Physical and Functional Interaction between Receptor-like Protein Tyrosine Phosphatase PCP-2 and β-Catenin†

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ABSTRACT: We have previously identified a human receptor protein tyrosine phosphatase of the MAM domain family, termed PCP-2, in human pancreatic adenocarcinoma cells and found that this protein was colocalized with β-catenin and E-cadherin at cell junctions [Wang, H.-Y., et al. (1996) *Oncogene 12*, 2555−2562]. Its intracellular part consists of two tandem phosphatase domains and a relatively large juxtamembrane region that is homologous to the conserved intracellular domain of cadherins, suggesting a role in the regulation of cell adhesion. This study reports that PCP-2 was endogenously expressed at the cell surface and upregulated with increased cell density. An in vivo binding assay revealed that PCP-2 could directly interact with β-catenin through a region in the juxtamembrane domain. Tyrosine phosphorylation of β-catenin by EGF or active SrcY527F did not disrupt the formation of the PCP-2−β-catenin complex, while PCP-2 in this complex could cause a significant reduction in the phosphorylation level in β-catenin. Finally, we showed that PCP-2 was a negative regulator for cell migration. In conclusion, interaction of PCP-2 with its substrate β-catenin is involved in the process of cell−cell contact.

The regulated phosphorylation of proteins on specific tyrosyl residues is a major mechanism of intracellular signaling that has been demonstrated to be involved in a large set of cellular events, including growth, differentiation, adhesion, and migration. Tyrosine phosphorylation is reversible and dynamic in vivo, and is controlled by the coordinated and competing actions of protein tyrosine kinases (PTKs)[1] that catalyze phosphate transfer and protein tyrosine phosphatases (PTPs) that are responsible for phosphate hydrolysis (*1*, *2*). PTPs are a large family that is broadly classified into receptor-like protein tyrosine phosphatases (RPTPs) and cytosolic PTPs. RPTPs are distinguished by their remarkable structural diversities in ectodomains, and all members possess at least one catalytic domain of approximately 230−280 amino acids containing the highly conserved active site of the consensus motif [I/V]HCXAGXXR[S/T]G (*3*, *4*). A subfamily of RPTPs containing an MAM domain in the ectodomain followed by an immunoglobulin-like (Ig) domain and fibronectin type III (FN-III) repeats is defined as the IIB RPTP family. There are at least four types of IIB phosphatases, i.e., PTPμ, PTPκ, PCP-2, and PTPρ (*5*). The similarities of their ectodomains to cell adhesion molecules (N-CAMs) lead to speculation that these RPTPs might be involved in cell−cell or cell−matrix interaction. To support this, two human RPTPs, PTPμ and PTPκ, have been demonstrated to mediate aggregation via calcium-independent homophilic binding (*6*, *7*). In addition, elevated levels of expression of these proteins significantly correlated with the increased density of cultured cells, and this expression was anticipated to be involved in the process of contact-dependent growth inhibition (*8*, *9*). It is well-known that the contact-based growth inhibition on cells is mainly regulated by a family of calcium-dependent adhesion molecules named cadherins. As a main component of cadherins, E-cadherin constitutes the major cell adhesion system in epithelia, playing a pivotal role in contact inhibition of growth of normal epithelial cells and contributing to the formation of stable adherent junctions (*10*). The function of E-cadherin is controlled via molecules from the cytoplasm, namely, α-, β-, and γ-catenins. β- and γ-catenins bind directly to the intracellular domain of cadherin, whereas α-catenin binds to β- or γ-catenin, thereby linking the cadherin−catenin complex to the cytoskeleton (*11*). Deletion or mutational inactivation of β-catenin has been implicated in the abolition of E-cadherin activity, causing the loss of cell adhesion and tumor invasion (*12*, *13*). Modification of the serine phosphorylation of β-catenin is involved in Wnt signaling (*14*−*17*), while tyrosine phosphorylation of β-catenin has been

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[1] Abbreviations: PTKs, protein tyrosine kinases; PTPs, protein tyrosine phosphatases; RTKs, receptor protein tyrosine kinases; RPTPs, receptor-like protein tyrosine phosphatases; LEF, lymphocyte enhancer factor; TCF, T cell factor; EGF, epidermal growth factor; GST, glutathione *S*-transferase; PBS, phosphate-buffered saline; RIPA, radioimmune precipitation buffer; PAGE, polyacrylamide gel electrophoresis; FBS, fetal bovine serum; NCS, new calf serum; APC, *Adenomatous polyposis coli*; RT, room temperature.

