A Homozygous Deletion within the Carbonic Anhydrase-like Domain of the Ptprg Gene in Murine L-Cells


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Abstract

Protein tyrosine phosphatases, on purely theoretical grounds, were suggested as possible tumor suppressor genes, and receptor protein tyrosine phosphatase γ (PTPRG) has been proposed, on the basis of its location at human chromosome region 3p14.2, specifically as a tumor suppressor gene for renal cell carcinoma.

Thus, mouse L-cells have lost one Ptprg allele and sustained an intragenic deletion in the other; such allele loss and mutation frequently occur at tumor suppressor gene loci.

Introduction

Mainly because of their potential to oppose or reverse effects of tyrosine kinases (for reviews see Refs. 1 to 3), protein tyrosine phosphatases were proposed as possible suppressor genes. In determining the chromosomal location of several human receptor protein tyrosine phosphatase (PTPR) genes (4, 5), we noted that the PTPRG4 gene mapped to the 3p14–21 chromosome region and might be a suppressor gene involved in kidney carcinomas (5).

Recently, full length cDNA clones for at least one isoform of human and murine Ptprg have been sequenced and analyzed (6). The human and murine predicted amino acid sequences for this isoform are greater than 90% identical; the Ptprg gene contains a single transmembrane domain and two tandem tyrosine phosphatase domains. The extracellular region contains one fibronectin type repeat and, like the PTPRG gene (7), an NH2-terminal region of 266 amino acids with approximately 30% sequence similarity to members of the carbonic anhydrase enzyme family (8).

During an investigation of the genomic organization of human and mouse Ptprg loci, we noted extensive polymorphism of the murine Ptprg locus and a homoygous deletion of a portion of the CA-like domain in murine L-cell lines, which we undertook to describe in detail, since homoygous deletion of a gene is one of the hallmarks of suppressor genes in tumors and tumor cell lines (for review, see Ref. 9); at the same time we determined the chromosomal location of the murine Ptprg locus.

Materials and Methods

Cultured Cells. The TK− cl-1D cell line is a clonal derivative of the parental L-strain (10), as are A9, LMTK−, and L929 cell lines. The parent L-cells were established in culture from normal s.c. areolar and adipose tissue of a 100-day-old male C3H mouse after treatment with methylcholanthrene (10). The L929 and A9 cell lines were purchased from the American Type Culture Collection, while cl-1D (originally from Saul Kit) and LMTK− cell lines were available in this laboratory, where they are routinely used in isolation of somatic cell hybrids. IT22 cells are a TK− derivative of a Swiss 3T3 fibroblast cell line. Mel cells were originally derived from the spleen of a virus-induced leukemic DBA/2J mouse (11).

Ptprg cDNA and Genomic Clones. Approximately 3 x 108 plaques of a B6/CBA mouse lung cDNA library (Stratagene) were screened by colony hybridization using the 5′-most 740 nucleotides of the human PTPRG cDNA as probe. cDNA clones obtained were subcloned into a pBluescript vector (SK+) vector. A mouse genomic library (B6/CBA strain, ~2 x 106 plaques in a Fix II vector, Stratagene) was screened with the same human probe; two Ptprg genomic clones, carrying a portion of the Ptprg CA domain 2nd exon and adjacent intron, were mapped. A 1.5-kilobase unique fragment, pKwml.5B, was subcloned into a pGEM vector (Promega) and used in murine backcross analysis.

DNA Sequence Analysis. Mouse Ptprg cDNA clones were sequenced using T-3 and T-7 primers and some synthetic oligonucleotides. Sequencing of the double-stranded plasmids was performed with the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.); reaction products were electrophoresed and recorded on the 373 DNA Sequencer (Applied Biosystems, Inc.). Sequence analyses were accomplished with GCG software.

