Normal prostate expresses the receptor protein-tyrosine phosphatase, PTP\(\mu\), whereas LNCaP prostate carcinoma cells do not. PTP\(\mu\) has been shown previously to interact with the E-cadherin complex. LNCaP cells express normal levels of E-cadherin and catenins but do not mediate either PTP\(\mu\)- or E-cadherin-dependent adhesion. Re-expression of PTP\(\mu\) restored cell adhesion to PTP\(\mu\)- and E-cadherin. A mutant form of PTP\(\mu\) that is catalytically inactive was re-expressed, and it also restored adhesion to PTP\(\mu\) and to E-cadherin. Expression of PTP\(\mu\)-extra (which lacks most of the cytoplasmic domain) induced adhesion to PTP\(\mu\) but not to E-cadherin, demonstrating a requirement for the presence of the intracellular domains of PTP\(\mu\) to restore E-cadherin-mediated adhesion. We previously observed a direct interaction between the intracellular domain of PTP\(\mu\) and RACK1, a receptor for activated protein kinase C (PKC). We demonstrate that RACK1 binds to both the catalytically active and inactive mutant form of PTP\(\mu\). In addition, we determined that RACK1 binds to the PKC\(\delta\) isoform in LNCaP cells. We tested whether PKC could be playing a role in the ability of PTP\(\mu\) to restore E-cadherin-dependent adhesion. Activation of PKC reversed the adhesion of PTP\(\mu\)WT-expressing cells to E-cadherin, whereas treatment of parental LNCaP cells with a PKC\(\delta\)-specific inhibitor induced adhesion to E-cadherin. Together, these studies suggest that PTP\(\mu\) regulates the PKC pathway to restore E-cadherin-dependent adhesion via its interaction with RACK1.

A diverse set of cellular behaviors including growth, differentiation, adhesion, and migration are regulated by protein tyrosine phosphorylation. Protein tyrosine kinases and protein-tyrosine phosphatases (PTP\(\mu\))

Expression of the Receptor Protein-tyrosine Phosphatase, PTP\(\mu\), Restores E-cadherin-dependent Adhesion in Human Prostate Carcinoma Cells*

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The abbreviations used are: PTP, protein-tyrosine phosphatase; PKC, protein kinase C; WT, wild type; RPTP, receptor-like PTP; GFP, green fluorescent protein; PBS, phosphate-buffered saline; GST, glutathione S-transferase; Pipes, 1,4-piperazinediethanesulfonic acid; C-S, PTP\(\mu\)C1095S; PMA, phorbol 12-myristate 13-acetate.
N-cadherin-mediated neurite outgrowth (16). In fact, expression of a catalytically inactive form of PTPα perturbed N-cadherin-mediated neurite outgrowth. This demonstrates that the phosphatase activity of PTPα is required for N-cadherin-mediated signal transduction and/or regulation of the cytoskeleton in neurons.

In this study, we employed the LNCaP prostate carcinoma cell line (17) to investigate the role of PTPα in E-cadherin-mediated adhesion. Unlike normal prostate epithelial cells, LNCaP cells do not express endogenous PTPα. Although these cells express the proteins in the cadherin-catenin complex, we found that they did not mediate E-cadherin-dependent adhesion in aggregation assays and in an in vitro adhesion assay. By using a retroviral/tetracycline-repressible system, we re-expressed wild type and mutant forms of PTPα in LNCaP cells and tested their effect on cell adhesion. Our data indicate that the cytoplasmic domain is important for restoring E-cadherin-dependent adhesion regardless of catalytic activity. In a recent paper (18), we isolated RACK1 as a PTPα-interacting protein using a two-hybrid screen. RACK1 is a scaffolding protein that was originally identified as a receptor for activated protein kinase C (19). Because RACK1 binds activated protein kinase C, we tested whether PKC may play a role in the ability of PTPα to regulate E-cadherin-mediated adhesion. Data presented here suggest that the cytoplasmic domain of PTPα regulates E-cadherin-mediated adhesion through modulating PKC via the PTPα/RACK1 interaction.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Reagents—**Monoclonal antibodies against the intracellular (SK7) and extracellular (BK2) domains of PTPα have been described (4, 6). A monoclonal antibody against γ-catenin (5172) was kindly provided by Pamela Cowin (New York University). Monoclonal antibodies against chick L1 (8D9) were generated in our laboratory. Monoclonal antibodies against E-cadherin, p120, α- and β-catenin, and RACK1 were purchased from Transduction Laboratories (Lexington, KY). A monoclonal antibody against vinculin and a monoclonal antibody against the extracellular domain of E-cadherin (D8C3; Sigma) were antibodies against chick L1 (8D9) generated in our laboratory. Goat anti-mouse IgG and IgM immunobeads were obtained from Zymed Laboratories Inc. Laboratories (San Francisco) or goat anti-mouse IgG immunobeads were alternatively obtained from Cappel (Coster Mesa, CA). Normal prostate epithelial cells were purchased from Clonetics (San Diego, CA). LY294002, PMA, rottlerin, chlorethylene chloride, GF109203X, and Go6976 were obtained from Calbiochem. RPMI 1640 medium, SMEM medium, and laminin were obtained from HyClone. Fetal bovine serum was obtained from HyClone (Logan, UT). Tetracycline was obtained from Fisher. All other reagents were obtained from Sigma.

