms the tissue distribution, showing strong expression in both meta­
nephric and mesonephric kidney and in the early gonad (29). These results are consistent with the hypothesis that the WT gene may have pleiotropic effects on both kidney and genital development. Since correct early gonadal function influences subsequent genital development, mutation in this gene could account for the high frequency of cryptorchidism and hypospadias in male WT patients with or without aniridia (1, 5).

In the case of aniridia-associated genitourinary abnormalities (four of five males among our patients with 11p13 deletion-associated aniridia, with or without WT, have crypt­
orchidism and/or hypospadias), the presence of a deletion (and therefore by definition a null allele) is implicated. However, the validity of the mouse model for human disease cannot be taken as proof. It appears that the mouse model is not as simple as a two-gene system, as suggested by C. A. Cavenee, W. K. (1988) Cancer Res. 48, 1653-1657.

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