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Profile and Differential Expression of Protein Tyrosine Phosphatases in Mouse Pancreatic Islet Tumor Cell Lines

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Summary: Protein tyrosine phosphatases (PTPs) play important roles in cell growth and differentiation of normal and tumor cells. In this study, we analyzed the PTP profile in two pancreatic islet tumor cell lines. Transcripts were isolated from αTC-1 (glucagon-secreting) and βTC-1 (insulin-secreting) cell lines for templates. A pair of degenerative primers, based on the conserved regions of known PTPs, was used to amplify the transcripts by polymerase chain reaction. A total of 1,620 clones was examined by restriction enzyme analysis and cDNA sequencing. Twenty-one PTPs were identified, including nine cytosolic PTPs (TcPTP, P19PTP, PTP1B, PTPMEG, PTP1C, SYP, PTPH1, PTPL1, and PTPD1), nine transmembrane PTPs (PTPδ, PTPγ, PTPκ, DEP-1, IA-2, LAR, PTPα, PTPNE3, and PTPε), and three new PTPs—PTPμ-like PTPκ-like, and IA-2β. An RNase protection assay demonstrated that some of these PTPs were expressed predominantly in glucagonoma (i.e., PTPδ and IA-2) and others in insulinoma (i.e., PTP1C, PTPκ, and PTPNE3) cells. In this report, we present the first profile of PTPs in α and β tumor cell lines. **Key Words:** Protein tyrosine phosphatase—Insulinoma—Glucagonoma—IA-2—Pancreatic islets.

The phosphorylation state of cellular proteins, regulated by protein tyrosine kinases and phosphatases (PTPs), plays an important role in signal transduction, cell growth, differentiation, and oncogenesis (1). PTPs dephosphorylate the tyrosine residues on proteins that are phosphorylated by protein tyrosine kinases. There are two types of PTPs, cytosolic and transmembrane (2). Tonks et al. (3) first isolated and characterized a cytosolic PTP, PTP1B, from placenta and subsequently showed that CD45 is a transmembrane PTP (4). Over the past several years, investigators have used PCR and differential cloning methods to isolate different PTPs from various tissues and species. There is, however, relatively little information on PTPs expressed in pancreatic islet tumors.

Recently, we isolated a novel transmembrane-type PTP, IA-2, from a human insulinoma subtraction library (5). The predicted open reading frame encodes a 979-amino acid protein that contains a leader sequence, an extracellular domain, a transmembrane segment, and an intracellular domain. IA-2 protein is found predominantly in islets, brain, and other neuroendocrine cells. IA-2 mRNA was found in the majority of human lung cancer cell lines with a neuroendocrine phenotype (6) and ~70% of sera from newly diagnosed IDDM patients have autoantibodies to IA-2 protein (7,8). To identify other PTPs expressed in pancreatic islet cells, two SV40 T antigen-transformed cell lines, αTC-1 and βTC-1, which secreted glucagon and insulin, respectively, were used to prepare cDNA libraries. cDNAs prepared from αTC-1 and βTC-1 islet cells served as templates and were amplified by PCR using degenerative primers based on conserved PTP sequences. Eighteen known PTPs and three new members of the PTP family were found in pancreatic islet cell lines.

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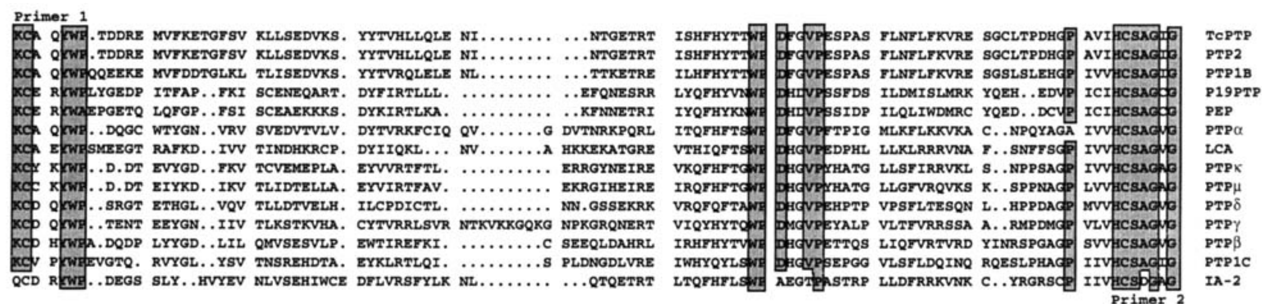
MATERIALS AND METHODS

