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Transcriptional map of 170-kb region at chromosome 11p15.5: Identification and mutational analysis of the *BWR1A* gene reveals the presence of mutations in tumor samples

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ABSTRACT **Chromosome region 11p15.5 harbors unidentified genes involved in neoplasms and in the genetic disease Beckwith–Wiedemann syndrome. The genetic analysis of a 170-kb region at 11p15.5 between loci *D11S601* and *D11S679* resulted in the identification of six transcriptional units. Three genes, *hNAP2*, *CDKN1C*, and *KVLQT1*, are well characterized, whereas three genes are novel. The three additional genes were designated *BWR1A*, *BWR1B*, and *BWR1C*. Full-length cDNAs for these three genes were cloned and nucleotide sequences were determined. While our work was in progress, *BWR1C* cDNA was described as *IPL* [Qian, N., Franck, D., O'Keefe, D., Dao, D., Zhao, L., Yuan, L., Wang, Q., Keating, M., Walsh, C. & Tycko, B. (1997) *Hum. Mol. Genet.* 6, 2021–2029]. The cloning and mapping of these genes together with the fine mapping of the three known genes indicates that the transcriptional map of this region is likely to be complete. Because this region frequently is altered in neoplasms and in the genetic disease Beckwith–Wiedemann syndrome, we carried out a mutational analysis in tumor cell lines and Beckwith–Wiedemann syndrome samples that resulted in the identification of genetic alterations in the *BWR1A* gene: an insertion that introduced a stop codon in the breast cancer cell line BT549 and a point mutation in the rhabdomyosarcoma cell line TE125-T. These results indicate that *BWR1A* may play a role in tumorigenesis.**

Chromosome region 11p15.5 has been actively studied since its recognition as a region of loss of heterozygosity in Wilms' tumor distinct from 11p13 (1, 2), where the WT-1 tumor suppressor gene was identified. Subsequent studies have associated this region with a variety of neoplasms, including other childhood malignancies (3, 4) as well as common neoplasms of adults, such as breast (5–7), lung (8, 9), ovary (10, 11), testes (12, 13), hepatocellular (14) and gastric carcinoma (15), astrocytoma (16), and medulloblastoma (17). The region also is associated with the Beckwith–Wiedemann syndrome (BWS) (1, 18). This overgrowth genetic disease is characterized by increased predisposition to neoplasms, suggesting that the BWS gene also could be a cancer-associated gene. In addition to molecular genetics studies, functional studies have indicated that this region contains tumor and growth suppressive functions (19–21). However, in spite of the intense efforts toward the identification of the critical tumor suppressor gene of the region, no clear picture has yet been delineated.

A number of genes at 11p15.5 have been associated with tumorigenesis or BWS. *H19* and *GOK* have been associated with the tumorigenic process by functional studies. Transfection of *H19* into G401 cells resulted in the suppression of the tumorigenic phenotype (22), whereas transfection of *GOK* into RD and G401 cells resulted in cell growth arrest (23). These results recall similar data obtained by transfer of subchromosomal fragments of region 11p15 that resulted in either tumor suppression (19, 20) or *in vitro* growth arrest (21). The region of overlap between these two subchromosomal regions includes the BWS cluster region 1 (BWSCR1) (24), suggesting that the region could harbor a gene involved in both the observed phenotypes. It should be noted, however, that *H19* maps within the region that induces tumor suppression, whereas *GOK* maps within the region that induces growth arrest, but none of these genes is included in the common region of overlap. Abnormal low expression characterizes both genes in tumor cells (23, 25), but no somatic mutation has yet been reported in either *H19* or *GOK* genes. Therefore, it seems that extinction or down-modulation of gene expression is the mechanism that associates these two genes with growth control and tumorigenesis. Two additional genes at 11p15.5, *CDKN1C* and *KVLQT1*, are associated with BWS (26, 27). Mutations in two of nine Japanese cases were found in the *CDKN1C* gene, and chromosomal aberrations occurring at 11p15.5 were found to disrupt the *KVLQT1* gene in five BWS cases and one rhabdoid tumor. These results indicate that alterations in these two genes can explain a small fraction, less than 20%, of BWS cases, and neither of these two genes has yet been clearly associated with the tumorigenic process. In summary, these data indicate that, although some genes at 11p15.5 already have been associated with the tumorigenic process or BWS, the presence of additional genes involved in tumorigenesis and BWS seems to be possible. With this hypothesis, we have constructed a complete DNA contig just centromeric to the BWSCR1 region and identified three additional genes. The analysis of one of them revealed the presence of somatic mutations in human tumor cell lines. Our results confirm the existence of additional genes at 11p15.5 involved in tumorigenesis and possibly in BWS.

