Pleiotrophin signals increased tyrosine phosphorylation of β-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase β/ζ

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Pleiotrophin (PTN) is a platelet-derived growth factor-inducible, 18-kDa heparin-binding cytokine that signals diverse phenotypes in normal and deregulated cellular growth and differentiation. To seek the mechanisms of PTN signaling, we studied the interactions of PTN with the receptor protein tyrosine phosphatase (RPTP) β/ζ in U373-MG cells. Our results suggest that PTN is a natural ligand for RPTP β/ζ. PTN signals through "ligand-dependent receptor inactivation" of RPTP β/ζ and disrupts its normal roles in the regulation of steady-state tyrosine phosphorylation of downstream signaling molecules. We have found that PTN binds to and functionally inactivates the catalytic activity of RPTP β/ζ. We also have found that an active site-containing domain of RPTP β/ζ both binds β-catenin and functionally reduces its levels of tyrosine phosphorylation when added to lysates of pervanidate-treated cells. In contrast, an (inactivating) active-site mutant of RPTP β/ζ also binds β-catenin but fails to reduce tyrosine phosphorylation of β-catenin. Finally, in parallel to its ability to inactivate endogenous RPTP β/ζ, PTN sharply increases tyrosine phosphorylation of β-catenin in PTN-treated cells. The results suggest that in unstimulated cells, RPTP β/ζ is intrinsically active and functions as an important regulator in the reciprocal control of the steady-state tyrosine phosphorylation levels of β-catenin by tyrosine kinases and phosphatases. The results also suggest that RPTP β/ζ is a functional receptor for PTN; PTN signals through ligand-dependent receptor inactivation of RPTP β/ζ to increase levels of tyrosine phosphorylation of β-catenin to initiate downstream signaling. PTN is the first natural ligand identified for any of the RPTP family; its identification provides a unique tool to pursue the novel signaling pathway activated by PTN and the relationship of PTN signaling with other pathways regulating β-catenin.

Pleiotrophin (PTN) is a platelet-derived growth factor-inducible heparin-binding growth and differentiation factor (1–4). PTN is nearly 50% identical with the retinoic acid-inducible factor midkine, which is also a growth and differentiation factor (3, 5). *Ptn* gene expression is limited to specific cell types at different times during development (3, 4, 6). However, in adults, *Ptn* gene expression is constitutive and limited to only a few cell populations (6), except in sites of injury, when its expression is sharply increased (7). PTN is mitogenic for fibroblasts (1–3), endothelial cells (8–10), epithelial cells (9–11), and trophoblasts (12). PTN also induces neurite outgrowth from neurons (2, 3) and glial process outgrowth from glial progenitor cells (H.-J. Yeh and T.F.D., unpublished data), suggesting that *Ptn* gene expression may influence a very broad range of functional activities.

Deregulated *Ptn* gene expression also may be very important in malignant transformation and progression of tumor growth. The *Ptn* gene is a protooncogene (13). It is expressed in many human tumors and cell lines derived from human tumors (9, 14–16). Recently, interruption of PTN signaling reversed the transformed phenotype of cultured human breast cancer cells that constitutively express the endogenous *Ptn* gene (17) and effectively reverted the malignant phenotype of cultured human melanoma cells (18), suggesting that mutations that establish stable expression of endogenous PTN in already transformed cells induce an incremental step in tumor progression.

Despite the apparent importance of PTN, the molecules through which PTN signals have not been established. Previously, PTN has been found to bind to heparin, heparin sulfate proteoglycans, and extracellular matrix (1–3, 19–21). PTN also induces tyrosine phosphorylation of a 190-kDa protein in PTN-treated murine fibroblasts (22), indicating the potential importance of tyrosine phosphorylation in PTN signaling. Recently, PTN was found to interact with the receptor (transmembrane) protein tyrosine phosphatase (RPTP) β/ζ (20, 23, 24) and syndecan-3 (19, 25). The expression of these molecules was found to correlate with PTN. However, the functional significance of these interactions has not been identified. Because PTN influences tyrosine phosphorylation (22) and the widespread importance of tyrosine phosphorylation in cell proliferation and differentiation (26, 27), we raised the possibility that RPTP β/ζ may be a functional receptor for PTN. This possibility is particularly interesting because there are no known soluble ligands for this class of transmembrane receptor tyrosine phosphatases, and thus PTN potentially is a unique probe to explore the receptor class of transmembrane tyrosine phosphatases and how they signal.

Materials and Methods

Cell Culture. U373-MG glioblastoma (American Type Culture Collection) cells were used in all experiments and cultured in DMEM and 10% FCS unless otherwise noted.

