Advances in Brief

Protein-Tyrosine Phosphatase Expression in Pre-B Cell NALM-6

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Abstract

Protein-tyrosine phosphatase (PTP)-related complementary DNAs from NALM-6 (pre-B cell line) were amplified by reverse transcriptase polymerase chain reaction using primers corresponding to the conserved catalytic domains of PTPs. Thirty-three polymerase chain reaction products, identified as PTP related complementary DNAs, were classified to RPTP-α, PTP1B, and 4 novel PTPs, which were designated as BPTP-1–4. Their expressions in NALM-6 and other cell lines were confirmed by Northern blot analysis. BPTP-1 and -2 exhibited extensive homology with the first and the second catalytic domains, respectively, of leukocyte-common antigen related molecule (LAR) and human PTPα. The transcriptional sizes of BPTP-1 and BPTP-2 are the same (7.2 kilobases) as that of LAR. The expression of BPTP-1 was abundant in lymphoid cell lines TALL-1 and NALM-6 but small in colon cell line BM314, which is in sharp contrast to the expression of LAR. These data suggest that the expression levels of BPTP-1 and LAR are altered in a cell-specific manner, probably making them cell type associated PTPs.

Introduction

A large number of PTKs have been identified as certain growth factor receptors and cellular oncogenes, thereby playing crucial roles in cell growth and oncogenesis (1–3). In some malignant cells, PTKs are abnormally activated and the cellular proteins are highly phosphorylated in tyrosine residues. Since the level of protein tyrosine phosphorylation is determined by the balance of PTK and PTP activities, regulation of FTP activity and identification of the associated proteins are becoming extremely important issues. The malfunction of HPTPγ, which maps to a region of human chromosome 3p21 (4), has been suggested to be crucial for the pathogenesis of small cell lung cancer and renal cell carcinoma, since the deletion of this area is tightly associated with these cancers. Recently many PTPs have been isolated from various cells. PTP1B (5), HPTPα, -β, -γ, -δ, -ε, and -ζ were cloned from human placenta cDNA (6). LCA (CD45) is exceptionally expressed in the cell surface of leukocytes and has PTP activity (7). LAR protein is expressed in epithelial cells (8). PTPH1 and MEG-01 have been cloned from the HeLa cell cDNA library (9) and from a human megakaryoblastic cell line (10), respectively. These data suggest that some PTPs are expressed in a tissue specific manner and may introduce tissue specific signal transduction. Thus, it is important to accumulate data detailing which PTPs are mainly expressed in each cell type. In this paper, we identify PTPs expressed in pre-B cell line NALM-6 by PCR amplification using primers corresponding to the nucleotide sequences within the conserved catalytic domain of the PTP family members. Furthermore, we identify four novel PTP cDNA clones, two of which had a high sequence similarity to the first and second catalytic domains of LAR and HPTPα, and two others were completely different from the PTPs thus far reported.

Materials and Methods

PCR Amplification and Sequencing. Oligonucleotide primers were synthesized based on the conserved amino acid sequences KCDQYWP (5'-CGAATTCAAGTGTGACCAGTACTGCCC-3', primer 1) and HCSAAGVG (5'-CCAATCCCCGACTGAGT-3', primer 2) within the catalytic domains of PTPs. Total RNA was prepared from NALM-6 (pre-B cell line) by the guanidinium thiocyanate/CsCl procedure. The RNA was converted to single strand cDNA with antisense primer 2 and avian myeloblastosis virus reverse transcriptase. The template cDNA from NALM-6 was amplified using Taq polymerase in the presence of 50 pmol of primers 1 and 2 on the basis of PCR protocol (11). The PCR cycle, repeated 30 times, consisted of denaturation at 94°C for 1 min and 20 s, annealing at 37°C, and extension at 72°C for 3 min. The PCR products were cloned into the EcoRI and PstI sites of pBlueScript SK (+) plasmid (Stratagene) and sequenced with a Sequenase kit using 7-deaza-2'-deoxy-CTP (USB).

Northern Blot Analysis. Total RNAs were prepared from NALM-6, TALL-1 (T-cell line), BM314 (colon carcinoma cell line), Jurkat (T-cell line), MT-1 (T-cell line), and normal peripheral mononuclear cells by the guanidinium/CsCl procedure. Polyadenylated RNA was prepared from NALM-6 by oligodeoxynucleotide selection using Oligotex (TAKARA), fractionated (5–10 µg/lane) on a formaldehyde containing 1% agarose gel, transferred to nitrocellulose using standard procedures, and probed with the PCR products.

Results

To identify the series of PTPs in pre-B cells, we utilized PCR amplification with primers corresponding to the conserved catalytic domains of PTPs (see "Materials and Methods"). Since we intended to isolate as many PTP-related cDNAs as possible, a mild annealing temperature (37°C) was chosen for the PCR amplification. Thus, almost one-half of the 67 PCR products we cloned possessed no distinct homology with conserved sequences within the catalytic domains of PTP molecules. The remaining 33 clones which encoded consensus amino acid sequences of PTPs are classified as 7 different PTP-related cDNAs (Table 1). B001, B002, and B003 are identical to HPTPα domain I, HPTPα domain II, and PTP1B, respectively, whereas the other 4 clones (BPTP-1–4) are novel. BPTP-1 encodes an amino acid sequence that is extremely similar to the first catalytic domains of LAR and HPTPα (Fig. 1A), while BPTP-2 encodes an amino acid sequence that is almost identical to their second catalytic domains (Fig. 1B). While the similarities of the nucleotide sequence of BPTP-1 with the first catalytic domains of LAR and HPTPα are 76 and 75%, respectively, those of BPTP-2...
Table 1  PTP-related cDNA clones in pre-B cell NALM-6