MATERIALS AND METHODS

Cell Lines, DNA, and RNA Samples. The following cell lines were used in the course of the study: 11 breast carcinomas

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Abbreviations: BAC, bacterial artificial chromosome; BWS, Beckwith–Wiedemann syndrome; BWSCR1, BWS cluster region 1.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AF030302 (*BWR1A*); AF035407 (*BWR1B*), and AF035444 (*BWR1C*)].

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(BT20, BT549, HBL100, MCF7, MDA-MB-175, MDA-MB-231, MDA-MB-415, MDA-MB-436, MDA-MB-453, SK-BR-3, and T-47D), three lung carcinomas (Ca-Lu-6, SK-LU-1, and A549), one lung mesothelioma (SK-Mes-1), seven rhabdomyosarcomas (TE617, A204, Hs729, A673, RD, SJCRH3, and TE125-T), one Wilms' tumor (SK-NEP-1), and one rhabdoid tumor (G401). Twelve additional samples were from lymphoblastoid cell lines of BWS samples (LL495, GM00539, GM13415, GM13416, GM13418, GM13419, BWS1485, BWS643, BWS594, BWS1634, BWS590, and BWS467). All tumor cell lines were obtained from the American Type Culture Collection. BWS lymphoblastoid cell lines were obtained from the Coriell Mutant Cell Repository (Camden, NJ; GM series) and from one of us (BWS series) (26). LL495 was obtained from C. Junien (Institut National de la Santé et de la Recherche Médicale, Paris) and was previously described (28). Genomic DNAs were purified from cell pellets using standard Hirt's/proteinase K lysis followed by organic extractions and ethanol/NaCl precipitation (29). RNA was purified from cell pellets using the RNA-Stat30 reagent (Tel-Test, Friendswood, TX).

Construction of DNA Contigs. A human bacterial artificial chromosome (BAC) library (30) release III was obtained from Research Genetics (Huntsville, AL) and was PCR-screened as suggested by manufacturer. The following sets of primers were used: 395T3-F/R (CATGGATGTGGGTTGGATG/AGAGATGTGCTCCGAACGG) and 469T3-F/R (CTTGGGACTGAGAATGGCTT/AGTGAGCCAGAATGTTCCAG).

Gene Identification. cDNA selection was carried out essentially as described (31, 32). cDNA specific probes were identified as described in the *Results* section.

cDNA Cloning and Sequencing. cDNA libraries from human fetal liver and human fetal kidney were obtained from CLONTECH. The library was screened with cDNA probes identified by cDNA selection using standard conditions (29). Phagemid auto-excision from the lambda vectors was performed as suggested by the manufacturer. Sequence analysis was carried out in an automated sequencing station Applied Biosystem model 377, using the dye terminator chemistry. Both strands were sequenced by using cDNA specific oligonucleotide primers. Nucleotide sequences were analyzed by using the Wisconsin (GCG) program package version Unix-8.1. Other programs for protein structure prediction were from the Institut Suisse de Recherches Expérimentales sur le Cancer (ISREC) (http://ulrec3.unil.ch/software/).

PCR–Single-Strand Conformation Polymorphisms (SSCP). The *BWR1A* gene was analyzed by reverse transcription–PCR–SSCP. PCR reactions were carried out with 1.0 μM of each primer, 100 μM of each dNTP, 1.5 mM MgCl2, 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 12.5% glycerol, and 0.5 unit of *Taq* polymerase (Perkin–Elmer Cetus) in a volume of 10 μl. The initial denaturation step was for 4 min at 94°C. PCR amplification was carried out for 30 cycles with a denaturing step at 94°C for 15 sec, annealing for 30 sec at temperature indicated in Table 2, and extension at 72°C for 30 sec. The SSCP analysis was performed essentially as described (33). PCR products were separated on 0.5× nondenaturing mutation detection enhancement gels (FMC) and visualized by silver staining. cDNA segments exhibiting band shifts were reamplified by using the same conditions. The PCR products were separated in a 2% agarose gels in 0.5× Tris/borate/EDTA buffer, gel purified, and sequenced.