Western Blot Analysis. U373-MG glioblastoma cells (≈10^6) were lysed in 50 mM Tris·HCl (pH 8.0)/150 mM NaCl/1 mM EDTA/1% Triton X-100/1 mM phenylmethylsulfonyl fluoride/0.5 μg/ml leupeptin/1 μM pepstatin/1 μg/ml aprotinin for 30 min at 4°C, boiled in SDS/PAGE sample buffer (25 mM Tris·HCl, pH 6.8/2.5% SDS/2.5% glycerol/5% 2-mercaptoetha-

Abbreviations: PTN, pleiotrophin; RPTP, receptor protein tyrosine phosphatase; PTN-Fc, PTN-Fc fragment of IgG; GST, glutathione *S*-transferase; D1, GST-juxtamembrane fragment.

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nol), separated by SDS/PAGE, transferred to poly(vinylidene difluoride) membranes, probed with antibodies as indicated, and illuminated with the enhanced chemiluminescence ECL-PLUS system (Amersham).

Chemical Cross-Linking. U373-MG cells ($\approx 10^6$)were incubated with the PTN-Fc fragment of IgG (PTN-Fc) for 30 min at 37°C, washed with PBS, and incubated with 1 mM of the reversible cross-linking agent 3,3′-dithiobis(sulfosuccinimidyl propionate) (Pierce) for 30 min at 37°C, and lysed.

PTN-Fc "Capture." U373-MG cells ($\approx 10^6$) were lysed as above, and proteins associated with PTN-Fc were bound to protein A Sepharose-4B and, after washing, eluted by boiling in sample buffer and analyzed in Western blots as above.

Glutathione *S*-Transferase (GST) "Capture" Assays. The GST-juxtamembrane (D1) fragment and the D1 fragment Cys-1925 → Ser were prepared by using a human RPTP β/ζ cDNA fragment to encode amino acids 1655–2018 fused with GST in the expression plasmid PGEX-KG (kindly provided by Z. Y. Wang), *Xho*I and *Xba*I sites. The constructs (or GST alone) were expressed in BL-21 competent cells from 5-ml overnight cultures, and the recombinant proteins were immobilized with 100 μl of glutathione-Sepharose-4B beads (Amersham Pharmacia). The beads were then incubated with U373-MG cell lysates from 60-mm confluent dishes, washed, eluted, and analyzed in Western blots as above.

Antibodies and Other Reagents. α-β-Catenin and α-RPTP β/ζ antibodies were obtained from Transduction Laboratories (Lexington, KY), and α-phosphotyrosine monoclonal antibodies (4G10) were obtained from Upstate Biotechnology (Lake Placid, NY). The cDNA of human RPTP β/ζ was the kind gift of H. Saito (Dana–Farber Cancer Institute, Boston), recombinant PTN was purchased from Sigma, and recombinant PTN-Fc was purified from conditioned media of human embryonic kidney (293) cells expressing a cDNA that encodes the full-length PTN molecule fused at its C terminus with the Fc fragment of IgG. For the dose responses of both PTN and PTN-Fc to inactivate RPTP β/ζ, see below. PTN was used at 50 ng/ml and PTN and PTN-Fc were established by using tyrosine phosphorylation of β-catenin and the ability PTN-Fc was used at 5 ng/ml, saturating levels of each, respectively.

RPTP β/ζ Tyrosine Phosphatase Activity. *PTN-treated U373-MG cells.* Confluent U373-MG cells were incubated either with DMEM alone or 50 ng/ml recombinant PTN (Sigma) at 37°C for 15 min, washed three times with PBS, lysed as described above, and cleared at 14,000 × *g* for 15 min at 4°C. Equal amounts of lysates were incubated with α-RPTP β/ζ antibodies or mouse IgG (control) at 4°C overnight, incubated with protein A Sepharose-4B at 4°C for 2 h, and washed three times in lysis buffer and once in assay buffer (20 mM imidazole, pH 7.2/0.1 mg/ml BSA). The phosphatase activity of the immobilized RPTP β/ζ protein was assayed as follows: 50 μl of either RPTP β/ζ or mouse IgG immobilized on protein-A beads was added to the assay buffer, the reaction mixture (25 mM imidazole, pH 7.2/0.1 mg/ml BSA/10 mM DTT/100 nM ^{32}P-labeled substrate Raytide) was added to a final volume of 80 μl, incubated at 30°C for various times, terminated, and the ^{32}P released was quantitated by a charcoal-binding assay. The synthetic peptide Raytide (Oncogene Science) was phosphorylated at its unique tyrosine residue by following the manufacturer's instructions.

