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Molecular cloning of a novel protein-tyrosine phosphatase SH-PTP3 with sequence similarity to the *src*-homology region 2*

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Protein-tyrosine phosphorylation and dephosphorylation are directly associated with cellular growth, signal transduction, and neoplastic transformation. Here we report the isolation of a complementary DNA (cDNA) clone encoding a novel protein-tyrosine phosphatase (PTP) from a human T cell PEER cDNA library. The predicted open reading frame encodes a ~68-kDa protein composed of 593 amino acids which contains two *src*-homology region 2's (SH2 domains) at the N terminus; this PTP is designated as SH-PTP3. Northern blot analysis revealed that SH-PTP3 mRNA was expressed throughout many tissues and the transcriptional size was consistent at about 6.0 kb. As with other SH2 domains in *src*-family kinases, the SH2 domains of SH-PTP3 may play a crucial role in interactions with tyrosine phosphorylated signaling proteins, including itself and protein tyrosine kinases (PTKs), to regulate targets' enzyme activity.

Protein-tyrosine phosphatase; Protein tyrosine kinase; *src*-Homology region 2

1. INTRODUCTION

A variety of growth factors can lead protein tyrosine phosphorylation through receptor-linked protein-tyrosine kinases (PTK) or their associated PTKs [1,2]. The signals are mostly initiated by autophosphorylation of the receptors [3] and subsequently transduced via intracellular signaling pathways. In these signalings, *src* homology region 2 (SH2) has a key role in the signaling process because tyrosine autophosphorylation of the receptors result in binding sites for proteins with SH2 regions [4], for example, phospholipase C-γ (PLC-γ), and GTPase-activating protein (GAP) [5,6].

During signal transduction, phosphorylated tyrosines within the regulatory domains in *src*-family kinases can be specifically dephosphorylated with corresponding protein-tyrosine phosphatases (PTP) so as, ultimately, to increase signal transduction; this has been demonstrated for interactions between CD45 and p56^{lck} [7] or p59^{fyn} [8]. Thus, cross-talk among PTKs and PTPs is likely to have a crucial role in the control of cellular growth and differentiation. Recently, a PTP (PTP1C [9], SH-PTP1 [10] or HCP [11]) which contains SH2 domains has been identified and demonstrated to form high-affinity complexes with the tyrosinephosphorylated epidermal growth factor receptor [9]. Although the biological functions of SH-PTP1 remain to be elucidated, the PTP may directly participate in signal transduction pathways.

We have previously identified several PTPs expressed in pre-B cell NALM-6 by the reverse transcriptase-polymerase chain reaction (RT-PCR) technique [12], and have characterized a non-transmembrane PTP gene, LC-PTP [13] or HePTP [14], by the use of the PCR cDNA probe (BPTP-4) [12]. Here, we report the full-length cDNA sequence of another novel non-transmembrane PTP (SH-PTP3), which has been cloned using another cDNA probe (BPTP-3) [12]. SH-PTP3 possesses two SH2 domains at the amino (N)-terminus and shows a strong homology with SH-PTP1, but a fairly different nucleotide sequence. Thus, our data provide another SH2-encoding PTP, supporting the earlier suggestion of complicated cross-talk between PTKs and PTPs.

2. MATERIALS AND METHODS

2.1. *Isolation of LC-PTP cDNA clones*

A PEER cDNA library (Clontech) was probed with a 0.26-kb BPTP-3 PCR product which had been isolated from total RNA of NALM-6 cells by RT-PCR [12]. Approximately 1.2 million plaques were transferred to nitrocellulose filters (Millipore) and screened. The hybridization conditions were 50% formamide/2 × SSC (1 × SSC is 150 mM NaCl/15 mM trisodium citrate)/2.5 × Denhardt's solution, 0.1% SDS, 2.5% dextran sulfate, and denatured calf thymus DNA (100 μg/ml). The filters were washed with 2 × SSC at 50°C and exposed to Amersham hyperfilms using an intensifying screen. Hybridizing

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Abbreviations: PTP, protein-tyrosine phosphatase; PTK, protein tyrosine kinase; PCR, polymerase chain reaction; cDNA, complementary DNA.

*The sequence reported in this paper have been deposited in the EMBL/GenBank data base under the accession number D13540.