RESULTS

BAC and Cosmid Contig. End sequences of cosmids cCI11-395 (*D11S648*) and cCI11-469 (*D11S679*) were determined. DNA probes and oligonucleotide primers were developed and used to isolate cosmids and BAC clones. Additional cosmids, cKIP-5D1 and c469-5A3, were identified by colony hybridization of a human cosmid library prepared from normal lymphocyte DNA, and the Research Genetics BAC library release III was screened by PCR using the primers described in *Materials and Methods*. The BACs positive for markers of the region and spanning the entire region are the following: 1-L-10, 178-J-24, 298-B-20, 20-G-16, 282-G-12, and 351-H-14. Fig. 1 summarizes the contig assembly.

Genes Identification and Cloning. cDNA probes were isolated by using a "cDNA selection" approach. Based on the homology to the BAC genomic segments, a pool of cDNAs were selected from a mixture of cDNAs synthesized from several human tissues, including brain, skeletal muscle, ovary, testis, and colon. The pool of selected cDNAs was used to identify nonrepetitive genomic fragments (data not shown) that were subcloned in pBluescript II. The subcloned genomic fragments then were used to isolate, from the initial pool, cDNA subclones specific for this region. The cDNA fragments identified were sequenced. Three known genes were recognized: *KVLQT1*, *hNAP2*, and *CDKN1C*. Additional cDNA segments, which were then identified to belong to three additional genes that we named *BWR1A*, *BWR1B*, and *BWR1C*, also were recognized. Full-length cDNAs of these three genes were cloned and sequenced.

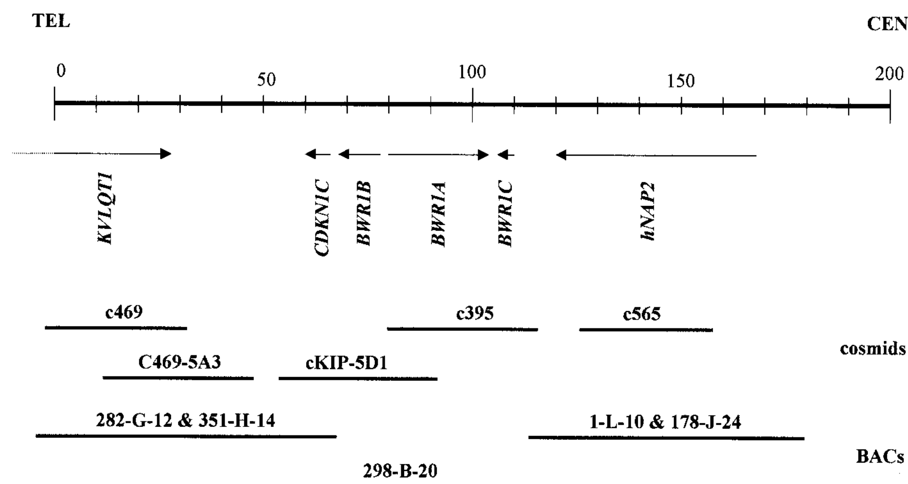

FIG. 1. Scheme of the transcriptional map of the region between loci *D11S601* and *D11S679*. Location and length of the six genes *hNAP2*, *KVLQT1*, *CDKN1C*, *BWR1A*, *BWR1B*, and *BWR1C/IPL* is shown. The map scale is based on the HTG phase III nucleotide sequence hsac001228. The contig of cosmid and BAC DNA clones is shown below the transcriptional map.