RPTP β/ζ activity in Sf9 cell membranes. The Bac-to-Bac Baculovirus Expression System (Life Technologies) was used to express RPTP β/ζ in Sf9 cells. A full-length human RPTP β/ζ cDNA was cloned into a pFastBac donor plasmid at *Not*I and *Xba*I sites (pFastBac-RPTP β/ζ), transformed into DH10Bac *Escherichia coli* which contains bacmid and helper virus, and plasmid DNA prepared. Sf9 cells were infected by the recombinant virus according to the manufacturer's instructions. To prepare membrane fractions, cells were sonicated in a hypotonic lysis buffer (25 mM Tris·HCl, pH 7.5/25 mM sucrose/0.1 mM EDTA/5 mM $MgCl_2$/5 mM DTT/1 mM phenylmethylsulfonyl fluoride/0.5 μg/ml leupeptin/1 μg/ml aprotinin), nuclei were removed by low-speed centrifugation, and membrane fractions were obtained by centrifugation at 100,000 × *g* for 60 min at 4°C. The resulting pellets were suspended by sonication in lysis buffer, brought to a concentration of 2 mg/ml, and used to measure PTPase activity as above.

The assays were linear with time and protein concentration.

Results

Both Exogenous PTN and the Endogenous *Ptn* Gene Product Interact with RPTP β/ζ. PTN-Fc was incubated with lysates of serum-starved, confluent U373-MG cells, and proteins associated with PTN-Fc were captured on protein A Sepharose and probed in Western blots with anti- (α)-RPTP β/ζ antibodies (Fig. 1*B*). Three major and other minor alternative-spliced forms of the single RPTP β/ζ gene have been identified (28–30); the results of the PTN-Fc capture were therefore compared with Western blots of immunoprecipitates from untreated U373-MG cell lysates incubated with α-RPTP β/ζ antibodies (Fig. 1*A*). Major bands of ≈230, ≈130, ≈85, and, variably, in other experiments, ≈250 kDa were identified (Fig. 1*A*), consistent with the known different spliced forms of RPTP β/ζ previously identified. Depending on the conditions of cell growth, different (presumably alternative-spliced) forms were identified (data not shown). In Western blots of proteins captured by PTN-Fc from U373-MG cell lysates, two major bands of ≈130 and ≈85 kDa were identified (Fig. 1*B*), suggesting that PTN-Fc preferentially associates with isoforms of ≈130 and ≈85 kDa. However, when the blots were exposed for longer times, a faint band at ≈230 kDa was also seen. When IgG alone was substituted for PTN-Fc, RPTP β/ζ was not captured by protein A Sepharose (Fig. 1*B*, left lane). When blots were reprobed with α-IgG antibodies that recognize the Fc portion of PTN-Fc or anti-PTN antibodies, it was established that PTN-Fc was present in the complex captured by protein A Sepharose (data not shown).

PTN itself is also expressed in U373-MG cells (data not shown) (E. Afrikanova and T.F.D., unpublished work). To establish that the endogenously expressed PTN and RPTP β/ζ interact with each other *in vivo*, untreated U373-MG cell lysates were immunoprecipitated with α-PTN antibodies and analyzed in Western blots. Anti-RPTP antibodies recognized protein bands at 130 and 85 kDa (faint) (Fig. 1*C*). The results thus establish that both exogenous and endogenous PTN physically interact with the major alternatively spliced products of RPTP β/ζ in U373-MG cells.

PTN Inactivates RPTP β/ζ Activity *in Vivo* and *in Vitro*. To directly determine if PTN affects the function of endogenous RPTP β/ζ, lysates of PTN-treated and control, untreated U373-MG glioblastoma cells were immunoprecipitated with α-RPTP β/ζ antibodies, incubated with protein A Sepharose, and directly assayed for protein tyrosine phosphatase activity as described above (Fig. 2*A*). The effects of PTN on the catalytic activity of recombinant RPTP β/ζ also were tested by using membrane fractions prepared from Sf9 insect cells infected with a baculovirus expressing recombinant RPTP β/ζ. Remarkably, the protein tyrosine phosphatase activity of the endogenous RPTP β/ζ in immunoprecipitates from PTN-treated cells was reduced by more than 90% when compared with RPTP β/ζ from untreated cells and when corrected for nonspecific background (IgG controls, Fig. 2*A*). PTN also strikingly reduced the catalytic