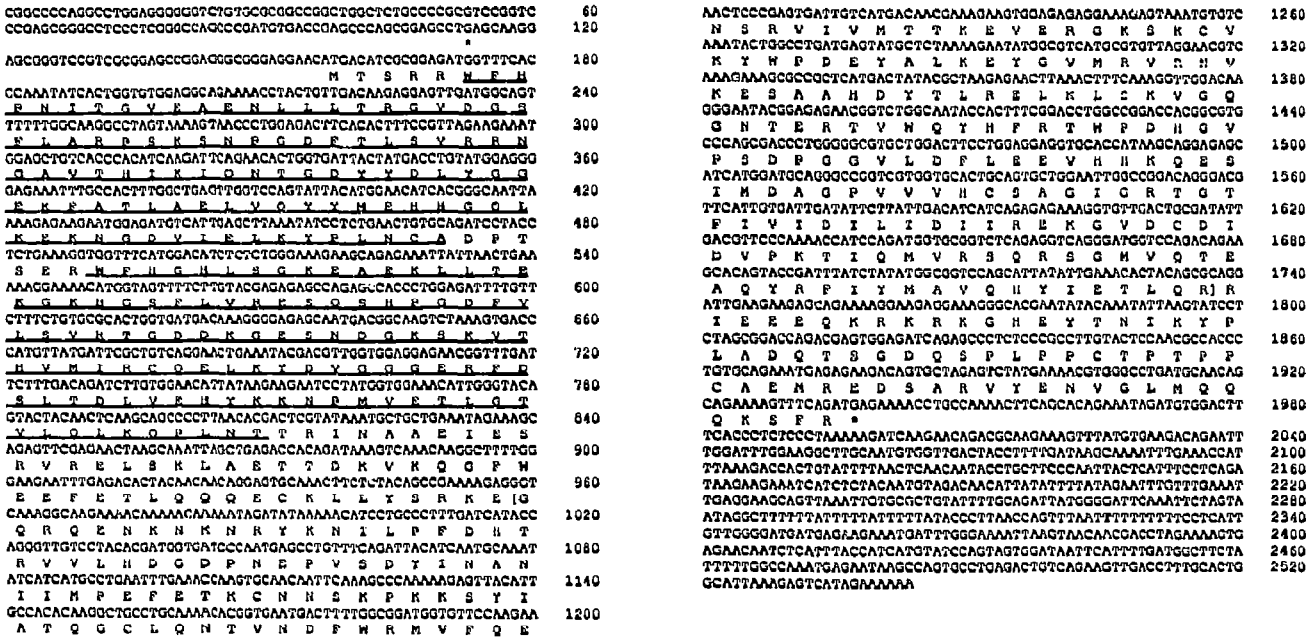

Fig. 1. Nucleotide and predicted amino acid sequence of the SH-PTP3 cDNA clone. The two SH2 domains are underlined, and a single PTP domain is indicated by brackets. Asterisks denote the stop codon. The single-letter amino acid code is used.

phages were purified, and the phage DNAs were prepared and subcloned into the pBlueScript SK(−) plasmid (Stratagene) using standard techniques.

2.2. *Nucleotide sequencing*

Closed circular plasmid DNA was purified by CsCl density centrifugation. For the sequencing of both strands, we generated overlapping deletion subclones of the longest SH-PTP3 cDNA clone with exonuclease III and Mung Bean Nuclease (Takara). The double stranded DNA was denatured with 0.2 M NaOH and the DNA sequence was determined by the dideoxynucleotide chain-termination procedure using either the manufacturer's primers or synthetic oligonucleotides derived from the existing sequences.

2.3. *RNA isolation and Northern blot analysis*

Total RNAs were extracted from human T cell leukemia lines (CEM, Jurkat, TALL-1, PEER), B cell malignant cells (BALL-1, RPMI8226, NALM-6), myeloid cell lines (HL60), glioblastoma (A-172, T98G), malignant melanoma (Mewo), fibroblast (WI-38), and colon carcinoma cells (BM314, COLO 201, CHC-Y1) by the guanidium/CsCl procedure. We also obtained total RNAs from various tissues of a patient under autopsy for cardiac failure (about 3 h after death). Ten micrograms of the total RNAs were separated in 1% agarose formaldehyde gels by electrophoresis and were transferred to nitrocellulose filters. The filters were hybridized with ^{32}P-labeled random primed cDNA fragments as probes. The probe for the SH-PTP3 gene was the 0.3-kb DNA fragment within the catalytic domain of the SH-PTP3 cDNA clone isolated from pre-B cell NALM-6 by RT-PCR [12]. The probe for β-actin was the 0.3-kb *Bam*HI–*Sal*I cDNA fragment of the PCR products, which was confirmed by sequencing and was used as control. The hybridization and wash conditions were the same as described for cDNA isolation. The same blots were used to test the SH-PTP3 and β-actin transcripts under identical conditions.