Table 1. Gene exons in 170 kb of the genomic sequence hsac001228

hsac001228 coordinates	Gene exons and cDNA coordinates
27291..28516	*KVLQT1*, 1409-2634; last exon
	gap 34,657 bp
63173..63453	*CDKN1C*, 1497..1217; exon 3
63537..63672	*CDKN1C*, 1216..1081; exon 2
64239..65300	*CDKN1C*, 1080..19; exon 1
	gap 2,050 bp
67350..68205	*BWR1B*, 1231..376; exon 2
78965..79340	*BWR1B*, 375..1; exon 1
	gap 2,652 bp
81992..82292	*BWR1A*, 1..300; exon 1
82788..83063	*BWR1A*, 301.575; exon 2
87812..87908	*BWR1A*, 576..673; exon 3
88775..88935	*BWR1A*, 674..834; exon 4
89156..89288	*BWR1A*, 835..967; exon 5
96200..96318	*BWR1A*, 968..1086; exon 6
97565..97672	*BWR1A*, 1087..1194; exon 7
98883..98983	*BWR1A*, 1195..1295; exon 8
101637..101738	*BWR1A*, 1296..1397; exon 9
101971..102091	*BWR1A*, 1398..1518; exon 10
104485..104720	*BWR1A*, 1519..1754; exon 11
	gap 3,187 bp
107907..108141	*IPL*, 760..526; exon 2
108370..108894	*IPL*, 525..1; exon 1
	gap 13,950 bp
122844-124053	*hNAP2*, 2518-1310; exon 16
127635-127672	*hNAP2*, 1309-1272; exon 15
129667-129723	*hNAP2*, 1271-1215; exon 14
130188-130217	*hNAP2*, 1214-1185; exon 13
132936-133055	*hNAP2*, 1184-1065; exon 12
134153-134175	*hNAP2*, 1064-1042; exon 11
136808-136954	*hNAP2*, 1041-896; exon 10
138181-138318	*hNAP2*, 895-756; exon 9
143091-143162	*hNAP2*, 755-684; exon 8
148227-148359	*hNAP2*, 683-552; exon 7
149875-149962	*hNAP2*, 551-463; exon 6
150530-150669	*hNAP2*, 462-323; exon 5
154452-154551	*hNAP2*, 322-223; exon 4
156713-156771	*hNAP2*, 222-164; exon 3
157636-157666	*hNAP2*, 163-133; exon 2
170683-170806	*hNAP2*, 132-9; exon 1

The nucleotide sequence of one of these, *BWR1C*, has been reported as *IPL* (34) while our work was in progress. Fig. 1 and Table 1 summarizes the transcriptional map and exons location of the different genes found in this region of 170 kb. A great help in the precise mapping of the exons of the three genes was the appearance in GenBank of the phase 3 HTG nucleotide sequence hsac001228 that spans the region we studied. With the help of this long genomic sequence, we also could determine, by computer search, that no expressed sequence tags exist in the intergenic regions between the six identified genes, suggesting that they represent all of the genes in the region. If additional genes exist, they must be expressed in a quite specific manner, for example in a narrow spatio-temporal expression window.

Characterization of the Three Additional Genes. *BWR1A* cDNA probes recognize a 1.7-kb mRNA transcript, which shows the highest level of expression in liver, heart, and kidney (Fig. 2*A*). The full-length cDNA was cloned from a human fetal liver cDNA library. The 1.7-kb nucleotide sequence reveals an ORF of 424 amino acids (GenBank accession no. AF030302). BLAST homology analysis determined that *BWR1A* gene product has a strong homology with tetracycline resistance efflux proteins, suggesting that this gene product may function as membrane pump-channel. Prediction of transmembrane helices with the TMpred program at the Institut Suisse de Recherches Expérimentales sur le Cancer (ISREC) (http://ulrec3.unil.ch/software/) indicated two possible models differing in the number of TM-helices, 9 or 10, with little difference in terms of stability, which might represent two different functional states of the protein buried within the cytoplasmic membrane.