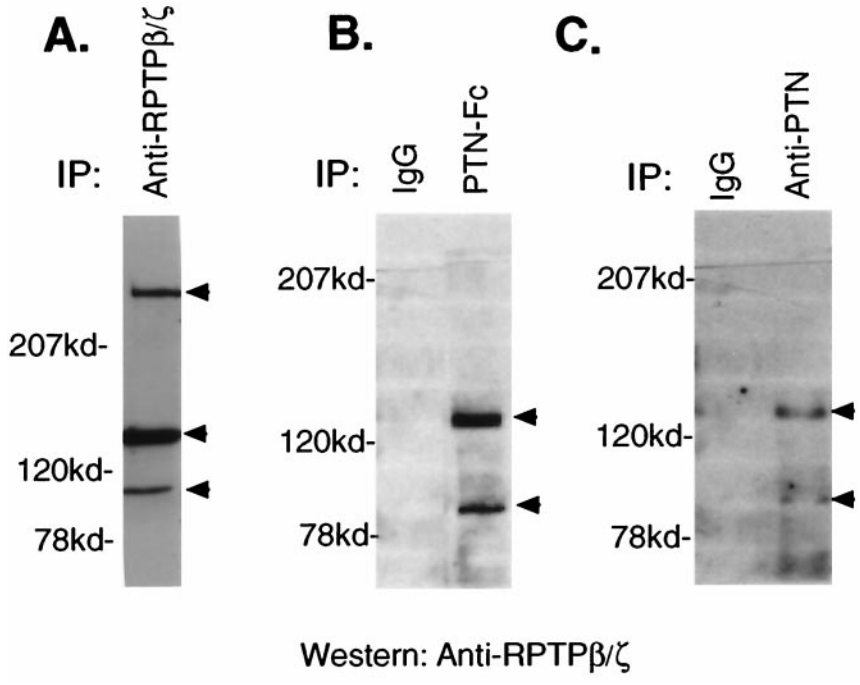

Fig. 1. Association of RPTP β/ζ with PTN. (*A*) Lysates of U373-MG glioblastoma cells were immunoprecipitated with anti-RPTP β/ζ monoclonal antibodies. The immunoprecipitates were separated on 6% acrylamide gel, transferred to a poly(vinylidene difluoride) membrane, and probed with anti-RPTP β/ζ antibodies. The arrowheads indicate the RPTP β/ζ spliced products of ≈230, 130, and 85 kDa. (*B*) Western analysis of RPTP β/ζ captured by PTN-Fc. Lysates of U373-MG cells were incubated with PTN-Fc and proteins interactive with PTN-Fc (right lane) were captured with protein A Sepharose-4B beads for 2 h. The beads were washed in cold lysis buffer, boiled in SDS/PAGE sample buffer, and the eluted proteins were separated on an 8% acrylamide gel and analyzed by Western blots probed with anti-RPTP β/ζ monoclonal antibodies. As a control, PTN-Fc was replaced with an equal amount of human IgG (left lane). The arrowheads indicate the ≈130- and ≈85-kDa spliced products of RPTP β/ζ. (*C*) Western analysis of RPTP β/ζ captured by endogenous PTN. Lysates of U373-MG cells were incubated with anti-PTN monoclonal antibodies (right lane) and the complexes were captured with protein A Sepharose-4B beads for 2 h. The beads were washed in cold lysis buffer and boiled in SDS/PAGE sample buffer, and the eluted proteins were separated on an 8% acrylamide gel and analyzed by Western blots probed with anti-RPTP β/ζ monoclonal antibodies. As a control, mouse IgG replaced the anti-PTN antibody (left lane). The arrowheads indicate the ≈130- and ≈85-kDa spliced products of RPTP β/ζ.

activity of recombinant RPTP β/ζ in Sf9 membranes when background phosphatase activity was again corrected (Fig. 2*B*). The inhibition by PTN is specific, because PTN inhibits tyrosine phosphatase activity only in Sf9 cell membranes that express RPTP β/ζ and it is rapid (Fig. 2*C*). Nearly 70% of the phosphatase activity of RPTP β/ζ is lost in 5 min. Thus, PTN not only physically associates with RPTP β/ζ but functionally, PTN profoundly reduces the catalytic activity of RPTP β/ζ. Furthermore, because PTN effectively reduces the endogenous RPTP β/ζ activity, it can be concluded that RPTP β/ζ is an intrinsically active tyrosine phosphatase, thereby suggesting that RPTP β/ζ may be an important regulator of steady-state tyrosine phosphorylation of compatible intracellular substrates that themselves are regulated by an intrinsically active tyrosine kinase activity.