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recently described in association with cell−cell contacts. It has been demonstrated that a large number of tyrosine kinases such as EGFR, Src, and ErbB2 in coordination with RPTPs such as PTPμ, PTPκ, and LAR are involved in the regulation of E-cadherin−β-catenin signaling, suggesting that tyrosine phosphorylation and dephosphorylation of β-catenin play an important role in E-cadherin and β-catenin stabilization (*9*, *18*−*20*). PCP-2, a new type of IIB RPTP we previously cloned from a human pancreatic adenocarcinoma cDNA library, has been shown to colocalize with the E-cadherin−β-catenin complex at areas of cell−cell contact (*1*). We have also demonstrated that the murine homologue of human PCP-2 served as a morphoregulatory molecule involved in embryogenesis (*21*). Structurally, the intracellular juxtamembrane domain of PCP-2 contains a region that is homologous to the conserved intracellular domain of cadherins (*22*). This structural arrangement suggests that PCP-2 directly sends signals in response to cell adhesion. This study reports that PCP-2 is expressed in a cell density-dependent fashion. We showed that PCP-2 directly interacted with β-catenin, and this interaction was independent of its phosphorylation state. We further mapped the regions of PCP-2 for β-catenin binding and discussed the potential signaling pathway that is involved.

EXPERIMENTAL PROCEDURES

Cells and Transfection. BHK-21 cells and COS-7 cells [American Type Culture Collection (ATCC)] were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 10% NCS, respectively. WRL-68 human embryomic liver cells (ATCC) were cultured in MEM containing 10% NCS and PC-12 cells in DMEM with 10% FBS and 5% horse serum. Transient transfection of BHK-21 and COS-7 cells was performed using lipofectamine reagent (GIBICO-BRL) according to the manufacturer's guidelines. Stable transfectants were selected by G418 resistance (GIBICO-BRL). For EGF stimulation, cells were serum-starved overnight and then incubated for 30 min in fresh medium with 100 ng/mL murine EGF (Sigma, catalog no. E1257).

Antibodies. The whole intracellular segment of PCP-2 (CT-Ab) and the fragment encoding amino acids 761−894 (JM-Ab) were subcloned into the fusion protein expression vector pGEX (pharmacia). The fusion protein was purified as described previously (*23*) and used for immunizing rabbits. NT-Ab, representing another polyclonal antiserum against a peptide sequence (residues 40−53) within the extracellular domain of PCP-2, was kindly provided by A. Ullrich. The anti-β-catenin antibody was raised against a His fusion protein containing amino acids 480−781 of β-catenin. The monoclonal antibody against phosphotyrosine (4G10) was obtained from Cell Signaling Technologies. The E-cadherin antibody was obtained from Santa Cruz Biotechnology, Inc. Irrelevant anti-MXR7 (generated by our laboratory) was served as a control antibody.

Preparation of RNA and Northern Blot Analysis. Total RNA was isolated from cultured cells at different densities. RNA samples (20 μg) were fractionated on a 1% formaldehyde/agarose gel by electrophoresis and transferred to nitrocellulose membranes. The blot was hybridized with a ^{32}P-labeled DNA probe corresponding to the sequence encoding the intracellular domain of PCP-2 under high-stringency conditions and exposed to X-ray film for 7 days at −80 °C. The 18S and 28S rRNAs were used to calibrate the relative quantities and quality of loaded RNAs (*24*).

DNA Construct. Different recombinants for full-length or mutant forms of PCP-2 were constructed using the PRK5RS or pcDNA3 vector as described previously (*1*). In vitro mutagenesis of PCP-2 was performed according to the method described by Gebbink et al. (*25*). Oligonucleotide primers used for the introduction of the Cys to Ser mutation in either the first PTP domain (PCP-2C1S) or the second PTP domain (PCP-2C2S) were 5′-CGCTGGAGTGGATGACAATGG-3′ and 5′-CACCATCGTGCACTCCCTAAAC-3′, respectively. The C-terminally truncated construct (PCP-2ΔC2) was generated by deletion of the second catalytic domain through Eam11041 digestion. The second PCP-2 deletion construct that lacks both PTPase domains (PCP-2ΔC1C2) but still contains the complete juxtamembrane region was obtained through Tth111I digestion. The PCP-2-EXT construct that encodes the extracellular domain and transmembrane region plus 12 additional amino acids was obtained by PCR using the primers 5′-GGAATTCGGCGACCTCCAACCAT-3′ and 5′-GCGTCGACGGCCTTGGTCATGTTCAC-3′. All sequences amplified by polymerase chain reaction were verified by DNA sequencing. A pLNCX expression plasmid encoding the active form of p60^{Src} (SrcY527F) was kindly provided by Dr. Liu (Cancer Institute, Chinese Academy of Medical Sciences).