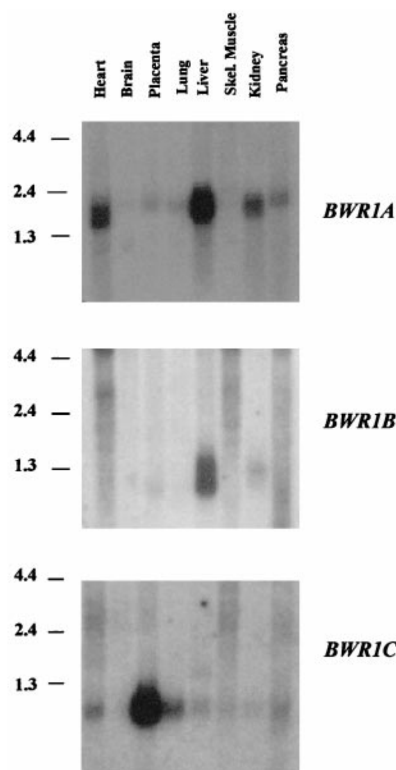

FIG. 2. Northern blot analysis of mRNA expression and cDNA nucleotide sequences of *BWR1A*, *BWR1B*, and *BWR1C*. Poly(A)RNA from multiple human adult tissues (CLONTECH) were analyzed with cDNA probes isolated by cDNA selection. Hybridization with *BWR1A* (*A*), *BWR1B* (*B*), and *BWR1C* (*C*) probes. Nucleotide sequences of *BWR1A*, *BWR1B*, and *BWR1C* cDNAs are deposited in GenBank with the accession nos. AF030302, AF035407, and AF035444, respectively.

BWR1B cDNA probes recognize a 1.2- to 1.3-kb mRNA transcript that is most abundant in the gastro-intestinal tract tissues, including small intestine, colon (data not shown), and liver, but its expression is detectable also in kidney and placenta (Fig. 2*B*). The full-length cDNA was cloned from a human fetal liver cDNA library. The 1.2-kb nucleotide sequence reveals an ORF of 253 amino acids (GenBank accession nos. AF035407). BLAST homology search with the SwissProt database revealed only a borderline homology (probability 4.7%) with the Sahara scorpion venom kaliotoxin-2 precursor, whose significance, if any, remains obscure, and does not provide any clue about the physiological function of the BWR1B protein product.

BWR1C cDNA probes recognize a 0.7–0.9 kb mRNA transcript that is highly expressed in placenta and very poorly in all adult tissues (Fig. 2*C*). The full-length *BWR1C* cDNA was isolated from a human fetal kidney cDNA library. This is in line with the evidence that, in fetal tissues, *BWR1C* is highly expressed in kidney (34). Nucleotide sequence of *BWR1C* cDNA (GenBank accession no. AF035444) revealed that it corresponds to *IPL* cDNA, the recently described gene imprinted in liver and placenta with high homology to TDAG51, whose protein is associated with the Fas apoptotic pathway (35). The full-length cDNA sequence is a little longer than *IPL*

Table 2. Oligonucleotide primers used to amplify *BWR1A* gene segments analyzed by PCR

Oligo name	Nucleotide sequence	Amplified segment	Product size	Annealing temperature, °C
592Tot F	CCTGCTTGGATCTCTCCTGG	388–587	200	62
592-1R	CCGAGACAGGTATGGCACGA			
592-2F	GCTGGCCGCCACAGAACTTA	524–748	225	62
592-2R	AGGAGCAGGTAGAGCGCCAA			
592-3F	TCACGCTCTCCTTCCTGGCT	706–866	161	62
592-3R	TGCCGACAGGTCCGTGATGA			
592-4F	TCTACCTGCTCTTCGCCTCG	781–1037	257	60
592-4R	GATGCAGGTGAAGCTGAGGA			
592-5F	ATTCAGTGCCCGGCCATCCT	969–1162	194	62
592-5R	AAGATCCTCGGGACGTCTGG			
592-6F	GGCCAGTGTGTTCGACCTGA	1097–1348	252	62
592-6R	ACCTCCTCCGAGAAGTGGCT			
592-7F	CCAAGCTGGCTACCTCATGT	1253–1433	181	60
592-7R	CAGGAGGCAGAAGTGGAAGA			
592-8F	GCTGGTCTTCATCGTGGTGG	1367–1569	203	62
592-8R	CCAGAGTTCGGAGCAGTGGT			
592-9F	TCAACGTGGTCACCGACAGC	1465–1726	261	61
592-Tot R	AGTCTGTGTCTGGGCAGCG			

(34) because of the use of a different polyadenylation site located farther 3′, but the coding region is identical.

The *BWR1A* gene consists of 11 exons spanning a genomic area of 23 kb, *BWR1B* consists of two exons spanning 12 kb, and *BWR1C* consists of two exons spanning 1.0 kb (Table 1).