β-Catenin Is a Potential Substrate for RPTP β/ζ. β-Catenin is known to associate with other RPTPs and be phosphorylated in tyrosine (31, 32). β-Catenin also is an important signaling molecule in development and in the *wnt*/APC−/− oncogenic pathways (33–35), suggesting that its signaling properties may be influenced by tyrosine phosphorylation and potentially be regulated by RPTP β/ζ and/or PTN. PTN-Fc-treated U373-MG cells were therefore incubated with 3,3′-dithiobis(sulfosuccinimidyl propionate) and lysed. Proteins cross-linked to PTN-Fc were captured with protein A Sepharose and analyzed in Western blots with either α-β-catenin (Fig. 3*A Right*) or α-RPTP β/ζ antibodies (control, Fig. 3*A Left*). In gels in which the captured proteins were not reduced before SDS/PAGE, a higher molecular weight complex with very limited migration was identified, and immunoreactive PTN and RPTP β/ζ were identified in this band (data not shown). In other control experiments, both β-catenin (Fig. 2*A Right*, lane 3) and RPTPβ/ζ (Fig. 3*A Left*, lane 3) were readily recognized in untreated U373-MG cell lysates. When the captured protein complex from PTN-Fc-treated cells cross-linked with 3,3′-dithiobis(sulfosuccinimidyl propionate) was reduced before SDS/PAGE and analyzed in Western blots probed with α-RPTP β/ζ antibodies, RPTP β/ζ spliced forms of ≈130 kDa, and more weakly, ≈230 kDa, were identified (Fig. 3*A Left*, lane 1). These forms were not identified in lysates of cells treated with the Fc fragment of IgG alone (Fig. 3*A Left*, lane 2). Remarkably, PTN-Fc also captured β-catenin, based on recognition by α-β-catenin antibodies and the migration of the band recognized by α-β-catenin at the estimated molecular mass of β-catenin (≈94 kDa) (Fig. 3*A Right*, lane 1). β-Catenin was not captured when cells were treated with the Fc fragment of IgG alone (Fig. 3*A Right*, lane 2). The results confirm that the extracellular domain of RPTP β/ζ interacts with PTN-Fc and suggest that β-catenin interacts with its intracellular domain. These results also raise the possibility that RPTP β/ζ links PTN signaling to β-catenin.

RPTP β/ζ has two phosphatase domains in its C-terminal cytoplasmic tail. The juxtamembrane-proximal D1 domain of RPTP β/ζ contains an active tyrosine phosphatase catalytic unit, whereas the juxtamembrane-distal D2 domain lacks the required cysteine residue and thus is inactive (36). To see whether β-catenin associates with the active site of RPTP β/ζ, the D1 domain and the D1 domain Cys-1925 → Ser (active site