Cell Lysis, Immunoprecipitation, and Immunoblotting. After being washed with PBS, cells were lysed in RIPA buffer [20 mM Tris (pH 7.5), 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM orthovanerdate, 10 μg/mL aprotinin, and 1 mM phenylmethanesulfonyl fluoride], and lysates were precleared by centrifugation at 13000*g* for 15 min at 4 °C. Supernatants with equal amounts of total protein were incubated with appropriate antibodies at 4 °C for 3 h, and with protein A for an additional 3 h. Immunoprecipitates were washed three times with lysis buffer, and beads were resuspended in SDS sample buffer. For subsequent Western blot analysis, immunoprecipitates were separated by SDS−PAGE, transferred to nitrocellulose (Schleicher and Schnell), and incubated with the respective antibodies. The ECL system (Amersham) in conjunction with horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit antibody (Calbiochem) was used to visualize proteins presented on the blot. For the reprobing assay, the blots were stripped by incubation in 68 mM Tris-HCl (pH 6.8), 2% SDS, and 0.1% β-mercaptoethanol at 50 °C for 1 h.

In Vivo Dephosphorylation Assay. In in vivo dephosphorylation assays, BHK-21 cells were transiently transfected with the expression plasmid for β-catenin together with vectors for wild-type PCP-2, PCP-2C1S, or PCP-2C2S. These cells were also cotransfected with the vector for active SrcY527F. Two days after transfection, cells were lysed in RIPA buffer containing phosphatase inhibitors. β-Catenin expressed in these cells was immunoprecipitated with the anti-β-catenin antibody. Total cell lysates were probed with NT-Ab targeted to N-terminal PCP-2. Anti-β-catenin immunoprecipitates were split and probed with 4G10. The stripped blot was then reprobed with anti-β-actin to control for equal loading.

Immunofluorescence. WRL-68 cells grown on coverslips were washed twice in TBS and then fixed with 4% paraformaldehyde for 5 min at room temperature (RT). The cells were permeabilized with 0.2% Triton in TBS (5 min), blocked in 10% normal goat serum, and detected with the primary antibody at 4 °C overnight. Coverslips were washed in TBS and incubated with the FITC-conjugated goat anti-rabbit antibody at RT for 45 min. After being washed in TBS, coverslips were mounted with 90% glycerol in TBS and analyzed with a conventional fluorescence microscope (Olympus IX70).

Migration Assay. For in vitro wound assays, BHK-21 cells were plated at a density of 7×10^4 cells/cm^2. Twenty-four hours later, the confluent monolayer was scratched with a pipet tip to create a cell-free area and incubated in serum-free DMEM for an additional 12 h at 37 °C in a humidified incubator containing 5% CO_2. Cells that had migrated into the wound area were photographed with a light microscope equipped with phase-contrast optics. Cell migration was also quantified using a modified Boyden chamber. The chamber was separated into two compartments (top and bottom) with a polycarbonate membrane (6.5 mm diameter, 8 μm pores, Transwell, Costar, Cambridge, MA) coated with 10 μg/mL laminin. DMEM containing 10% FBS was added in both compartments. BHK-21 cells (5×10^5) were seeded in the top compartment and incubated at 37 °C in a 5% CO_2 atmosphere. Six hours later, cells were stained with Giemsa, and the number of cells that migrated into the bottom compartment was counted and photographed under 400× magnification. Experiments were performed in triplicate.

RESULTS

Analysis of the PCP-2 Gene Product. A number of previous studies have described that various RPTPs, including PTPμ, PTPκ, and LAR, have a regulated, specific proteolytic cleavage that contributes to shedding of the ectodomain (*8*, *9*). To investigate the properties of the PCP-2 gene product, we examined the processing of PCP-2 in WRL-68 cells (Figure 1A) and transiently transfected COS-7 cells (Figure 1B) using immunoprecipitation and Western blot assays. Unexpectedly, we failed to detect any cleavage products. As shown in Figure 1, the NT-Ab antibody precipitated only the 180 kDa protein, and the results were further confirmed in the same way with antibodies against the juxtamembrane domain (JM-Ab) or against the carboxyl terminus (CT-Ab) (data not shown).

Elevated PCP-2 Expression Levels with Increased Cell Density. Several RPTPs such as PTPμ and PTPκ have been shown to be upregulated by cell−cell contact (*8*, *9*). To investigate the possible effect of cell adhesion on PCP-2 expression, WRL-68 cells were harvested at different densities that were grouped as low (sparse cultures with little or no intercellular contacts), medium (near confluence), and high (confluent with maximal intercellular contacts). High-stringency Northern blotting was employed to analyze the expression of PCP-2. As shown in Figure 2A (top), PCP-2 mRNA was significantly upregulated with an increased cell density. An approximately 3-fold higher level of PCP-2 mRNA was found in cells with a high density compared to those with a low density. The amount of 18S and 28S rRNA indicated equal loading of the RNA samples that were not affected by cell−cell contact (Figure 2A, bottom). To confirm the protein level, cells at different densities were collected, lysed, and analyzed on a Western blot with the NT-Ab antibody. The results revealed a more profound elevation of the level of the PCP-2 protein in high-density cells as compared with mRNA level (Figure 2B, top). An equal amount of protein in each loading was indicated by the β-actin level (Figure 2B, bottom). Immunofluorescence experiments revealed that weak and dispersive staining for PCP-2 was observed in cells with fewer intercellular contacts and that intensive PCP-2 staining was seen at cell membrane when cells reached 100% confluency (Figure 2C).