Mutational Analysis of *BWR1A* in Tumor Cell Lines and BWS Samples. Using nine sets of primers (Table 2), mutational analysis of *BWR1A* coding region was carried out on cDNAs from cell lines derived from 11 breast carcinomas (BT20, BT549, HBL100, MCF7, MDA-MB-175, MDA-MB-231, MDA-MB-415, MDA-MB-436, MDA-MB-453, SK-BR-3, and T-47D), three lung carcinomas (Ca-Lu-6, SK-LU-1, and A549), one lung mesothelioma (SK-Mes-1), seven rhabdomyosarcomas (TE617, A204, Hs729, A673, RD, SJCRH3, and TE125-T), one Wilms' tumor (SK-NEP-1), and one rhabdoid tumor (G401). Twelve additional samples were from lymphoblastoid cell lines of BWS samples (LL495, GM00539, GM13415, GM13416, GM13418, GM13419, BWS1485, BWS643, BWS594, BWS1634, BWS590, and BWS467). Somatic changes were detected in two tumor cell lines.

In the breast cancer cell line BT549, an insertion of 111 nucleotides between nucleotides 1295–1296 of the cDNA sequence was found (Fig. 3). These nucleotides represent the junction between exon 8 (nucleotides 98883–98983 of sequence hsac001228) and exon 9 (nucleotides 101637–101738 of sequence hsac001228) of the *BWR1A* gene. Therefore, this inserted segment behaves like a new exon. Indeed, the newly added 111 nucleotides segment is found within intron 8, at 331 nucleotides from the 3′ end of exon 8 between nucleotides 99314 and 99424 of the genomic sequence hsac001228. The presence of the canonical splicing sites at the boundaries of the genomic sequence confirms that the segment has been inserted in the normal *BWR1A* transcript by a mRNA splicing event. However, the newly inserted segment is uniquely found in the breast cancer cell line BT549 and, although it does not introduce a frame shift, it does introduce an in-frame stop codon after 18 nucleotides, removing the last 136 amino acids of the normal polypeptide sequence. These results indicate that this finding represents an aberrant mRNA and not an alternatively splicing event. The presence of the insertion was detected with two different sets of oligonucleotides primers, 592–6F/6R and 592–7F/7R (Table 2), and the absence of the normal mRNA is obvious from agarose gel ethidium bromide stain (Fig. 3).

A second alteration, a point mutation changing the encoded amino acid was detected in the rhabdomyosarcoma cell line TE125.T (Fig. 4). The nucleotide substitution, a G to A at nucleotide 688, introduces an arginine in place of a cysteine.

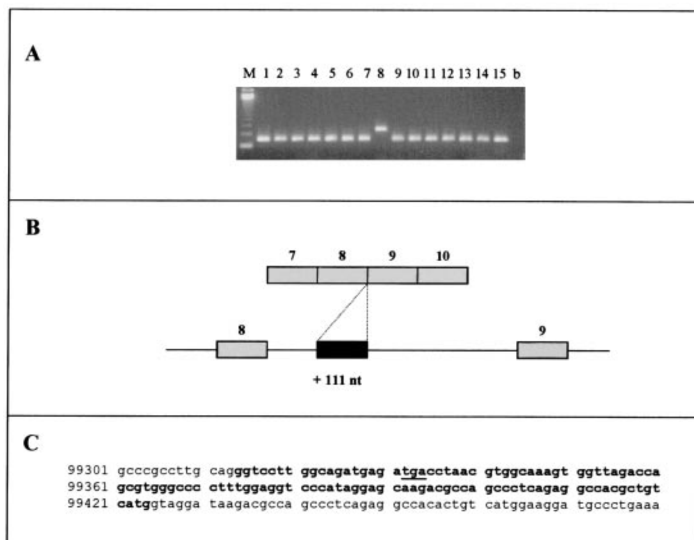

FIG. 3. Insertion of 111 nucleotides between exon 8 and 9 of the *BWR1A* mRNA in the breast cancer cell line BT549. (*A*) RNA PCR products obtained by amplification with oligonucleotides 592–7F/7R from various tumor cell lines: sample 11 showing a larger fragment is from the breast cancer cell line BT549. The same result was obtained with oligonucleotides 592–6F/6R. (*B* and *C*) Determination of the nucleotide sequences of the abnormal PCR products indicates that the inserted segment derives from nucleotides 98984–99314 of the genomic sequence hsac001228 (shown in bold) is found in intron 8 of the gene and that an in-frame stop codon is present after 18 nucleotides of the newly inserted sequence, which determines the replacement of the last 136 amino acids with six different amino acids. This abnormal *BWR1A* transcript was observed only in the BT549 cells.