<table>
<thead>
<tr>
<th>Clones</th>
<th>PTP type</th>
<th>Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B001</td>
<td>HPTPα(α)</td>
<td>1</td>
</tr>
<tr>
<td>B002</td>
<td>HPTPα(β)</td>
<td>2</td>
</tr>
<tr>
<td>B003</td>
<td>PTPβB</td>
<td>5</td>
</tr>
<tr>
<td>B004</td>
<td>Novel (BPTP1)</td>
<td>5</td>
</tr>
<tr>
<td>B005</td>
<td>Novel (BPTP2)</td>
<td>6</td>
</tr>
<tr>
<td>B006</td>
<td>Novel (BPTP3)</td>
<td>6</td>
</tr>
<tr>
<td>B007</td>
<td>Novel (BPTP4)</td>
<td>2</td>
</tr>
</tbody>
</table>

Discussion

Protein tyrosine phosphorylation levels in B-cells are known to be elevated by stimulation of membrane IgM (12) and the interleukin 6 receptor (13). Recently it has been demonstrated that membrane IgM is associated with lyn protein-tyrosine kinase (14) and LCA (CD45) (15). These observations suggest that protein-tyrosine phosphorylation plays a crucial role in biological function, differentiation, and proliferation in B-cells. However, little information about PTPs in B-cells has been presented. Here we show 7 different cDNA clones expressed in the pre-B cell line. Four of the clones, designated as BPTP-1–4, encoded conserved catalytic domain segments of PTP and are not identical to any known PTPs. BPTP-1 showed a relatively high expression level of transcription in T-cell line TALL-1 and in pre-B cell line NALM-6. However, little expression of BPTP-1 was detected in colon cell line BM314 which expresses abundant LAR (Fig. 3A), suggesting that BPTP-1 is preferentially expressed in lymphoid cells. Furthermore, BPTP-1 and -2 have the same transcriptional sizes (7.2 kilobases) and are very homologous to conserved segments within the first and second catalytic domains of LAR or HPTPβ, respectively (Figs. 1 and 2). Taken together, BPTP-1 and BPTP-2 may be the first and the second domains, respectively, of a member of the LAR-HPTPβ-like PTPs. Cloning of the full-length of the BPTP cDNA is under way in our laboratory and will demonstrate whether our idea is correct. In any case, the discovery of a LAR

A LAR cyl CAGCTGCCACAGACGACGTCCTGCGCTGCTGCGCTAGTGCCAGACGATAC
BPTP-1 CAGCTGCCACAGACGACGTCCTGCGCTGCTGCGCTAGTGCCAGACGATAC
HPTPβ cyl CAGCTGCCACAGACGACGTCCTGCGCTGCTGCGCTAGTGCCAGACGATAC

HPTPα cyl CAGGAGGACGACGTCCTGCGCTGCTGCGCTAGTGCCAGACGATAC

BPTP-2 CAGGAGGACGACGTCCTGCGCTGCTGCGCTAGTGCCAGACGATAC

Discussion

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isomorph expands the family of PTPs and suggests the idea that BPTP1, BPTP2, LAR, and HPTPα genes have arisen from duplication of an ancestral gene. BPTP-3 is widely expressed in lymphoid cells MT-1, TALL-1, and NALM-6 and normal peripheral mononuclear cells as well as epithelial cells BM314 and Colo201 (data not shown). Since the transcriptional size is large (~6.4 kilobases), BPTP-3 may be a novel receptor-linked PTP (Fig. 3B). BPTP-4 is expressed in a variety of lymphoid cells, NALM-6, MT-1, Jurkat (Fig. 3C), and the transcriptional size is ~3.8 kilobases.

HPTPα has been shown to be a receptor-linked PTP and is expressed throughout many tissues, most abundantly in the fetal brain and liver (16). PTP1B has been shown to be a cytoplasmic PTP with a function of growth regulation (17) and is expressed especially in the kidney, placenta, spleen, and brain (18). We have demonstrated that these two PTPs were isolated from pre-B cell NALM-6 as major fractions, indicating that the pre-B cells express abundant HPTPα and PTP1B.

Our primers were designed to isolate most PTP cDNAs. The comparison of the nucleotide sequences of the primers with the corresponding conserved sequences of known PTPs shows that only 0–4 nucleotide mismatches are found, except for the far greater mismatched cases of PTP1B, LCA (domains I and II), and HPTPα (domain II) in the case of the sense primer and LCA (domain II), HPTPα (domain II), HPTPγ (domain II), and HPTPα (domain II) in the case of antisense primer. Although our system could not isolate all of the PTP cDNAs expressed in NALM-6, PTP1B clone, one of the most difficult PTP cDNAs to be isolated with the primers we used, was in fact isolated as a major fraction, suggesting that our PCR amplification has the potential to isolate PTPs with 4–5 nucleotide mismatches at the annealing sites. In addition, LAR and HPTPα cDNA clones have been isolated from colon and placenta cDNA in our laboratory using the same approach (data not shown), suggesting that there is little or no expression of these mRNAs in pre-B cell NALM-6. Taken together, our data may reflect the population of PTPs (HPTPα, PTP1B, and BPTP-1–4 as major fractions) expressed in pre-B cell NALM-6. As an interesting hypothesis, our novel BPTPs (BPTP-1 and BPTP-2), which exhibit extensive homology with LAR and HPTPα, might be lymphoid associated PTP(s) in place of LAR which is mainly expressed in epithelial cells (8). It remains to be elucidated why two very homologous PTPs, BPTP-1,2, and LAR, are expressed differently in lymphoid cells and in epithelial cells. Functional analysis of these PTPs including LAR and BPTP-1,2, seems to be crucial to answer this question.

Acknowledgments

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