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Short Communication

cDNA Cloning of the Human *Polybromo-1* Gene on Chromosome 3p21*

IZUMI HORIKAWA† and J. CARL BARRETT

Laboratory of Biosystems and Cancer, Center for Cancer Research, National Cancer Institute, 9000 Rockville Pike, Bldg. 37, Rm. 5046, MSC-4264, Bethesda, MD 20892, USA

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The bromodomain is suggested as a chromatin-targeting domain involved in regulation of gene expression and chromatin structure. We here report the cDNA cloning of the human *polybromo-1* (*hPB1*) gene that encodes a 1634-amino acid polypeptide containing six bromodomains. The hPB1 polypeptide shows 91% identity to the previously identified chicken polybromo-1 with conservation of the bromodomains and other characteristic features. Northern blot analysis detected the ubiquitous expression of hPB1 mRNA in a variety of human tissues. Four alternative splicings were found within the hPB1 coding region: three making in-frame deletions and one resulting in a C-terminal truncation. The *hPB1* gene is located on chromosome 3p21, where the tumor suppressor genes for breast, lung and kidney cancers have been mapped.

Keywords: Bromodomain; Chromosome 3p21; *hPB1* gene; *Polybromo-1* gene

The bromodomain, which presumably mediates protein–protein interaction, is found in a number of proteins involved in regulation of gene transcription and/or chromatin structure (Jeanmougin *et al.*, 1997; Winston and Allis, 1999). The chicken *polybromo-1* (*cPB1*) gene encodes a 1633-amino acid polypeptide containing five bromodomains (Nicolas and Goodwin, 1996), while other known bromodomain-containing proteins have only one or two bromodomains (Jeanmougin *et al.*, 1997). We here report the cDNA cloning of the human homologue of the *cPB1* gene. Sequence similarity search using the Basic Local Alignment Search Tool (BLAST: http://www.ncbi.nlm.nih.gov/BLAST) identified a number of human expressed sequence tags (ESTs) that show high similarity to the *cPB1* gene, and some of the human ESTs appeared to contain a translation initiation codon (UniGene cluster Hs.44143; http://www.ncbi.nlm.nih.gov/UniGene/index.html) or a translation termination codon (UniGene cluster Hs.179680). Based on these EST sequences, we designed the following primers to amplify the entire coding region of the human *polybromo-1* (*hPB1*) cDNA: 5′-TCC ATG GGT TCC AAG AGA AGA-3′ and 5′-ACC CCC AGT AAC TAA AAT GGC T-3′. The reverse transcription (RT)-PCR with these primers and the Advantage cDNA PCR kit (BD Bioscience Clontech, Palo Alto, CA) generated ~5-kb products from human diploid fibroblasts (MRC-5)-derived cDNA and human testis cDNA (Marathon-Ready cDNA from BD Bioscience Clontech). The products were cloned into the plasmid pCR-XL-TOPO by using the TOPO XL PCR Cloning kit (Invitrogen, San Diego, CA), and multiple clones (four each from MRC-5 or testis) were fully sequenced in both strands with M13 reverse primer, T7 primer and 20 internal primers. As shown in Fig. 1, the longest cDNA sequence (clone 6 in Table I; GenBank accession number AF177387; nucleotide numbers hereafter follow this cDNA sequence) has an open reading frame encoding a 1634-amino acid polypeptide

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†Corresponding author. Tel.: +1-301-594-8510. Fax: +1-301-480-2772. E-mail: horikawi@mail.nih.gov

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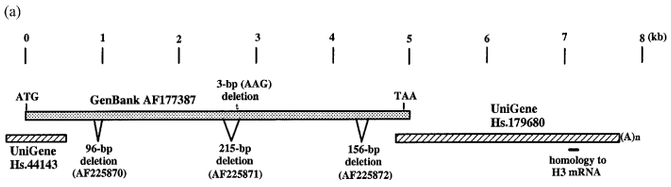

TABLE I Splicing patterns in 8 cDNA clones derived from MRC-5 fibroblasts and testis

cDNA clone	96 bps (903–998)†	215 bps/3 bps* (2571–2785/2783–2785)†	156 bps (4292–4447)†
MRC-5 fibroblasts			
Clone 1	Present	3-bp Deleted	Deleted
Clone 2	Deleted	3-bp Deleted	Deleted
Clone 3	Present	Present	Deleted
Clone 4	Present	Present	Deleted
Testis			
Clone 5	Present	215-bp Deleted	Present
Clone 6	Present	Present	Present
Clone 7	Present	3-bp Deleted	Present
Clone 8	Present	Present	Deleted

*The last three nucleotides of 215-bp deletion correspond to 3-bp deletion. †Nucleotide numbers in GenBank AF177387 are shown in parentheses.

with high similarity to the cPB1 protein (identity 91.2%; similarity 93.9%). The five bromodomains (bromo 1–5 in Fig. 1b) are well conserved in the hPB1 protein. Our ProfileScan (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html) identified the sixth bromodomain (bromo 6), which is truncated and matches less to the consensus pattern than the other five. The human and chicken proteins also share a truncated high-mobility-group (HMG) box, two bromo-associated homology (BAH) domains and a sequence with similarity to the regulatory domain of the DNA cytosine-5-methyltransferase (DMTase).