PCR Probe Generation. The oligonucleotides for generating Ptprg probes were designed, based on the mouse Ptprg sequence (see Fig. 1), using the computer program Oligo 4.0 (National Biosciences). For Southern blots, probes A, B, C, D, and G (see Fig. 2A) were produced by PCR amplification using primers A-1 plus A-2, B-1 plus B-2, C-1 plus C-2, D-1 plus D-2, and G-1 plus G-2, respectively. Primers R-1 and R-2 were synthesized for RT-PCR; probe R, generated using primers R-3 and R-4, was used for detection of

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4 The abbreviations used are: PTPRG, receptor protein tyrosine phosphatase γ; cDNA, complementary DNA; PCR, polymerase chain reaction; RT, reverse transcription; CA, carbonic anhydrase; Mel, murine erythroleukemia; TK−, thymidine kinase deficient.

5 Unpublished data.
Fig. 1. Partial sequence of the Ptprg CA domain aligned with mouse carbonic anhydrase II sequence. The nucleotide (nt) sequence of the murine Ptprg CA-like domain and some upstream sequence is shown. The deduced amino acid (aa) sequence in standard one-letter code is above the nucleotide sequence. The sequence of mouse carbonic anhydrase II (12) is aligned on the top. The approximate positions of the putative introns in the Ptprg sequence are shown by arrowheads, based on the structure of the CA II gene. The corresponding exon numbers of the mouse CA II gene are also indicated on either side of each arrowhead. The positions of oligonucleotides for generating probes or for reverse transcription are indicated with dashed lines and directional arrows.
Genomic DNA Preparation and Southern Blot Hybridization. Cellular DNAs were isolated and Southern blots were prepared by conventional methods. Probes were labeled by random priming with [32P]dCTP (New England Nuclear) and hybridized to membranes in 0.75 M NaCl-50% formamide at 42°C overnight. Final washes of membranes were in 0.1 x standard saline-citrate and 0.1% sodium dodecyl sulfate at 65°C for 30 min.

**RNA Extraction, Reverse Transcription, and RT-PCR Reaction.** cl-1D mRNA was isolated from IT22 and cl-1D cells after treatment with 4 μM guanidinium isothiocyanate, followed by phenol-chloroform extraction and isopropyl alcohol precipitation. Reverse transcription was performed in 20 μl final volume of 50 mM Tris-HCl (pH 8.3), 60 mM KCl, 1 mM MgCl2, 10 mM dithiothreitol, 1 mM deoxyribonucleotide triphosphates, 0.5 mM oligodeoxythymidylate, murine leukemia virus-RT (200 units; BRL), RNasin (40 units; Promega), and 2 μg RNA at 37°C for 30 min. The RT-PCR reaction was carried out with primers R-1 and R-2 under conditions as described above for PCR probe generation. The products were run in 1.5% agarose gels, blotted to membranes, and hybridized with appropriate probes.

**Murine Backcross Analysis.** The interspecific mouse backcross used for chromosome mapping was described previously (14). The pKWM1.5B probe used to map the Ptprg locus detected a 4.2-kilobase HindIII fragment in AEJ/Gn and a 4.0-kilobase HindIII fragment in Mus spretus DNA. The PCR oligomers and simple sequence-length polymorphism detecting the Plau locus were as described (15). The probes and restriction fragment length polymorphisms detecting the Psp-2 and Tpi-rs9 loci were as described (16, 17).

**Results and Discussion.**

A probe representing the 5'-740 nucleotides of the human PTPRG gene was used to screen for murine cDNA and genomic Ptprg clones. A 1.5-kilobase genomic fragment was used in linkage analysis of interspecific backcross mice to determine the chromosomal location of the murine gene. The results of the interspecific backcross analysis show that the Ptprg locus maps to the proximal portion of mouse chromosome 14 (Fig. 2). The localization of Ptprg to mouse chromosome 14 is consistent with its position as determined by mapping of the recombinant inbred strains (6). Specifically, Ptprg maps just proximal to Plau; Rab was shown to be mapped proximal to Plau as well (18), suggesting close linkage between Ptprg and Rab. The human homologues of Rab and Ptprg reside on human chromosome 3p24 and 3p14.2, respectively. However, several human genes lie between RARB and PTPRG on human chromosome 3p, such as ACY1, MYL3, and GNAI2B; their mouse homologues (Acy-1, Myl3, and Gna12b) are located on mouse chromosome 9 (19). In addition, CCHL1A2 maps to human chromosome 3p14.3 and mouse chromosome 14 (Fig. 2). Although Rab, Ptprg, and Cchla2 map to both chromosome 14 and human chromosome 3, current data indicate that they most likely represent three distinct homology segments, rather than defining a conserved linkage group. Directly determining the genetic distances between Rab, Ptprg, and Chila2 in the mouse would shed light on the evolutionary relationship of these regions on mouse chromosome 14 and human chromosome 3.