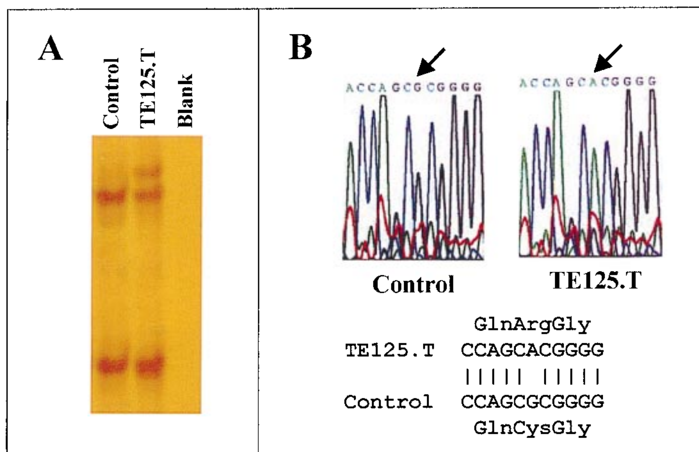

FIG. 4. *BWR1A* nucleotide and amino acid change in the rhabdomyosarcoma cell line TE125.T. (*A*) Band shift detected by PCR–single-strand conformation polymorphisms analysis detected with primers 592–2F/592–2R in a 0.5× mutation detection enhancement gel. (*B*) Nucleotide, 688-G to A, and amino acid, Cys to Arg, changes.

The change was found only in this sample and is present in homozygosity, suggesting that loss of the normal allele occurred during the tumorigenic conversion. Loss of a normal allele can be deduced by the fact that this amino acid change was not detected in any other of the 38 samples, indicating that the normal allele must have been deleted during the tumorigenic process. The substitution of a cysteine appears to be an important amino acid change that could potentially affect protein structure and function.

DISCUSSION

The identification and precise localization of six genes in a 170-kb region just centromeric to the BWSCR1 area at chromosome 11p15.5 defines the transcriptional structure of this important region. The cloning of the *BWR1A*, *BWR1B*, and *BWR1C* genes together with the fine mapping of the exons belonging to the other three known genes, *KVLQT1*, *CDKN1C*, and *hNAP2*, leaves very little space for the presence of other genes in the region. If present, they must be located within regions 28500–63170 or 108900–122800 of the genomic sequence hsac001228. However, computer search indicated that no known expressed sequence tag is present in these intergenic regions, strongly suggesting that the transcriptional map of this 170-kb region is likely to be complete. If other genes exist, they are to be expressed in a quite specific and narrow spatiotemporal window. Functional study of this important chromosomal region now can be approached with a clear picture of its genetic structure.

Three genes, *hNAP2*, *CDKN1C*, and *KVLQT1* were previously characterized. In two of them, *CDKN1C* and *KVLQT1*, point mutations or gross chromosomal aberrations were detected in BWS samples. Of the three additional genes, *BWR1A*, *BWR1B*, and *BWR1C*, identified and molecularly characterized in this work, at least one, *BWR1A*, is mutated in tumor cell lines. This evidence indicates that the BWSCR1 region contains a cluster of genes involved in cell growth control and tumorigenesis. Although not yet directly implicated, other genes in the region also might be involved in tumorigenesis. For example, BWR1C protein product, recently reported as IPL, is homologous to TDAG51 protein (35), which couples TCR signaling to Fas expression in activation-induced cell death. The *BWR1C/IPL* gene is preferentially expressed in placenta and fetal kidney and, if involved in an apoptotic pathway, it may be suggestive to speculate that lack of its expression may cause kidney cells to become resistant to apoptotic signals during organogenesis. Could this promote tumorigenesis? As *BCL-2* studies indicate, resistance to apoptosis may indeed favor the development of tumor cell clones.