2.4. *Southern blot analysis of SH-PTP2*

Human genomic DNA was extracted from human T cell leukemia cells MOLT-3 and cleaved with the restriction endonucleases *Bam*HI, *Eco*RI and *Hin*dIII. The blot was hybridized with the ^{32}P-labeled cDNA 0.3-kb probe (PCR cDNA fragment). Hybridization and wash conditions were the same as those described for the screening of the library.

3. RESULTS

3.1. *Isolation of SH-PTP2 cDNA clones*

The 274 bp PCR cDNA fragment (BPTP-3) was used to isolate three cDNA clones from a human T cell leukemia PEER cDNA library. The sequence analysis of the longest cDNA fragment showed that this clone contained a single open reading frame (ORF) of 1,779 bps encoding a polypeptide of 593 amino acids and a 3′ untranslated region (Fig. 1). The nucleotides located at positions 154 to 160 around the first available ATG/methionine match well with the Kozac consensus sequence for eukaryotic translation initiation [15]. There is an upstream termination codon TGA at position 112 in this frame. The ORF encodes a protein of approximately 68 kDa and terminates with a TGA stop codon at position 1,936. SH-PTP3 lacks a signal sequence or potential transmembrane region, indicating SH-PTP3 is a non-transmembrane PTP. The carboxyl region, amino acids (a.a.) 202 to 455, contains a single catalytic domain of PTP molecules with the highly conserved consensus sequence HCSAGIGR, which is commonly seen in non-transmembrane PTPs [13]. SH-PTP3 is most closely related to CD45 [16] and HPTPα [17] (these PTPs are 38.6% identical over 324 a.a.). The most