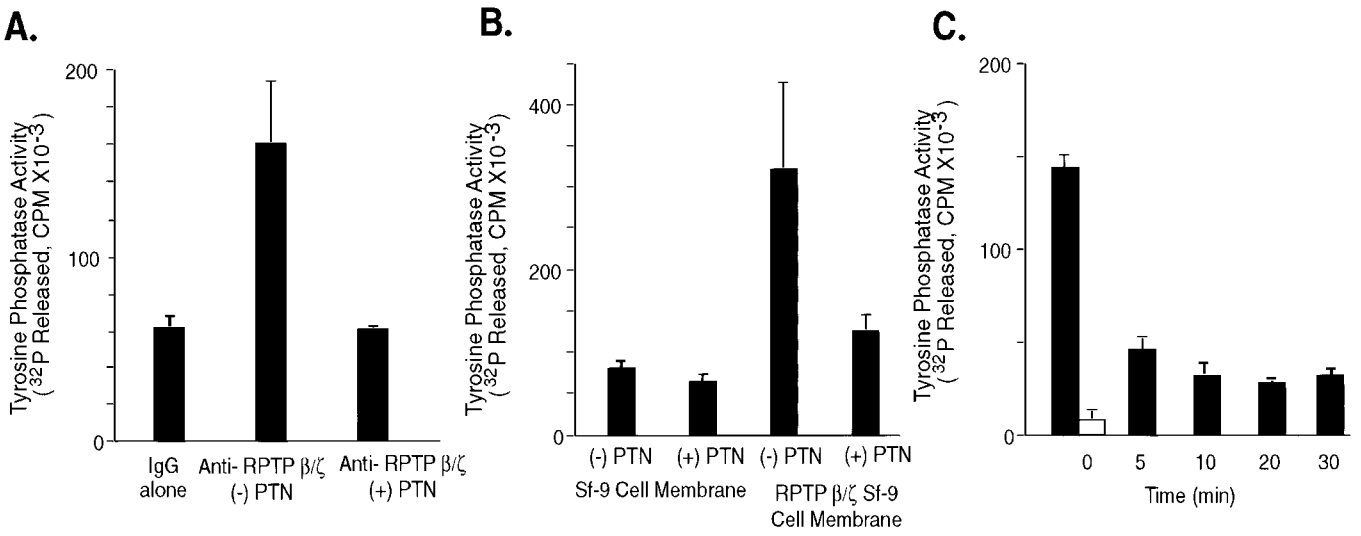

Fig. 2. PTN-dependent inhibition of the intrinsic tyrosine phosphatase activity of RPTP β/ζ. (*A*) Inhibition of the endogenous RPTP β/ζ tyrosine phosphatase activity in PTN-treated U373-MG cells. The left bar represents tyrosine phosphatase activity in immunoprecipitates from lysates of untreated cells with mouse IgG (control) to replace the anti-RPTP β/ζ antibodies. The center bar represents tyrosine phosphatase activity in immunoprecipitates with anti-RPTP β/ζ antibodies from lysates of untreated cells, and the right bar represents tyrosine phosphatase activity of immunoprecipitates with anti-RPTP β/ζ antibodies from lysates of cells treated with recombinant PTN (50 ng/ml.) (*B*) Inhibition of recombinant RPTP β/ζ phosphatase activity in Sf9 cell membranes. Membrane fractions of Sf9 cells that were infected by a baculovirus containing a cDNA-encoding RPTP β/ζ (right two bars), or uninfected (left two bars) that were untreated (− PTN) or treated (+ PTN) with 50 ng/ml PTN were assayed as described in *Materials and Methods*. (*C*). Time course of PTN-dependent inactivation of RPTP β/ζ in PTN-treated (50 ng/ml) Sf9 cell membranes expressing RPTP β/ζ (solid bars) and Sf9 cell membranes without RPTP β/ζ (open bar, $t = 0$ only).

inactivating) mutation were coupled with GST, incubated for 15 min with U373-MG cell lysates from cells pretreated with pervanidate, and analyzed in Western blots. Both the active and inactive D1 domains of RPTP β/ζ captured β-catenin (Fig. 3*B Upper*) at essentially equal levels. However, when the Western blots were reprobed with α-phosphotyrosine antibodies, the levels of tyrosine phosphorylation of β-catenin were sharply reduced in lysates incubated with the active (wt) D1 domain compared with the D1 domain Cys-1925 → Ser (Fig. 3*B Lower*), localizing the association of β-catenin to the active site-

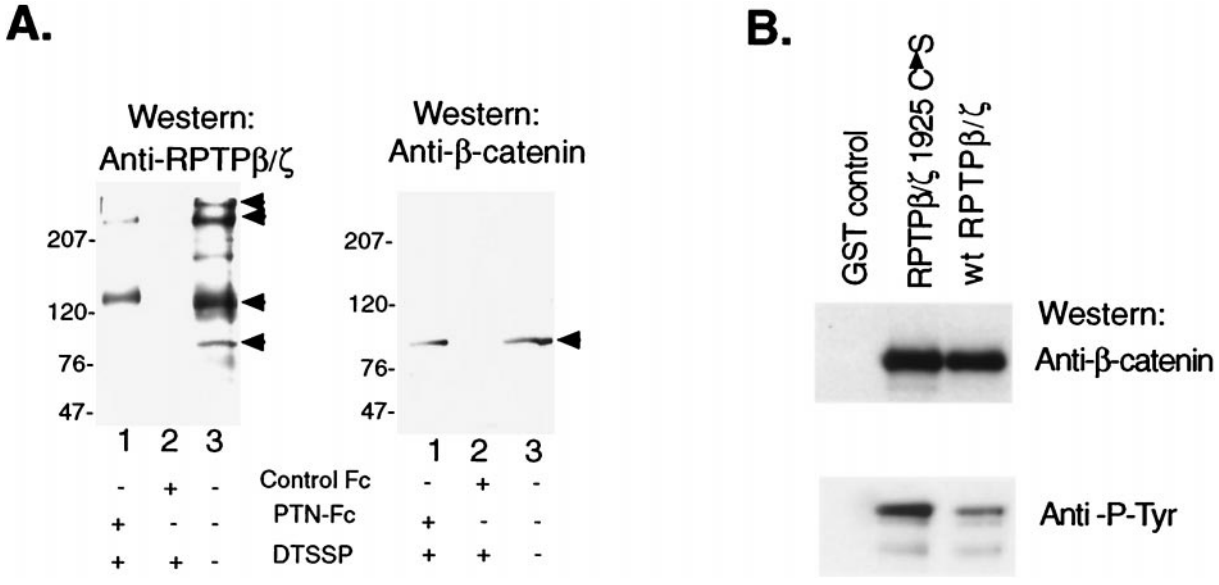

Fig. 3. Physical and functional association of β-catenin with PTN/RPTP β/ζ. (*A*). PTN-Fc is in complex with RPTP β/ζ and β-catenin. PTN-Fc-treated confluent U373-MG cells from 60-mm dish were chemically cross-linked with 3,3′-dithiobis(sulfosuccinimidyl propionate). Lysates from PTN-Fc-treated, chemically cross-linked cells (lanes 1) or Fc- (alone) treated (control) U373-MG cells (lanes 2) were incubated with protein A Sepharose, washed, eluted with SDS sample buffer with 5% 2-mercaptoethanol, and analyzed in 6% SDS gels and Western blots. Lysates from untreated U373-MG cells alone (lanes 3) were also analyzed as a control. Western blots were analyzed with anti-β-catenin (*Right*) or anti-RPTP β/ζ antibodies (*Left*). Arrowheads identify RPTP β/ζ spliced products of ≈250, 230, 130, and 85 kDa (*Left*) and β-catenin (94 kDa) (*Right*). (*B*) β-Catenin interacts with proximal (catalytic) domain of RPTP β/ζ. The GST-D1-RPTP β/ζ wild-type, GST-D1-Cys-1925 → Ser (inactivating) mutant fusion protein or GST alone were expressed and immobilized with glutathione-Sepharose-4B beads, incubated with U373-MG cell lysates, washed, and analyzed in Western analysis with the α-phosphotyrosine antibodies and visualized with the enhanced chemiluminescence ECL-PLUS system (*Lower*). The same blot was reprobed with α-β-catenin antibodies and detected as above (*Upper*).