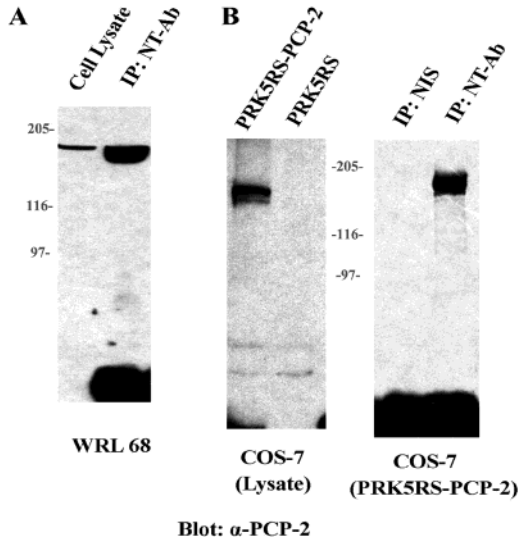

FIGURE 1: No proteolytic cleavage for PCP-2 in either endogenous expressed or overexpressed cells. (A) Confluent WRL-68 cells were collected and lysed in RIPA buffer. Endogenous expression of PCP-2 in these cells was detected on a Western blot using the antibody against a region (residues 40−53) of the extracellular domain of PCP-2 (NT-Ab). (B) COS-7 cells were transiently transfected with a PCP-2 expression vector (PRK5RS-PCP-2) or an empty vector (PRK5RS). Anti-PCP-2 immunoprecipitates from the cell lysates were subjected to Western blot analysis for detection of overexpression of PCP-2 in these cells using the same NT-Ab.

PCP-2 Interacted with β-Catenin, and the Association Was Independent of the Tyrosine Phosphorylation State of β-Catenin. We have previously found that PCP-2 was colocalized with β-catenin and E-cadherin at cell−cell contact (*1*). In this study, we investigated the possible interaction among these proteins through the co-immunoprecipitation experiment. As observed in Figure 3A, PCP-2 could be detected in anti-β-catenin immunoprecipitates from PC-12 cells, though the level was low. However, the level of E-cadherin in these cells was too low for it to be present in the same immunoprecipitates. To come to an in-depth understanding, BHK-21 cells that expressed low levels of β-catenin and E-cadherin were used in transfection experiments. The expression vector for full-length PCP-2 was cotransfected into BHK-21 cells with β-catenin or E-cadherin. Immunoprecipitates from these cells with antibodies of either anti-β-catenin or anti-E-cadherin were split and assayed on a Western blot for association among PCP-2, β-catenin, and E-cadherin (Figure 3B). We confirmed the association between PCP-2 and β-catenin; however, we failed to detect PCP-2 in anti-E-cadherin immunoprecipitates (data not shown). We next tested whether PCP-2−β-catenin interaction was affected by the tyrosine phosphorylation state