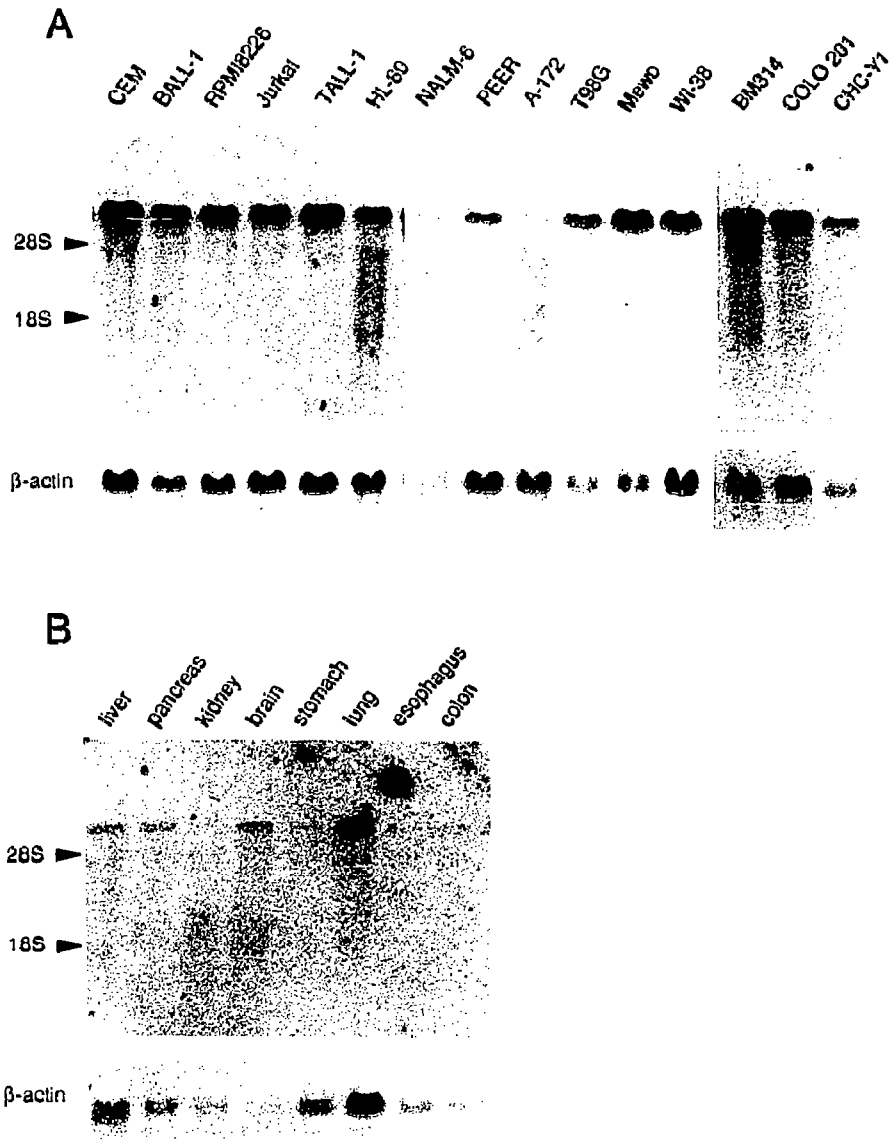

Fig. 2. RNA transfer blot analysis of SH-PTP3 transcript. (A) Total RNA samples were extracted from human T cell leukemia lines (CEM, Jurkat, TALL-1, PEER), B cell malignant cells (BALL-1, RPMI8226, NALM-6), myeloid cell lines (HL60), glioblastoma (A-172, T98G), malignant melanoma (Mewo), pulmonary fibroblast (WI-38), and colon carcinoma cells (BM314, COLO 201, CHC-Y1), and (B) from human tissues from an autopsy subject. Ten μg of total RNAs were electrophoresed (1% agarose gel), transferred to nitrocellulose filters, and hybridized with the ^{32}P-labelled SH-PTP3 cDNA probe. After the first autoradiography, the same filters were rehybridized with ^{32}P-labeled β-actin cDNA probe to confirm equal loading (bottom). The positions of ribosomal RNA markers are indicated to the left of the blots.

striking feature of SH-PTP3 is that this contains two SH2 domains at the N-terminus (Fig. 1). It is noteworthy that the SH2 domains contain the invariant residues found in SH2 domains of other molecules PTK, PLC-γ and GAP. In addition, there are two FLVRESES motifs which have been suggested to be involved in phosphotyrosine binding [18], FLVRESQS (a.a. 135 to 142) and FLARPSKS (a.a. 29 to 36) segments (Fig. 1). Interestingly, carboxyl (C) terminus contains nuclear localization-like motif [19], KRKRK (a.a. 534 to 538) and the prolin-rich domain (a.a. 551 to 568). Furthermore, there is a consensus sequence for ATP binding site (GXXXXGKS) [20] within the N-terminal SH2 domain (a.a. 158 to 165).

3.2. *Broad expression of SH-PTP3 transcripts*

Northern blot analysis of SH-PTP3 mRNA revealed approximately 6.0-kb transcripts. Initial studies of some cell lines showed SH-PTP3 transcripts in all the lines examined. A wide variety of cell lines and human tissues were then analyzed; SH-PTP3 was found in every kind of tissue we examined, though at considerably differing

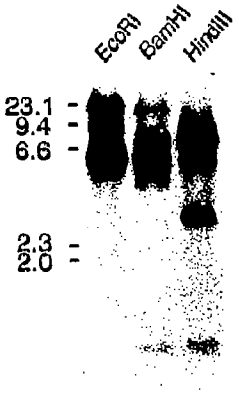

Fig. 3. Southern blot analysis of SH-PTP3. Ten μg of genomic DNA from MOLT-3 was digested with the indicated restriction enzymes, electrophoresed (0.7% agarose gel), transferred to nitrocellulose filters, and hybridized with the ^{32}P-labelled SH-PTP3 cDNA probe. Mobility of DNA standards (kb) is shown at left.

Fig. 4. Amino acid sequence comparison between SH-PTP3 (rat and human) and SH-PTP1. Two FLVRESES motifs were shown by boldface type. Amino acid sequence available for rat SH-PTP3 (L1) is overlined and the arginine (R) indicates an amino acid which is different from human SH-PTP3 sequence. The putative ATP binding domain is overlined with a broken line and the consensus sequence for nuclear localization signal is double overlined. The proline-rich domain is indicated by asterisks. Gaps (space) were introduced to optimize alignments.

levels of expression (Fig. 2). It was present at somewhat high levels in our one sample of brain tissue (Fig. 2B).