Primer design

Fourteen known mouse PTP sequences from the GenBank were aligned using the PILEUP program (9). The



Primer 1: 5'-CAGTGGATCC AA(A/G) TG(C/T) (T/G)(C/A)N (C/A)(A/G)(A/G) TA(C/T) TGG CC-3'
BamHI

Primer 2: 5'-CTAGGAAT TCC N(A/G)(C/T) (A/G)CC NGC (A/G)CT GCA (A/G)TG-3'
EcoRI

FIG. 1. Sequence alignment and determination of PTP degenerative primers. Each segment from the catalytic domain of 14 known mouse PTP alignments was generated by the PILEUP program. Highly conserved residues are shown in the shaded areas. Primers 1 and 2 were designed from the highly conserved regions. N represents all four nucleotides. Two restriction enzyme sites, *Bam* HI and *Eco*RI, were added at the 5′-end of the primers for cloning.

amino acid sequences between two conserved regions, KCXXYWP and HCSXGXG in the PTP domain, were selected for polymerase chain reaction (PCR) amplification. Two degenerative primers were designed with the least mismatches between the primers and the known PTP sequences: primer, 1,5′-CAGT**GGATCC**AA(A/G)TG(C/T)(T/G)(C/A)N(C/A)(A/G)(A/G)TA(C/T)TGGCC-3′; and primer 2,5′-CTAG**GAATTC**CN(A/G)(C/T)(A/G)CCNGC(A/G)CTGCA(A/G)TG-3′ (N represents all four nucleotides). The primers contain 1,024 and 512 combinations, respectively, and the maximum mismatches between any of the known PTPs and the best-fit primer is 2 (average mismatch, 0.71 and 0.5). *Bam*HI or *Eco*RI restriction enzyme sites were added to flank the 5′ end of each primer (underlined) for subsequent cloning.

Construction of PCR-amplified libraries

Mouse pancreatic islet cell lines, αTC-1 and βTC-1, which secrete glucagon and insulin respectively, were kindly provided by Dr. E. H. Leiter (Jackson Laboratory, Bar Harbor, ME, U.S.A.) and cultured in low-glucose modified Eagles' medium supplemented with 10% fetal calf serum. Total RNAs were purified by the acid guanidinium thiocyanate method (10). Poly(A)$^+$ mRNA was isolated with an Oligotex-dT kit (Qiagen, Chatsworth, CA, U.S.A.). Five micrograms of poly(A)$^+$ mRNA was used to synthesize the first-strand cDNA (Clontech, Palo Alto, CA, U.S.A.). One-tenth of the synthesized cDNA served as template for the PCR. PCR conditions were as follows: 3 min at 94°C for denaturing; 1 min at 94°C, 1 min at 42°C, and 1 min at 72°C for 35 cycles; and 7 min at 72°C for extension. A single band of ~300 bp was isolated from agarose gel and further digested with the restriction enzymes *Bam*HI and *Eco*RI, then subcloned into the pBlueScript II (SK+) vector (Stratagene, LaJolla, CA, U.S.A.). A total of 1,620 clones was obtained from both the αTC-1 and the βTC-1 libraries. Plasmid DNA from each clone was prepared with a mini-preparation kit (Promega, Madison, WI, U.S.A.). Sequencing analysis revealed that several PTPs, such as TcPTP, P19PTP, PTP1B, PTPδ, PTPγ, and IA-2 PTP, represented a relatively high percentage of clones in the libraries. Since each PTP displays a different restriction enzyme pattern, abundant clones were first screened by digestion with at least two restriction enzymes, whereas rare clones were characterized by direct sequencing. The combination of DNA sequencing and/or restriction enzyme mapping was used to identify the clones in the two libraries.