**During analysis of the murine Ptprg locus in murine cell lines, we noted possible murine restriction fragment length polymorphisms.** Thus, DNA from various cell lines, derived from different mouse strains, were more systematically investigated with control tissue DNAs from the respective parental mouse strains, using individual regions of the murine Ptprg cDNA. Results indicated that the mouse strains tested could be divided into two groups, based upon insertion/deletion type polymorphic loci occurring in a number of introns of the Ptprg locus; BALB/c, AKR/J, C57BL/6, and Swiss were in one group and DBA/2J, C3H/HeJ, 129/J, and BXH-2 were in the other group (data not shown). When various L-cell derived clones such as A9, cl-1D, LMTK−, and L929 were compared to C3H tissue DNA using human or mouse Ptprg cDNA probes, it was clear that a portion of the gene was entirely missing in all the L-cell lines examined (not shown).

In order to determine the position and extent of the homozygous deletion, we positioned oligonucleotide primers along the length of the deletion type polymorphic loci occurring in a number of introns of the Ptprg locus.
the 5' end of the Ptprg cDNA, as shown in Fig. 1, for preparation of region-specific probes by PCR amplification, using the entire cDNA as template. The probes generated using various primer pairs are sketched in Fig. 3A, which shows the relationship between the probes and exons of the Ptprg CA domain. The respective probes were hybridized to filters carrying restriction enzyme-cleaved DNAs of the cl-1D and Mel cell lines as shown in Fig. 3B. Fig. 3B, Panel A, shows the DNAs, Mel and cl-1D, in alternating lanes cut with BamHI (Lanes 1 and 2), EcoRI (Lanes 3 and 4), and HindIII (Lanes 5 and 6), hybridized to probe A, which covers two exons. None of the three enzymes cut within either of the exons or the intervening intron; therefore only one fragment is detected for each of the enzymes. The Mel and cl-1D patterns are the same, showing that the region detected by probe A is intact in L-cells. In Fig. 3B, Panel B, a similar filter was hybridized to probe B, illustrating that this region of the Ptprg gene is entirely missing in cl-1D DNA (see Fig. 3B, Panel 2, 4, and 6); filters were rehybridized to other murine probes to show equivalent murine DNA in all lanes (not shown). Next, it was observed that probe C covering exons CA5 and CA6 was intact in cl-1D DNA (see Fig. 3B, Panel C, in which cl-1D lanes are identical to Mel lanes). Thus from Fig. 3B, Panels A, B, and C, using probes A, B, and C, we know that exon CA1 and upstream exons are intact in L-cells, exons CA2 and CA3 are at least partially missing (the portion covered by probe B), and exons CA5 and CA6 are intact in L-cells. Similar results were obtained using DNA from A9 and L929 DNAs. To locate the 5' end of the deletion, probe D was hybridized to DNA from the two cell lines (see Fig. 3B, Panel D); probe D overlaps exon CA1 and its upstream exon (like probe A), but sees in addition exon CA2 (see Fig. 3A). Probe D (compare Panel D to Panel A) detects two bands in Mel cells but only 1 in cl-1D, showing that the 5' end of the deletion is within the first CA intron because none of the exons have recognition sites for the enzymes used. To find the 3' end of the deletion, probe