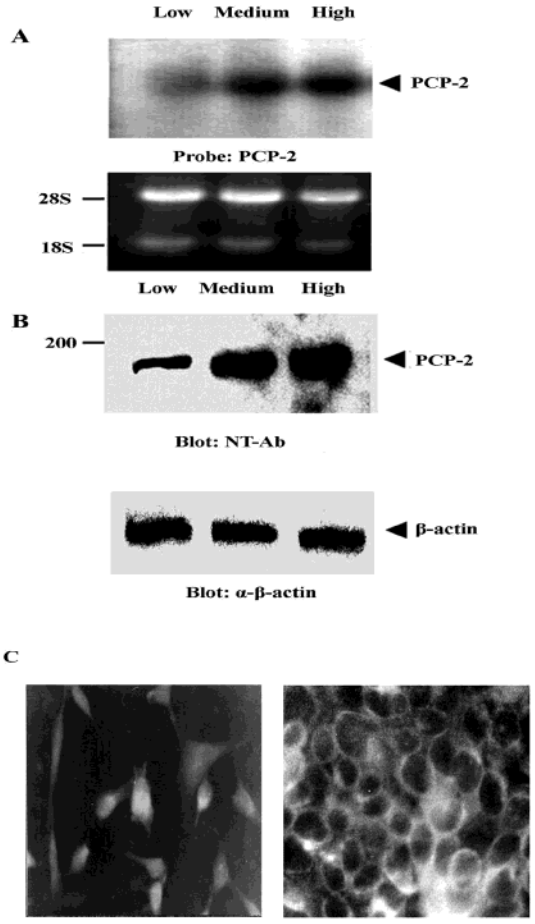

FIGURE 2: PCP-2 expression was upregulated by cell density. (A) The level of PCP-2 mRNA expression increased with cell growth. Total RNAs (20 μg/lane) prepared from WRL-68 cells at different densities (low, medium, and high) were separated by formaldehyde/agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with ^{32}P-labeled cDNA targeted to the intracellular domain of PCP-2 (A, top). 18S and 28S rRNA signals presented the equivalent sample loading (A, bottom). (B) Correlation of the PCP-2 protein expression level with cell density. Lysates (50 μg of total protein) from WRL-68 cells were split and probed on a Western blot with NT-Ab (B, top). To control for an equal amount of loaded protein, the blot was reprobed with the anti-β-actin antibody (B, bottom). (C) In situ detection of PCP-2. Fluorescence analysis showed that PCP-2 expression in WRL-68 cells was stained weakly and dispersely at low cell densities. An increased level of expression of PCP-2 was observed when the cells grew at a high density, and more intensive fluorescence staining for PCP-2 was seen at points of cell−cell contact.

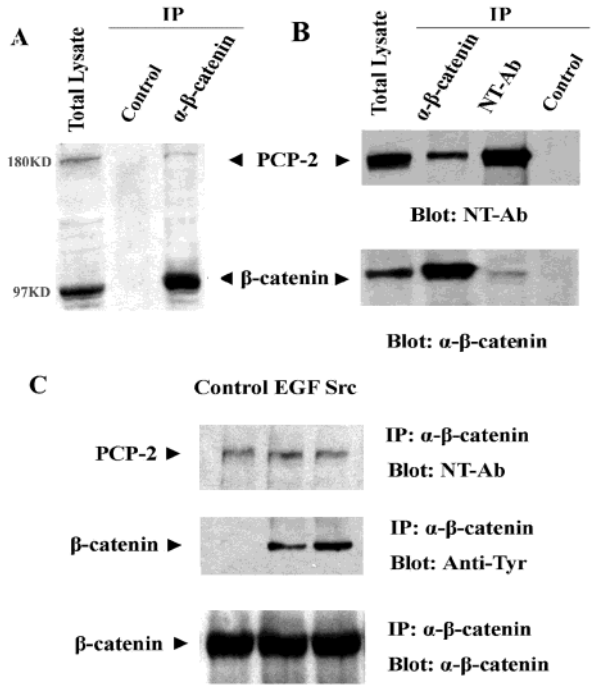

FIGURE 3: Association between PCP-2 and β-catenin. (A) Total lysates as well as anti-β-catenin immunoprecipitates from PC-12 cells were separated via 8% SDS−PAGE for Western blot analysis. As indicated, PCP-2 and β-catenin could be detected in the total lysates (lane 1), and the presence of these two proteins was recognized in β-catenin immunoprecipitates (lane 3). Neither PCP-2 nor β-catenin could be precipitated by the irrelevant antibody (lane 2). (B) Expression vectors for PCP-2 (PRK5RS-PCP-2) and β-catenin (PCDNA3.1-β-catenin) were cotransfected into BHK-21 cells. As indicated, PCP-2 and β-catenin were again co-immunoprecipitated in these cells via the anti-PCP-2 or anti-β-catenin antibody. Total cell lysates for the same detection were also provided (lane 1). (C) To determine if tyrosine phosphorylation of β-catenin disturbed its interaction with PCP-2 in vivo, the SrcY527F expression vector was cotransfected into BHK-21 cells together with β-catenin and PCP-2 (lane 3). In addition, BHK-21 cells cotransfected with β-catenin and PCP-2 without the SrcY527F expression vector were treated with EGF (100 ng/mL) for 30 min after an overnight starvation (lane 2). Tyrosine phosphorylation was analyzed with the anti-Tyr antibody (4G10). As observed, tyrosine phosphorylation in β-catenin was strongly induced by either EGF or Src, but this did not affect its interaction with PCP-2.

of β-catenin. For this purpose, BHK-21 cells were cotransfected with vectors for PCP-2 and β-catenin. To induce tyrosine phosphorylation in β-catenin, BHK-21 cells were treated with EGF or cotransfected with a vector for expression of active SrcY527F. As expected, β-catenin was heavily phosphorylated by either treatment of EGF or transfection with SrcY527F in BHK-21 cells, but this did not disturb the interaction between PCP-2 and β-catenin (Figure 3C).