RNase protection assay

PCR-amplified PTP fragments in the pBlueScript II (SK+) vector were used as template DNAs. The plasmid DNA was linearized by *Xba*I digestion and purified from agarose gel by GeneClean I (Bio 101, Vista, CA, U.S.A.). Antisense riboprobe was radiolabeled by in vitro transcription (Ambion, Austin, TX, U.S.A.). Briefly, 0.5 μg of linearized DNA was used for in vitro transcription with T7 RNA polymerase at 37°C for 1 h. RNase-free DNase I (2 U) was added to the reaction mixture for 15 min at 37°C to digest the template DNA. Radiolabeled riboprobe was separated from free isotope with a G-50 desalting column. The riboprobe (1 × 10^5 cpm) was mixed with various concentrations of RNA in

a hybridization buffer (80% formamide, 100 m*M* sodium citrate, pH 6.4, 300 m*M* sodium acetate, pH 6.4, 1 m*M* EDTA). The reaction mixture was heated to 90°C for 5 min and slowly cooled down to 44°C for overnight incubation. RNase digestion was carried out by adding 200 μl of RNase solution (300 m*M* sodium chloride, 10 m*M* Tris–Cl, pH 7.4, 5 m*M* EDTA, 2 μg/ml RNaseT1, 40 μg/ml RNaseA) at 37°C for 30 min. The reaction was stopped by adding 300 μl of 1% sodium-dodecyl sulfate (SDS) and 5 μl of 10 mg/ml protease K at 37°C for 15 min and ethanol precipitated. The protected fragment was analyzed on an 8% sequencing gel.

RESULTS

Identification of 21 different PTP fragments in pancreatic islet cells

Oligonucleotide primers were synthesized based on the PTP domain of 14 known mouse PTPs that were aligned using the PILEUP program (9). A segment ~100 amino acids in length was selected for PCR amplification (Fig. 1). Highly conserved residues, KCXXYWP and HCSXGXG, at both ends of the fragment were selected for designing the two degenerative primers. Low-stringency conditions were applied for the PCR reaction to ensure maximum amplification of all the PTP members that matched with the primers. Transcripts isolated from two clonal cell lines, αTC-1 and βTC-1, were used as templates to construct the PCR-amplified PTP libraries. A large number of clones were picked from each library to cover most, if not all, of the PTPs that were expressed in the cells. Each clone was first screened by restriction enzyme digestion and verified by sequence analysis. As shown in Table 1, the αTC-1 library yielded 1,025 clones including eight cytosolic PTPs, nine transmembrane PTPs, and three new PTPs. The major PTPs found in αTC-1 were TcPTP, P19PTP, PTP1B, PTPMEG, PTPδ, PTPγ, and IA-2 PTP (5,11–16). Other PTPs were found in relatively low numbers of clones, especially PTPH1, PTPL1, PTPD1, PTPNE3 (17–20), and two of the three new PTPs (IA-2β and PTP κ-like). The βTC-1 library yielded 595 clones including eight cytosolic PTPs, eight transmembrane PTPs, and two of the three new PTPs.

Differential expression of PTPs in α and β cell lines

The frequency of detection of PTP members in the α and β libraries is shown as a percentage in Table 1. Certain PTPs were highly expressed in both α and β cells (e.g., TcPTP, P19PTP, PTP1B, PTPMEG, and PTPγ). Others were expressed predominantly in α cells (e.g., PTPδ and IA-2) (5,15), and still others predominantly in β cells (e.g., PTP1C, PTPκ, and PTPNE3) (16,20–22). A number of PTPs were expressed only rarely in either cell line (e.g., PTPH1, PTPL1, PTPD1, IA-2β, PTPμ-like, and PTPκ-like).

TABLE 1. *PCR-amplified PTP fragments from αTC-1 and βTC-1 cell lines*

PTP	No. of isolates α-TC-1	No. of isolates βTC-1	% αTC-1	% βTC-1	Ref. no.
Cytosolic PTP					
TcPTP	284	274	27.7	46.1	11
P19PTP	93	94	9.1	15.8	12
PTP1B	89	57	8.7	9.6	13
PTPMEG	56	15	5.4	2.5	14
PTP1C	0	7	0.0	1.2	21
SYP	8	4	0.8	0.7	26
PTPH1	1	1	0.1	0.2	17
PTPL1	1	1	0.1	0.2	18
PTPD1	1	0	0.1	0.0	19
Transmembrane PTP					
PTPδ	289	0	28.2	0.0	15
PTPγ	40	66	3.9	11.0	16
PTPκ	16	37	1.5	6.2	22
DEP-1	11	11	1.1	1.8	34
IA-2	113	6	11.0	1.0	5
LAR	4	6	0.4	1.0	32
PTPα	10	5	1.0	0.8	31
PTPNE3	1	3	0.1	0.5	20
PTPε	3	3	0.3	0.5	31
Unknown PTP					
IA-2β	1	4	0.1	0.7	—
PTPμ-like	3	1	0.3	0.2	—
PTPκ-like	1	0	0.1	0.0	—
Total	1,025	595	100	100	