We found four alternative splicings within the coding region of *hPB1* gene (Fig. 1 and Table I). A 96-bp deletion (deletion of nucleotides 903–998; GenBank AF225870) results in an in-frame deletion of 32 amino acid residues (301–332 in Fig. 1b) with a change of arginine to serine at the residue 300. The corresponding alternative splicing was also observed in the chicken gene (Nicolas and Goodwin, 1996). Another conserved alternative splicing was the 156-bp deletion (deletion of nucleotides 4292–4447; GenBank AF225872) resulting in an in-frame deletion of 52 amino acids (1431–1482 in Fig. 1b). The other two we found in the human gene have not been reported in the chicken gene: a 3-bp deletion (AAG; nucleotides 2783–2785) resulting in the deletion of a glutamic acid at the position 927; and a 215-bp deletion (deletion of nucleotides 2571–2785; GenBank AF225871), 3′ end of which is identical to the 3-bp deletion. The 215-bp deletion changes the reading frame, making a C-terminally truncated form of hPB1 protein (amino acid residues 1–856, followed by a premature stop codon immediately downstream) that lacks the DMTase-related domain, the BAH domains and the HMG-box. Interestingly, the alternative splicing at a different position in the chicken gene also produced a C-terminally truncated protein (amino acids 1–987, followed by 21 unrelated residues; Nicolas and Goodwin, 1996), suggesting a conserved role of the C-terminally truncated polybromo proteins. Xue *et al.* (2000) also isolated a cDNA clone encoding the hPB1 protein from Jurkat T cells. Their cDNA clone had two in-frame deletions, which were different from the deletions identified in this study, resulting in the loss

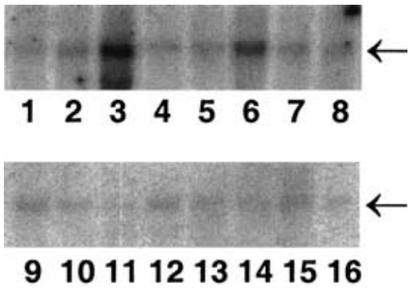

FIGURE 2 Northern blot analysis of hPB1 mRNA. Human MTN Blot and Human MTN Blot II (BD Bioscience Clontech, Palo Alto, CA) were hybridized with the hPB1 cDNA probe. 1, pancreas; 2, kidney; 3, skeletal muscle; 4, liver; 5, lung; 6, placenta; 7, brain; 8, heart; 9, peripheral blood leukocytes; 10, colon; 11, small intestine; 12, ovary; 13, testis; 14, prostate; 15, thymus; 16, spleen. Arrows indicate ~8-kb hPB1 mRNA.

FIGURE 1 (a) Schematic representation of the hPB1 cDNA. The entire coding region obtained by RT-PCR in this study (GenBank AF177387) and the two overlapping UniGene clusters (Hs.44143 and Hs.179680) are shown. ATG, translation initiation codon; TAA, translation termination codon; (A)n, poly(A) tail. The positions of four alternative splicings are indicated. GenBank accession numbers of the splicing variants are shown in parentheses. The bar shows a 3′UTR sequence with high similarity to the H3 mRNA (Rastinejad and Blau, 1993). (b) The predicted amino acid sequences of human (GenBank AF177387) and chicken (GenBank X90849) *polybromo-1*. Vertical lines and dots show identical and similar amino acids, respectively. Bromodomains (bromo 1 to 6), BAH domains (BAH 1 and 2), a sequence with similarity to the regulatory domain of the DNA cytosine-5-methyltransferase (DMTase), and a truncated HMG box are indicated by rectangles. Identification of these domains was based on the ProfileScan (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html). Underlined amino acid sequences are lost in-frame in accordance with the alternative splicing (96-bp and 156-bp deletions in human; GenBank AF225870 and AF225872, respectively). A glutamic acid (E) marked by triangle is removed from the human protein by the 3-bp (AAG) deletion. A closed arrow indicates the position where the human protein prematurely terminates because of 215 bp of out-of-frame deletion (GenBank AF225871). An open arrow indicates the position of the C-terminal truncation in the chicken protein.

of amino acids 989–1013 and 1337–1363. Of great interest will be whether and how each of our and their splicing variants differ in function.