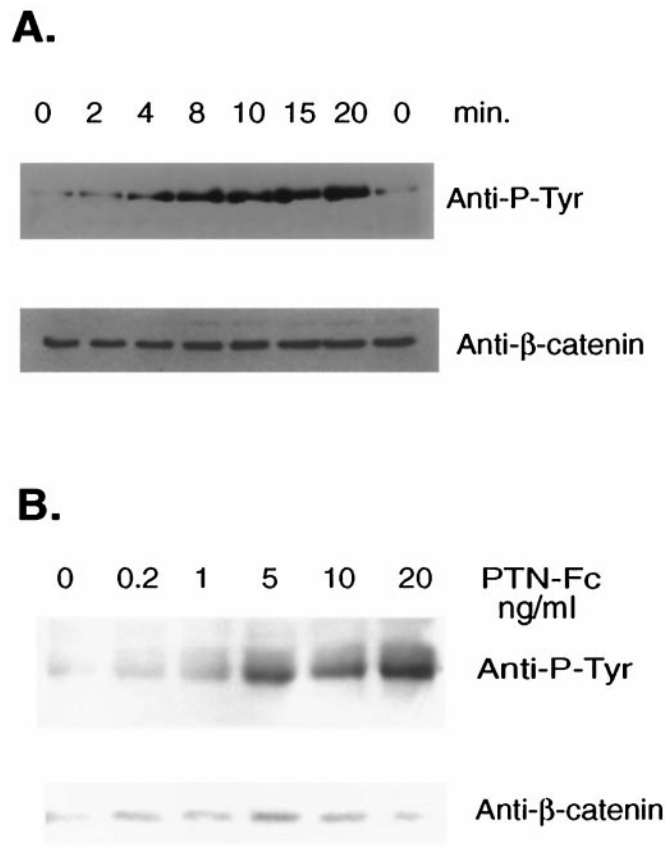

Fig. 4. Increased β-catenin tyrosine phosphorylation. (*A*) Time course of the tyrosine phosphorylation of β-catenin in response to PTN-Fc treatment. Cells were treated with 10 ng/ml PTN-Fc for the times indicated. Lysates were immunoprecipitated with α-β-catenin antibodies and analyzed in Western blots probed with α-phosphotyrosine antibodies (*Upper*) and the blots were reprobed with α-β-catenin antibodies (*Lower*). (*B*) U373-MG cells were treated with different doses of PTN-Fc for 20 min. Cells were grown to near confluence, and then were serum-starved for 48 h. PTN-Fc was added up to the indicated concentrations. The Fc fragment alone (20 ng/ml) was added as a control. Lysates were immunoprecipitated and analyzed in Western blots with antiphosphotyrosine antibodies as described above. Parallel immunoblots were probed for β-catenin.

containing D1 domain and strongly suggesting that β-catenin is a substrate of RPTP β/ζ.

PTN Stimulates Tyrosine Phosphorylation of β-Catenin. To pursue the possibility that β-catenin is a substrate of RPTP β/ζ in intact cells and that PTN-dependent inactivation of RPTP β/ζ influences tyrosine phosphorylation of β-catenin, it was examined temporally after the addition of PTN-Fc to intact U373-MG cells. Tyrosine phosphorylation of β-catenin increased within 2 min of addition of PTN and reached peak levels within 8 min (Fig. 4*A*). The levels of β-catenin itself were essentially identical; indicating that PTN-Fc had no detectable influence on the levels of β-catenin protein. Furthermore, the response was PTN-Fc dose-dependent between 0.2 and 5 ng/ml (Fig. 4*B*).