Since the numbers of isolates for certain of the PTPs were very low and the sequences of the three new PTPs were not taken into account in designing our degenerative primers, we evaluated for expression some of the rare and/or differentially expressed PTPs in α and β cells by RNase protection assays. As shown in Fig. 2, PTPδ and IA-2 were expressed more in α cells than β cells. In contrast, PTPκ-like, PTPNE3, PTP1C, and IA-2β were expressed more in β cells than α cells. PTPD1 and PTPμ-like were expressed to approximately the same degree in both cell types. The results from RNase protection experiments are consistent with those obtained with the PCR libraries except for PTPD1 and PTPκ-like, which were expressed in extremely low amounts in the PCR libraries.

New PTPs found in islet cell lines

Restriction enzyme analysis and sequencing revealed three new PTPs. As shown in Fig. 3, these PTPs have high similarities to PTPκ, PTPμ, and IA-2. PCR-amplified segments of PTPκ-like, PTPμ-like, and IA-2β showed 72% identity with PTPκ, 79% identity with PTPμ, and 89% identity with IA-2, respectively.

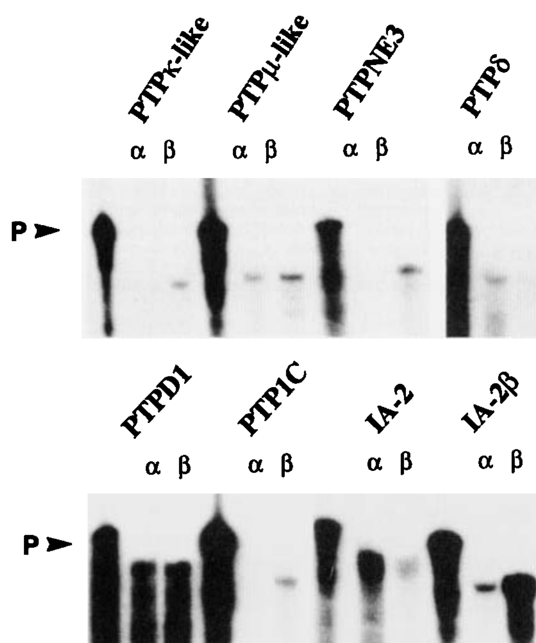


FIG. 2. RNase protection assay. Total RNA isolated from αTC-1 and βTC-1 was hybridized with ^{32}P-labeled riboprobes. Different concentrations of RNA were used in each lane: 10 μg, PTPNE3; 20 μg, PTPκ-like, PTPδ, IA-2, and IA-2β; 40 μg, PTPμ-like and PTPD1; and 50 μg PTP1C. "P" represents the riboprobe synthesized from an individual cDNA clone isolated from the PCR-amplified library. The abundance of transcripts is estimated from the protected fragments.

DISCUSSION

To study PTPs expressed in the pancreatic islet cells, we constructed a pair of PCR-based libraries from the α and β pancreatic islet cell lines, αTC-1 and βTC-1. These are clonal cell lines derived as a result of SV40 T antigen transformation (23). Analysis of the PCR-based libraries showed that >95% of the inserts belonged to PTP fragments, indicating that our degenerative primers properly matched islet cell PTP sequences. Although PCR methods have been used to amplify PTP from other cell lines and tissues (19,24) only a limited number of clones were analyzed in each of these studies. Analysis of the 1,620 clones described here is the largest known effort of this type and revealed a remarkable number of PTPs (21 in total) in islet cells including 3 previously unknown PTPs. It should be emphasized, however, that the number of PTPs recognized by this method is determined by the combination of message abundance and the matches between designed primers and cellular PTP sequences. The presence of 21 different PTPs identified in the pancreatic islets may still not represent the entire population of islet PTPs.