An approximately 8-kb hPB1 mRNA was detected by Northern blot analysis in all the human tissues examined (Fig. 2). A high expression was observed in skeletal muscle (lane 3) and placenta (lane 6). The ubiquitous expression of hPB1 suggests that it may play a fundamental role in maintaining cell integrity, for example, by regulating gene transcription and/or chromatin structure as suggested for other bromodomain-containing proteins (Jeanmougin *et al.*, 1997; Winston and Allis, 1999).

As shown in Fig. 1a, one of the interesting features of the *hPB1* gene is a sequence within its 3′ untranslated region (3′UTR) with high similarity (115 out of 122 bps) to the H3 mRNA, which was reported as a 3′UTR sequence that augmented the differentiation of muscle cells and suppressed the proliferation of fibroblasts (Rastinejad and Blau, 1993). The *hPB1* gene may play a role in cell growth and differentiation through the activity of its 3′UTR sequence, in addition to the function of the hPB1 protein it encodes.

Based on radiation hybrid mapping data available at the GeneMap'98 (http://www.ncbi.nlm.nih.gov/genemap98/), the UniGene cluster Hs.179680, which represents the 3′UTR of *hPB1* gene, was mapped to an interval between D3S3582 (65.1 cM) and D3S1588 (67.7 cM) at or near the chromosome band 3p21.1. Our PCR mapping using two independent primer pairs (one designed within the coding region and another designed within the 3′UTR) and a panel of mouse × human monochromosomal hybrids (Koi *et al.*, 1989) confirmed the localization of *hPB1* gene on chromosome 3p (data not shown). This chromosomal region has been suggested by loss of heterozygosity (LOH) studies to contain tumor suppressor genes for human cancers of the breast, lung and kidney (Mitsudomi *et al.*, 1996; Matsumoto *et al.*, 1997; Orikasa *et al.*, 1998). By means of microcell-mediated chromosome transfer, we also revealed that the 3p14.2-p21.1 region carries a gene that functions as a telomerase repressor through transcriptional repression of the telomerase catalytic subunit gene in a subset of renal cell carcinoma cells (e.g. RCC23 cell line; Horikawa *et al.*, 1998; 1999; Tanaka *et al.*, 1998). To test whether the *hPB1* gene is a telomerase repressor gene on 3p14.2-p21.1, we examined the expression level, nucleotide sequences and splicing variants of this gene in telomerase-positive RCC23 cells. The RCC23 cells expressed the wild-type hPB1 mRNA at a comparable level to the telomerase-negative counterparts (i.e. normal kidney tissues and RCC23 with a transferred normal chromosome 3), without any RCC23-specific abnormality of splicing, making it unlikely that the *hPB1* gene is primarily responsible for telomerase repression.

Dhalluin *et al.* (1999) revealed that bromodomain interacts specifically with acetylated lysine, a major modification of histones, providing direct evidence for its role in chromatin remodeling (Winston and Allis, 1999). The two human bromodomain-containing proteins, BRG-1 and hbrm, both of which are human homologs of a yeast SWI2/SNF2 subunit of the SWI/SNF chromatin remodeling complex, can associate and cooperate with a tumor suppressor protein RB to repress the *S*-phase-inducing transcription factor E2F1 and induce cell cycle arrest (Dunaief *et al.*, 1994; Trouche *et al.*, 1997). Another human subunit of the SWI/SNF complex, hSNF5/INI1, was shown to be inactivated by biallelic mutation in aggressive pediatric cancers (Versteege *et al.*, 1998). These findings suggest that some protein complexes containing a bromodomain protein(s) are involved in control of cell growth and carcinogenesis, and that, taken together with the chromosomal location described above, the *hPB1* gene may be an attractive candidate for the tumor suppressor on chromosome 3p. It will be of interest to examine whether inactivating mutation or loss of expression of the *hPB1* gene contributes to the pathogenesis of renal cell carcinomas other than RCC23 cells, as well as other types of human cancers showing LOH at the loci on chromosome 3p.

The function of polybromo-1 protein is still unknown, although one can imagine that multiple bromodomains may be responsible for the association with multiple proteins, at least some of which are acetylated at lysine residues (e.g. histones), to form a large protein complex involved in regulation of chromatin structure and gene expression. Indeed, the cPB1 protein was found to be part of an approximately 2-million dalton complex in the cell nucleus (Nicolas and Goodwin, 1996). The cDNA cloning of the entire coding region of *hPB1* gene in this report enables us to investigate the effect of enforced expression of the hPB1 protein and to identify the binding partners of the protein, facilitating the understanding of its functions in human cells. These analyses of the hPB1 protein, which unusually contains many bromodomains, may also provide clues to a generalized and specific roles of each bromodomain found in a variety of proteins.

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