Discussion

Recently, Maeda *et al.* (20, 23) reported that 6B4 proteoglycan/phosphacan, the extracellular domain of RPTP β/ζ, binds PTN, whereas acidic fibroblast growth factor fibronectin, and laminin do not. Two different sites with K_d of 0.5 and 3 nM were identified, and after enzymatic cleavage of chondroitin sulfate from RPTP β/ζ, the binding affinity was found to be 13 nM, suggesting that the binding of PTN to phosphacan is specific. Our data demonstrate that both exogenous PTN and endogenously expressed PTN interact with both endogenous and recombinant RPTP β/ζ and that exogenous PTN inactivates the intrinsic tyrosine phosphatase activity of both the endogenous and recombinant RPTP β/ζ. These results are consistent with a recently proposed model based on the analysis of the crystal structure of the membrane-proximal catalytic domain (D1) of the murine RPTPα (37). The analysis suggested that the dimerization of the D1 domain induced a conformationally determined block ("a wedge") of access of substrate protein tyrosine phosphates to the active site and consequent loss of intrinsic tyrosine phosphatase activity of RPTP α (37–39). In support of this model, it has recently been reported that ligand-induced signaling may be established through dimerization of a heterologous extracellular domain of chimeric RPTP α (40). The model is consistent with what we have found. Our results suggest that PTN is a natural ligand of RPTP β/ζ, it induces ligand-dependent dimerization, and it inactivates the catalytic activity of RPTP β/ζ, presumably denying the access of substrate(s) to its catalytic site. Because we also found that β-catenin interacts with the catalytically active D1 domain of RPTP β/ζ and that addition of the D1 domain of RPTP β/ζ with an active tyrosine phosphatase catalytic site to lysates of cells previously treated with pervanidate sharply reduces levels of tyrosine phosphorylation of β-catenin, it is highly likely that β-catenin is a substrate for the tyrosine phosphatase activity of RPTP β/ζ. Furthermore, because PTN rapidly signals tyrosine phosphorylation of β-catenin in intact U373-MG cells, it is also very likely that inactivation of RPTP β/ζ is directly responsible for the increase in tyrosine phosphorylation of β-catenin, as a result of the disruption of the normal balance of tyrosine kinase and phosphatase activities. We suggest a model in which RPTP β/ζ is intrinsically active and a principal regulator of tyrosine phosphorylation levels of β-catenin. In PTN-stimulated cells, the model envisions that RPTP β/ζ is functionally inactivated, steady-state levels of β-catenin tyrosine phosphorylation and perhaps other downstream signaling molecules are increased, and a PTN-dependent downstream signaling cascade is initiated. We suggest that β-catenin not only is an endogenous substrate for RPTP β/ζ but also a downstream mediator of PTN signaling. To the best of our knowledge, PTN is the first natural soluble ligand to be identified for any of the known transmembrane class (receptor-type) tyrosine phosphatases. The results that we present appear to validate a unique mechanism of receptor signaling, what we now call "ligand-dependent receptor inactivation."

The family of RPTPs are single-chain transmembrane proteins with either one or two intracellular tyrosine phosphatase domains, single transmembrane domains, and variable extracellular domains that contain motifs that are important in cell-cell or cell-matrix adhesion (36, 41, 42). β-Catenin with α- and γ-catenin (plakoglobin) is a major member of the catenin family and is an essential factor in the *wnt/wingless* (33, 34) pathway. In *Drosophila*, *wingless* negatively regulates *Armadillo* (the *Drosophila* homologue of β-catenin) phosphorylation on Ser and Thr residues (33, 34). β-Catenin also is required for hTCF-4 to transactivate transcription in nuclei of APC$-/-$ colon carcinoma cells that contain a stable β-catenin hTCf-4 complex that is constitutively active (35). Whether PTN also signals nuclear translocation and transactivation of genes signaling oncogenic pathways is not known. Cell–cell adhesion requires members of the cadherin–catenin families to link the highly conserved cadherin cytoplasmic domain to the actin-based cytoskeleton and to connect adjacent cells via the cadherin extracellular domains (31, 32, 43). Balsamo *et al.* (44) demonstrated that the association of β-catenin with E-cadherin is inversely related to tyrosine phosphorylation

levels of β-catenin in pervanidate-treated cells, raising the distinct possibility that through its ability to increase tyrosine phosphorylation of β-catenin, PTN disrupts the normal association of β-catenin and E-cadherin, underscoring the need for reciprocal control of tyrosine phosphorylation of β-catenin (31, 45–48). Because constitutive expression of PTN itself transforms cells with striking loss of contact inhibition, cell adhesion, and striking disruption of cytoskeletal architecture, the ability of PTN to disrupt the reciprocal control of tyrosine phosphorylation of β-catenin by tyrosine kinases and phosphatases is likely to be extremely important and may account for many of the properties of PTN-transformed cells and those human cancer cells which constitutively express PTN.

The experiments in this manuscript provide compelling evidence that (*i*) PTN is a soluble ligand for RPTPβ/ζ; (*ii*) PTN signals through its ability to inactivate the intrinsically active protein tyrosine phosphatase of RPTPβ/ζ; and (*iii*) PTN increases levels of tyrosine phosphorylation of the intracellular signaling molecule β-catenin in PTN-stimulated cells. We propose that PTN may modulate a number of downstream biological functions through regulation of tyrosine phosphorylation of β-catenin, including cell adhesion, tumor invasiveness, and metastasis.

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