The Juxtamembrane Domain of PCP-2 Was Sufficient for PCP-2−β-Catenin Interaction. To determine which part of PCP-2 was involved in its interaction with β-catenin, several PCP-2 deletion mutant constructs were cotransfected into BHK-21 cells with the vector for β-catenin. As shown in Figure 4, wild-type PCP-2 as well as its short forms without the second (PCP-2ΔC2) or both PTP domains (PCP-2ΔC1C2) could be detected in anti-β-catenin immunoprecipitates, while a deleted PCP-2 without cytoplasmic termini (PCP-2-EXT) was absent in the same immunoprecipitates. This implied that interaction of PCP-2 with β-catenin was mediated by a region of the juxtamembrane domain in PCP-2, and the process was independent of the catalytic activity of PCP-2.

β-Catenin Was a Substrate of PCP-2 in Vivo. Although PTP domains in PCP-2 were not involved in the interaction of PCP-2 with β-catenin, its catalytic activity for dephosphorylation of β-catenin remained to be determined. For this purpose, we introduced a point mutation (Cys to Ser) into the first PTP domain (PCP-2C1S) or the second PTP domain (PCP-2C2S) of PCP-2. BHK-21 cells were transiently transfected with β-catenin together with SrcY527F and either of the expression vectors for wild-type PCP-2, PCP-2C1S, or PCP-2C2S. The results showed that full-length or mutant PCP-2 could be detected in each transfection, although the level of endogenous expression was very low. β-Catenin expressed in confluent BHK-21 cells was slightly tyrosine phosphorylated in confluent cells and became highly phos-

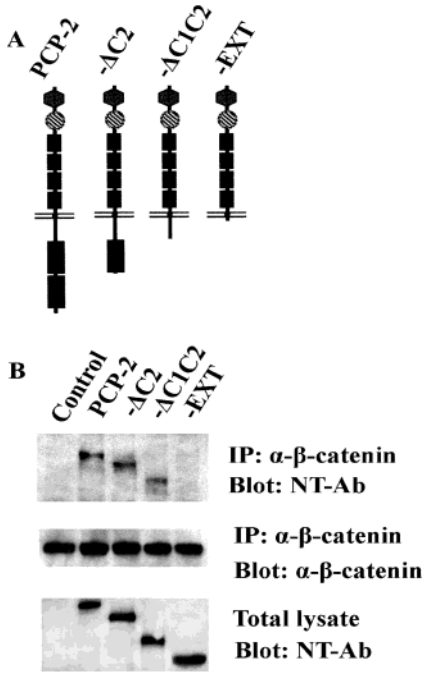

FIGURE 4: Juxtamembrane region of PCP-2 that is essential for β-catenin binding. (A) A schematic presentation of the wild-type and different mutant forms of PCP-2. −ΔC2 indicates a lack of the second PTP domain in PCP-2. −ΔC1C2 indicates both PTP domains are absent. −EXT was a short form of PCP-2 without the cytoplasmic domain. (B) Constructs for wild-type PCP-2 or its mutant forms were cotransfected into BHK-21 cells with β-catenin. Expression of either wild-type PCP-2 or mutant PCP-2 proteins without PTP domains could be detected in every transfection; however, a C-terminally truncated PCP-2 protein was missing in anti-β-catenin immunoprecipitates.

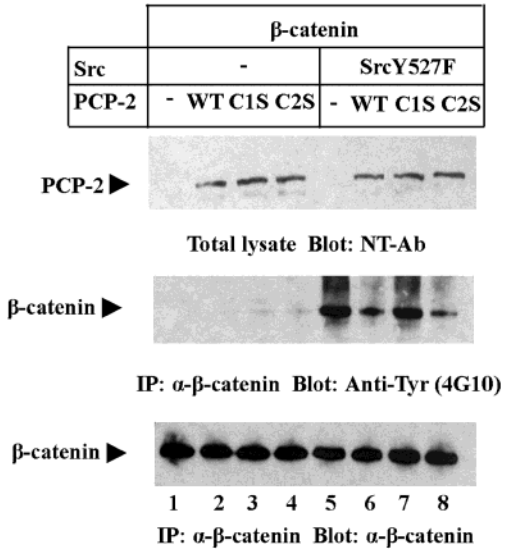

FIGURE 5: Dephosphorylation of β-catenin by PCP-2 in vivo. BHK-21 cells were cotransfected with pcDNA3.1-β-catenin, PLNCX-SrcY527F, and one of the different PCP-2 constructs (WT for wild-type PCP-2, C1S for the Cys to Ser mutation in the first PTP domain, and C2S for the same mutation in the second PTP domain). Empty vectors served as controls. The anti-β-catenin antibody was used for immunoprecipitation as well as for β-catenin dection; tyrosine phosphorylation was assayed with the anti-Tyr mAb (4G10), and PCP-2 expression was monitored with NT-Ab against the PCP-2 ectodomain.

phorylated when the cells were cotransfected with SrcY527F. The tyrosine phosphorylation level of β-catenin was significantly reduced in cells coexpressed with PCP-2 or PCP-2-C2S, but not in cells expressed with the mutant PCP-2C1S, suggesting that β-catenin is a substrate for PCP-2 and the first PTP domain in PCP-2 is responsible for β-catenin dephosphorylation.