**Construction and Expression of the PTPα Retroviruses—**The retroviral system used is a tetracycline-repressible ("tet-off") promoter-based system (20). By using the pBSTR1 vector generously provided by Dr. Steven Reeves (Harvard Medical School, Charlestown, MA), the following constructs were generated: wild type PTPα, the C-S mutant form of PTPα, and PTPα-extra. The wild type PTPα plasmid (PTPα-WT) and the PTPα-C109FS (C-S) catalytically inactive mutant have been described previously (16). Briefly, the wild type PTPα plasmid contained almost the entire coding sequence of PTPα (base pairs 1–1435), i.e. it only lacked the last two amino acids and the stop codon. This was done to create an in-frame fusion with the green fluorescence protein at the C terminus (PTPα–GFP). The mutant form of PTPα is also GFP-tagged and contains a cysteine to serine mutation at residue 1095. A functional system used is a tetracycline-repressible ("tet-off") promoter-based system (20). By using the pBSTR1 vector generously provided by Dr. Steven Reeves (Harvard Medical School, Charlestown, MA), the following constructs were generated: wild type PTPα, the C-S mutant form of PTPα, and PTPα-extra. The wild type PTPα plasmid (PTPα-WT) and the PTPα-C109FS (C-S) catalytically inactive mutant have been described previously (16). Briefly, the wild type PTPα plasmid contained almost the entire coding sequence of PTPα (base pairs 1–1435), i.e. it only lacked the last two amino acids and the stop codon. This was done to create an in-frame fusion with the green fluorescence protein at the C terminus (PTPα–GFP). The mutant form of PTPα is also GFP-tagged and contains a cysteine to serine mutation at residue 1095. A construct containing the extracellular, the transmembrane, and 55 amino acids of the intracellular domains has been previously described (4). Note: PTPα-extra is not GFP-tagged. This construct was cloned into the tetracycline-regulatable retroviral vector, pBSTR1. Integration-defective amphotrophic retroviruses were made by transfecting the PA317 helper cell line (ATCC CRL-9078) with the respective PTPα-containing plasmids. Control virus was generated by transfecting PA317 helper cells with the pBSTR1 plasmid.