3.3. *Southern blot analysis of SH-PTP3*

Southern blot of human genomic DNA was hybridized with SH-PTP3 cDNA probe containing the catalytic domain. The blots revealed several bands of hybridization (Fig. 3), indicating that either the gene is huge with many introns or that there are multiple genes for SH-PTPs. Since the Northern blot analysis revealed a single transcript (~6.0 kb), it is likely that the SH-PTP3 gene is very large and consists of complicated exon–intron organizations. These multiple bands constantly appeared in at least three independent experiments with high stringency (data not shown).

4. DISCUSSION

We have isolated a novel non-transmembrane PTP (SH-PTP3) with two SH2 domains from a human T cell leukemia cell PEER cDNA library. Recently, a PTP with two SH2 domains has been identified in many laboratories [9–11]; it has been dubbed with a correspondingly wide variety of names, but because of some striking features of its structure and preliminary data suggesting additional SH2-encoding PTPs, the name SH-PTP1 [10] is used in this presentation. Overall, our novel SH-PTP3 is highly homologous to the SH-PTP1 (71% identity). Nucleotide sequence similarity in the coding regions is not so high (39.6% identity), which appears to have been the reason we could not detect transcripts of SH-PTP1 (2.6 kb) with SH-PTP3 probes under our conditions. Interestingly, SH-PTP3 is expressed throughout many tissues (Fig. 2), whereas SH-PTP1 is predominantly expressed in hematopoietic cells [11]. These different expression patterns may be associated with their biological functions.

The evidence summarized above conclusively demonstrates, we believe, that there is a subfamily of PTPs with SH2 domains. SH2 domains contain approximately 100 amino acids that are highly conserved among the *src* family members, and recently have also been found in cytoplasmic signaling proteins including PLC-γ, GAP, an 85-kDa subunit of PI3′-kinase [21], and the *v-crk* oncogene [22]. The roles of the SH2 domains of these signaling proteins suggest several possible functions for the SH2 domains in SH-PTP3. First, as the SH2 domains of *src*-like kinases [23], SH2 domains of SH-PTP3 may interact with its own tyrosine phosphorylation sites to negatively regulate enzyme activity. It is necessary to investigate whether SH-PTP3 itself can be tyrosine phosphorylated. Second, SH2 domains directly interact with signaling proteins and the resultant complexes may activate them or make them accessible to specific substrates by tyrosine dephosphorylation. Third, SH2 domains can target activated signaling proteins for signal termination by reversing tyrosine phosphorylation.

Recently, PTP L1 has been purified from rat liver cytosol; the amino acid sequence of the peptides indi-

cates that PTP L1 [24] has extensive similarity (only one a.a. difference within the available sequence) to SH-PTP3 (Fig. 4), implying that they are species homologs. The molecular mass of purified L1 is estimated to be 67 kDa by SDS-PAGE [24], which is similar to the molecular weight (68 kDa) of the predicted protein for SH-PTP3. Interestingly, L1 preferentially dephosphorylates tubulin [24], suggesting L1 (SH-PTP3) can be associated with microtubule-based motility. In view of the nucleotide-dependent interaction of SH-PTP3 with microtubules, it is of interest that SH-PTP3 possesses the consensus sequence for ATP binding, GXXXXGKS [20], which is found in SH-PTP3 sequence beginning at Gly^{158}.

Interestingly, SH-PTP3 has a nuclear localization signal-like segment. So far two PTKs (c-*abl* and c-*fer*) have been localized to the nucleus [25,26]. Their biological functions are being studied extensively, but are still obscure. Recently, loss of phosphotyrosine in $p34^{cdc2}$ was shown to be associated with activation of intrinsic serine kinase activity and appears to be a necessary step in the G2 to M transition in cell cycle [27]. Importantly, $p35^{cdc25}$ has been demonstrated to manifest intrinsic PTP activity; it dephosphorylates the phosphotyrosine of $p34^{cdc2}$ [28]. Thus, the enzyme activity of some serine/threonine kinases is also likely to be regulated by their own tyrosine-phosphorylation and dephosphorylation, suggesting important roles of SH2 domains. SH-PTP3 is ubiquitously expressed in human tissues and possesses a nuclear localization signal. These data suggest the attractive prospect that SH-PTP3 can participate in the modification of phosphotyrosine, a crucial step in the cell cycle.

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