PCP-2 Inhibited Cell Migration. We finally investigated if PCP-2 is involved in regulation of cell migration. In an in vitro wound assay, we noticed that BHK-21 cells transfected with PCP-2 migrated more slowly into the wounded cell-free area than the cells transfected with the empty vector. The impaired motility of cells expressing PCP-2 was confirmed in a Boyden chamber assay. As observed in Figure 6, the number of BHK-21 cells that migrated into the bottom chamber 6 h after plating was significantly reduced compared to the vector control. Together, these data suggested that PCP-2 served as a negative regulator for cell migration.

DISCUSSION

Epithelial cell−cell adhesion plays a major role in the establishment and maintenance of cell morphology and cell proliferation. Strong cell−cell junctions within epithelial colonies are maintained primarily by E-cadherin together with its associated intracellular molecules, the catenins. Alteration of cadherin-mediated cell−cell adhesion and cell proliferation is frequently associated with tyrosine phosphorylation of β-catenin (*26*−*28*). The correlation was initially observed in V-Src transformation of cells, leading to the loss of adhesive properties accompanied by tyrosine phosphorylation of β-catenin (*29*). In addition to V-Src, stimulation of EGFR or ErbB-2 has been described in adherent destabilization, loss of cell polarity, and reduced intercellular adhesion through tyrosine phosphorylation of β-catenin (*30*, *31*). Roura and colleagues further confirmed this connection by mapping the phosphorylation site on the Tyr-654 residue of β-catenin (*19*). Tyrosine phosphorylation of β-catenin may perturb its access to E-cadherin or cause its disassociation from the E-caherin−β-catenin complex, hence resulting in unstructured cadherin and promoting migration. Destabilization of adherent junctions is usually correlated with tumor invasion and malignant progression (*32*).

However, the molecular mechanisms underlying the loss of expression or the functionality of individual components of the cadherin−catenin complex is still only partly understood. We previously observed that PCP-2 was colocalized with the E-cadherin−β-catenin complex at areas of cell−cell contact, suggesting that PTPs play a role in the regulation of the stabilization of this complex. Supportingly, several other membrane-associated protein tyrosine phosphatases such as PTPμ, PTPκ, and PTP-LAR have been linked to E-cadherin−β-catenin function at the adherent junction (*9*, *18*, *20*, *33*−*35*). The investigation presented here reported an approximately 3-fold increase in the level of expression of PCP-2 with respect to the mRNA level and a more significant elevation of the protein level when cells grew to be highly intensive, suggesting that PCP-2 was regulated both transcriptionally and posttranscriptionally in a cell density-dependent manner. This expression pattern of PCP-2 may correlate with changes in tyrosine phosphorylation states of β-catenin during the process of cell growth as in the case of PTPκ (*9*, *36*). We confirmed the association between PCP-2 and β-catenin through co-immunoprecipitation. Since the sequences of juxtamembrane regions of RPTPs such as PTPκ