Our study showed that TcPTP, P19PTP, PTP1B, and PTPMEG were the dominant cytosolic PTP species in the islet cells. All of the cytosolic PTPs contained a single PTP domain, and in addition, some contained sequences that bear similarity to motifs such as PEST, src-homology 2 (SH2), and cytoskeletal protein 4.1 (17, 21,25,26). PEST is known to play a role in G protein receptor signaling and in cross-talk between G proteins and tyrosine kinase receptor pathways (27). SH2 domains can serve as binding sites for the activation of certain PTPs (e.g., PTP1C) (28) and the carboxyl termini of some PTPs (e.g., TcPTP and PTP1B) are important in determining the localization and regulation of PTP activity (29,30).

PTPδ, PTPγ, PTPκ, and IA-2 were the dominant transmembrane PTP species in the islet cells. In contrast to the cytosolic PTPs, most transmembrane PTPs are characterized by two tandem-repeat PTP domains. IA-2 is the exception and has only a single PTP domain (5). The sequences of the extracellular domains of transmembrane PTPs are quite diverse. Some (e.g., PTPα and PTPε) (31) have short extracellular domains, whereas others (e.g., PTPδ, PTPκ, LAR, and PTPNE3) possess multiple immunoglobulin-like and fibronectin-like motifs (13,18,20,32). Still others (e.g., PTPκ) possess the additional MAM motif, which exhibits homophilic binding for cell–cell aggregation (33), and DEP-1 is a density-dependent PTP (34). Why islet cells need so many different PTPs is still not clear. Perhaps the intracellular PTP catalytic domains are more substrate specific than has generally been appreciated. Alternatively, the different extracellular PTP motifs may be required for proper localization, signaling, and regulation of PTP activity.

The PCR-amplified libraries and the RNase protection experiments showed that certain PTPs are expressed primarily in β cells and others in α cells. It is interesting to speculate on whether these PTPs may be involved in the unique function of these cell types such as insulin and glucagon secretion and/or glucose sensing. IA-2 is preferentially expressed in α cells and is a major autoantigen in insulin-dependent diabetes mellitus (IDDM) (7,8) and a neuroendocrine marker in human lung tumor (6). Two of the three new PTPs identified here, PTPκ-like and IA-2β, appear to be expressed preferentially in β cells. We now have succeeded in sequencing and expressing both mouse and human IA-2β and find that nearly 50% of sera from newly diagnosed IDDM patients have autoantibodies to this protein (35,36).

In conclusion, by using a sensitive PCR amplification method we found that 21 different members of the PTP family, including 3 new PTPs, were expressed in pancreatic islet cell lines. The demonstration that some of these PTPs were differentially expressed provides the first profile of PTP activity in α and β tumor cell lines.

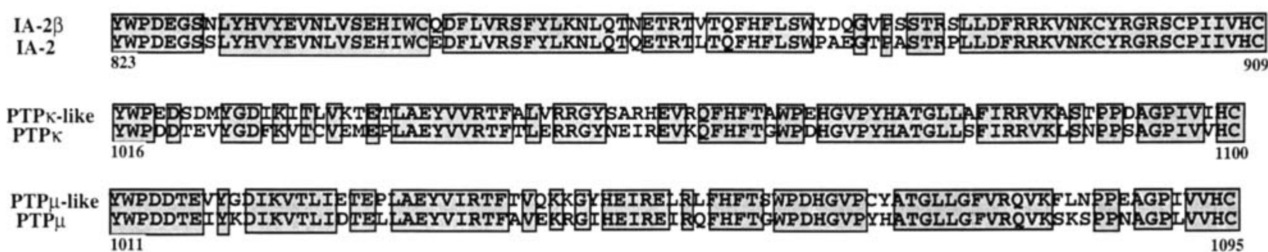


FIG. 3. Amino acid sequence alignment of the three new PTPs isolated from αTC-1 and βTC-1 cell lines. Predicted amino acid sequences from PCR-amplified and sequenced cDNA fragments were used to search the Swiss Protein data bank, and the closest alignments are shown. Each new sequence was named after the sequence to which it was most similar. Shaded boxes represent identical residues. Residue numbers represent the amino acid sequences of IA-2, PTPκ, and PTPμ, respectively.

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