The relevance of this region in tumorigenesis is supported by loss of heterozygosity studies that have defined the region between *D11S988* and *D11S1318*, which contains the BWSCR1 region, as a common area of deletion in a variety of human neoplasms. The presence of recurrent deletions in tumor cells is considered to pinpoint the location of tumor suppressor genes. However, in spite of the multiple evidence implicating this region in tumor suppression and the effort of several laboratories throughout the world, no mutations in genes of this region were described in tumor cells. The mutations in the *BWR1A* cDNA detected in the breast carcinoma cell line BT549 and in the rhabdomyosarcoma cell line TE215.T are mutations detected in tumor cells in a gene at 11p15.5. However, the number of cases in which mutations were detected is low. This raises some questions. Is this because of the unselected nature of the cases analyzed? Are there other genes at 11p15.5 involved in tumorigenesis? Are there mechanisms other than the "two-hits" model that could explain the association of genes of this regions with the tumorigenic process? All of these hypotheses could be true. With the identification of genetic alterations in the *BWR1A* gene, five genes at 11p15.5 are associated with growth control and/or tumorigenesis by loss-of-function genetic alterations. Functions of *H19* and *GOK* appear to be functionally inactivated by down-modulation of RNA expression (23, 25), whereas point mutations and gross chromosomal rearrangements affect the *CDKN1C* and *KVLQT1* genes in the overgrowth genetic disorder BWS (26, 27), but not tumor cells. It seems also conceivable that the different genes of the region may be differentially implicated in the tumorigenesis of various cell types. In this study, we describe two genes, *BWR1A* and *BWR1B* that are expressed at the highest level in liver, and a third, *BWR1C/IPL*, that is expressed at highest level in placenta and fetal kidney. Previously, we have described that *GOK*, also found at 11p15.5, is expressed at high level in skeletal muscle (23). Expression of these genes appear to be, at least in part, tissue specific, and it is conceivable that they might have important functions in the tissues where they are expressed at the highest level. With these considerations in mind, it is intriguing to note that tumors associated with deletions at region 11p15.5, such as Wilms' tumor, hepatoblastoma, and rhabdomyosarcoma derive from the tissues where the mentioned genes are highly expressed. We already have shown that *GOK*, highly expressed in skeletal muscle cells, can inhibit growth of rhabdomyosarcoma cells, but not breast cancer cells, suggesting its involvement in rhabdomyosarcoma tumorigenesis (23). Could this suggest the involvement of *BWR1A* or *BWR1B* in hepatoblastoma and *BWR1C/IPL* in Wilms' tumor and, occasionally, in adult neoplasms? The analysis of these rare pediatric neoplasms for the presence

of mutations in these genes is certainly important. In summary, although the complexity of the picture is obvious, it also is becoming clear that chromosomal region 11p15.5 is not the location of a single pleiotropic tumor suppressor gene, but instead it contains a cluster of genes controlling *in vitro* and *in vivo* cell growth. These genes, when altered, may promote tumorigenesis. The apparently minimal involvement of these genes in tumorigenesis may increase if selected types of tumors are chosen. Furthermore, to understand the role of these genes in tumorigenesis, it also must be considered that most of the genes in the region are imprinted. *BWR1A* (unpublished work) and *BWR1C/IPL* (34) are imprinted in fetal tissues, and it seems conceivable that *BWR1B* also is imprinted. As demonstrated in BWS, where mutations in the single active cyclin-dependent kinase inhibitor *CDKN1C* has been associated with the pathogenesis of the genetic disease (26), it also seems possible that the same mechanism could take place in tumor development. The inactivation by mutation of the single active allele could be sufficient to promote tumorigenesis. However, although this model seems reasonable, it must be viewed with caution, because of the many faces imprinting may assume. Imprinting can be tissue or developmental specific, loss of imprinting may occur (36, 37), and interdependence of genomic imprinting between genes at 11p15.5 exists (38, 39). These factors may complicate the analysis of imprinted genes. Furthermore, although it is conceivable that genomic imprinting could play a role in tumors of embryonal origin, where imprinting is thought to have a role, less clear is its role in adult common neoplams that originate from tissues in which imprinting often cannot be demonstrated. The evidence that imprinting occurs in clustered genes indicates the existence of cis-acting elements in the region and raises the possibility that genetic alterations not directly affecting coding regions of local genes could upset their balanced regulation, altering in the end their growth promoting and/or inhibiting properties. Although it has been partly demonstrated for *H19* and *IGF2* (39), and it has been suggested that abnormalities at the untranslated transcripts of *KVLQT1* might affect the entire chromosomal imprinted domain (27), the extent of the role played by genomic imprinting in tumorigenesis is not yet known. Once the specific role of each individual gene at 11p15.5 in tumorigenesis and BWS is clarified, it will be critical to determine their functional relationship and understand the role of region 11p15.5 as a whole.

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