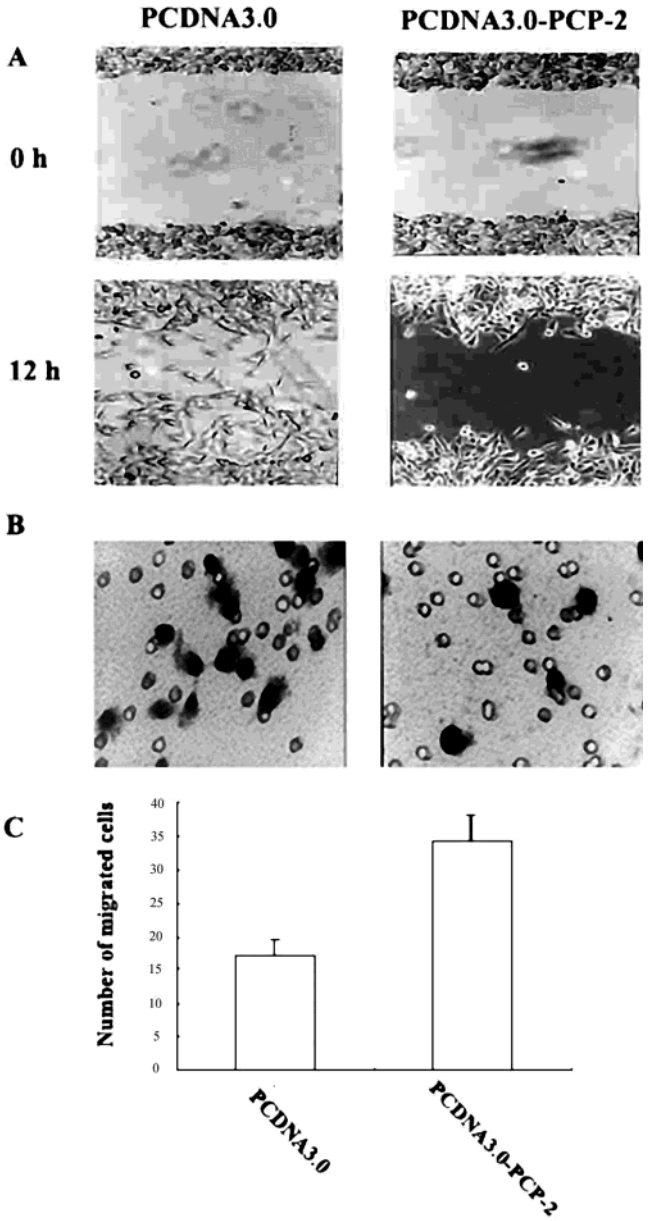

FIGURE 6: Ectopic expression of PCP-2-inhibited cell migration. (A) Monolayers of BHK-21 cells stably transfected with the empty vector PCDNA3.0 (left) or PCP-2 were wounded and maintained in the absence of serum. Cell migration into the wound area was examined at 0 and 12 h with a light microscope equipped with phase-contrast optics (original magnification of 100×). (B) BHK-21 clones with the expression vector for PCP-2 or the empty vector were seeded on porous membranes in Boyden chambers in the absence of a chemoattractant. Six hours later, cells were stained with Giemsa and photographed (original magnification of 400×). (C) Graphical data representing the means ± the standard deviation of the number of migrated cells, each done in triplicate.

and PCP-2 are homologous to that of the intracellular domain of cadherin, it is assumed that PCP-2 is involved in the regulation of β-catenin function through direct interaction. To confirm this, we transfected BHK-21 cells with wild-type or mutant PCP-2 either lacking PTP domains (PCP-2ΔC2 and/or PCP-2ΔC1C2) or the whole intracellular domain, including the juxtamembrane region (PCP-2-EXT). We found that PCP-2 is connected with β-catenin through the juxtamembrane region regardless of its catalytic activity. We further demonstrated that interaction of PCP-2 with β-catenin was not disturbed by EGF or SrcY527F treatment, indicating that this interaction was independent of the tyrosine phophorylation state of β-catenin. Although PTP domains in PCP-2 were not involved in the interaction of PCP-2 with β-catenin, we provided evidence that the active catalytic domain of PCP-2 is essential for its dephosphorylation of β-catenin. Unexpectedly, we failed to detect E-cadherin in the PCP-2−β-catenin complex from BHK-21 cells. We supposed that this phenomenon might due to a low level of E-cadherin expression in the cells that were employed.

Interestingly, cross talk between the E-cadherin−β-catenin signal and the Wnt−β-catenin signal has recently been established. Gottardi and colleagues (*37*) reported that ectopic expression of E-cadherin in the SW480 colorectal tumor cell line significantly inhibits cell growth by binding and antagonizing the nuclear signaling function of β-catenin. Consistently, Stochinger and colleagues found that transient and stable expression of E-cadherin in both epithelial and fibroblastic cells significantly decreased β-catenin activity, causing arrest of cell growth or promoting apoptosis (*38*). Muller and colleagues demonstrated that PTP-LAR plays a role in the regulation of the free pool of signaling β-catenin (*18*), indicating that PTP-LAR may serve as a mediator for this cross talk. In an in vitro wound assay as well as in the Boyden chamber assay, we observed that ectopic over-expression of PCP-2 significantly inhibited cell migration. Similar situations were also found in PTP-LAR and PTPμ (*18*, *39*). It is possible that RPTPs play a role in inhibiting cell migration partly through downregulation of the free pool of signaling β-catenin by a mechanism of cross talk (*18*).

Another interesting finding in our experiment is that no proteolytic cleavage of PCP-2 was found either in intact cells or in transient transfection assays using different truncated constructs. Sequence analysis reveals that the absence of a furin-like cleavage (RLRR) in the fourth fibronectin domain of PCP-2, which is highly conserved in other two members of this receptor family (PTPμ and PTPκ) (*7*, *9*, *40*), may be responsible for the absence of proteolysis. It is assumed that shedding of the extracellular domain may serve to regulate the cell-associated form of the enzyme and thereby modulate growth behavior (*7*); however, the molecular mechanism for different processing on PTPμ, PTPκ, and PCP-2 needs to be further determined.

In summary, we provided here the evidence that PCP-2 plays an important role in the regulation of cell adhesion through direct interaction with β-catenin. The detailed molecular basis for this regulation needs to be further investigated.

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