Fig. 3. Analysis of the homozygous deletion within the Ptprg gene in the cl-1D cell line. (A) The putative exons of the CA-like domain of mouse Ptprg (CA 1–CA 6) are shown by boxes, based on the mouse CA II gene structure; thin lines indicate introns but are not to scale. Probes A, B, C, D, G, and R are shown below the exons, indicating the regions of Ptprg sequence covered. Probe A covers exon CA 1 plus some of the upstream exon. Probe B includes a portion of CA2 and CA3. Probe C covers most of CA5 and the full CA6. Probe D overlaps probe A and carries the 5' end of CA2. Probe G covers most of CA4 and a portion of 5' CA5. Probe R covers CA1 through CA6. Probes were generated from cDNA and contain no intron sequence. (B) Hybridizations illustrating the extent of deletion using probes A, B, C, D, and G, respectively, are shown in the five blots. The 5 probes cover different regions of the Ptprg CA-like domain as described in A. Each pair of lanes contained restriction enzyme-digested DNAs from Mel and cl-1D cell lines. Lane 1, BamHI-Mel; Lane 2, BamHI-cl-1D; Lane 3, EcoRI-Mel; Lane 4, EcoRI-cl-1D; Lane 5, HindIII-Mel; Lane 6, HindIII-cl-1D. Probe A detected the same bands with each enzyme in Mel and cl-1D (Panel A). Probe C also detected the same fragments in both cell lines (Panel C). In Panel B, each cl-1D lane shows that probe B, covering exons 2 and 3, is deleted. Probe D, crossing exons 1 and 2, detected the presence of exon CA1 and absence of exon CA2 in cl-1D (Panel D). Probe G, spanning exons CA4 and CA5, detected presence of exon 5 and absence of exon CA4 (Panel G). Position of DNA size markers (kilobase pairs) are indicated on the right of Panel G. bp, base pairs.
G was hybridized as shown in Fig. 3B, Panel G: this panel shows that exon CA4 is missing in L-cells. The 3' end of the deletion is, therefore, in intron 4 of the CA domain, leaving the 5' end and regulatory regions for this gene intact in L-cells.

To determine if the partially deleted gene was expressed at the mRNA level and might, therefore, encode a functionally abnormal protein, RT-PCR was performed using mRNA from L-cells, IT22 cells, and Mel cells. RT-PCR products were size fractionated on agarose gels, blotted, and hybridized to probe R or B (see Fig. 3A and Fig. 4A for position of probes). Results demonstrate (see Fig. 4B) that a novel transcript is present in L-cell mRNA which is 400 bases shorter than the IT22 product (Fig. 4B, Lanes 1 and 2); the Mel product and the product from control cDNA appeared identical to the IT22 product (not shown). The cl-1D RT-PCR product is not detected by probe B (Fig. 4B, Lane 3), which detects exons CA2 and CA3 in the IT22 RT-PCR product. These results confirm that the L-cell Ptpg gene is expressed and could, therefore, code for an aberrant protein product. Since the CA domain of the extracellular portion of this receptor tyrosine phosphatase gene is very likely involved in ligand binding (6) and the L-cell deletion removes amino acids 12–145, including the putative active site of this CA domain (6), the L-cell Ptpg gene product could not transmit signals normally.

It is interesting that the deletion end points are within introns; in fact, the actual DNA break points must be very far away from the exons because we have never seen a DNA rearrangement using cDNA-derived probes and a large battery of standard restriction enzymes; we are currently using infrequently cutting enzymes and pulsed field gel electrophoresis to detect the rearrangement and plan its cloning. Preliminary results indicate that the deletion involves more than 200 kilobase pairs. In nearly 50 years of tissue culture, this deletion has remained the same in all L-cell lines, an unlikely occurrence if the gene did not serve some function. We also speculate that this deletion could be involved in the tumorigenicity of the L-cell clones and could have arisen due to methylcholanthrene treatment during establishment of the original L-cell strain. Interestingly, Pathak et al. (20) have reported that structural abnormalities in murine chromosome 14, leading to deletion near the Plau locus, are associated with high metastatic potential of K-1735 melanoma cells. It will be important to determine if the Ptpg locus could be the target of the deletion in this tumor model.

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References


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