**Expression and Purification of GST Fusion Proteins—**An E-cadherin GST fusion protein construct containing amino acids 9–139 of mouse E-cadherin was obtained from Dr. Robert Brackenbury (University of Cincinnati, Cincinnati, OH). The E-cadherin GST fusion protein was constructed by restriction digest of pBATEM2 with PvuII and HincII. The fragment was ligated into the Small site of pGEX-KG, which results in a fusion protein containing amino acids 9–139 of E-cadherin with GST in the C-terminus. The GST-fusion protein expression of the entire extracellular domain of PTPα has been described previously (4). Expression of GST-tagged proteins in E. coli was induced by isopropyl-1-thio-β-D-galactopyranoside. The bacteria were collected by centrifugation at 3000 x g for 10 min and lyed in PBS containing 1% Triton X-100, 5 µg/ml leupeptin, 5 µg/ml aprotinin, and 1 mM benzamidine. The bacteria were disrupted again at 3000 x g for 10 min to remove debris. The supernatant was passed over glutathione-Sepharose beads (Amersham Biosciences) and washed, and the bound protein was eluted with 10 mM glutathione as described previously (4).
centation of 75 μg/ml for PTPμ and E-cadherin, respectively, and 40 μg/ml for laminin. To identify the individual protein spots on the coverslips, the protein solutions were supplemented with 20 μg/ml Texas Red BSA (Sigma). Three distinct spots, each containing a single adhesion molecule (laminin, E-cadherin, and PTPμ), were generated by spotting 20 μl of each protein solution on one coverslip. After a 20-min incubation at room temperature, the protein solutions were aspirated, and this procedure was repeated once. Remaining binding sites on the nitrocellulose were blocked with 2% BSA in PBS, and the dishes were rinsed with RPMI 1640 medium. LNCaP cells infected with the indicated retrovirus were fully trypsinized with 0.05% trypsin, 0.53 mM EDTA (Invitrogen), and 3 × 10² cells were added to coverslips. The cells were allowed to adhere overnight to regenerate cell surface proteins. In the control experiments, function-blocking antibodies to either PTPμ (BK2, 10 μg/ml ascites) or E-cadherin (DECMA, 1: dilution of culture supernatant), or 5 mM EDTA (final concentration) was added to the dishes just prior to the addition of cells. In some experiments, the overnight incubation was followed by a 15-min incubation with 20 mM PMA or an equal volume of Me 2SO. Alternatively, uninfected LNCaP cells were added to coverslips and incubated overnight followed by a 45-min incubation with either 5 μM rottlerin, 10 μM chelerythrine chloride, 0.5 μM GF109203X, 15 mM G68976, 10 μM LY294002, or ME SO alone. At the concentrations used, chelerythrine chloride (IC50 = 0.66 μM) and GF109203X (IC50 ranges between 8 and 5.5 μM for different isoforms of PKC) are specific for PKC, whereas rottlerin is specific for PKCδ (IC50 = 6 μM), and G68976 is specific for PKCα and β (IC50 = 2.3 and 6 μM, respectively). LY294002 inhibits the phosphatidylinositol 3-kinase (IC50 = 1.4 μM). The medium was then removed, and the coverslips were rinsed once in PBS to remove unattached cells. The cells were subsequently fixed with 4% paraformaldehyde, 0.01% glutaraldehyde in PEM buffer (80 mM Pipes, 5 mM EGTA, 1 mM MgCl2, 3% glycerol, 0.1% aqueous glycerol, 0.05% SDS-PAGE, transferred to nitrocellulose, and immunoblotted with monoclonal antibodies to PTPμ. To verify that protein expression is under tetracycline control, 4 μg/ml tetracycline was added daily to cells infected with retrovirus containing PTPμ.WT (WT), a mutant form of PTPμ containing a single point mutation in the catalytic site (C-S), or a construct containing the extracellular, transmembrane, and 55 amino acids of the intracellular domains of PTPμ (Extra). To assess the functional role of PTPμ catalytic activity in the regulation of E-cadherin-mediated adhesion, we have generated tetracycline-repressible retrovirus encoding a mutant form of PTPμ-GFP containing a single amino acid mutation in the catalytic site (16). Mutation of the conserved cysteine residue PTPμ.C1095S (C-S) results in a catalytically inactive enzyme. Immunoblot analysis showed that the C-S mutant was expressed at a similar level to PTPμWT in LNCaP cells (Fig. 1a, C-S). Fluorescence microscopy confirmed that infection with the C-S mutant (Fig. 2, G and H) resulted in expression at the plasma membrane at a similar level as PTPμWT, demonstrating that the expression and subcellular localization are not affected by the loss of catalytic activity.

Re-expression of PTPμ—The receptor tyrosine phosphatase PTPμ has been shown previously to interact with E-cadherin in a variety of tissues by immunoprecipitation (13–15). To investigate whether PTPμ plays a functional role in E-cadherin-mediated adhesion, we employed the LNCaP prostate carcinoma cell line (17). Unlike normal prostate epithelial cells (NPr), these cells do not express PTPμ (Fig. 1a, VEC). To re-express PTPμ in LNCaP cells, we generated a tetracycline-regulatable retrovirus encoding the PTPμ cDNA sequence tagged with the green fluorescence protein (PTPμ-GFP) (16). By using this retrovirus, we re-expressed wild type PTPμ (PTPμWT) in the LNCaP cells. Five days after retroviral infection, the cells were analyzed for expression of PTPμWT-GFP by immunoblot and by fluorescence microscopy. Immunoblot analysis showed that LNCaP cells infected with retrovirus containing an empty vector do not express PTPμ (Fig. 1a, VEC). Cells infected with retrovirus containing PTPμWT (Fig. 1a, WT) expressed both the full-length protein (200 kDa) as well as the proteolytically processed forms (~100 kDa) (6). Due to the GFP tag, both the full-length and the proteolytically processed form of the re-expressed PTPμWT migrated at a higher molecular weight than the PTPμ expressed in the normal prostate cells (Fig. 1a, NPr). The retroviral system we used is a tet-off system, and the number of adherent cells per imaging area was calculated. The data obtained in 4–6 separate experiments were analyzed by Student’s t test (Statview 4.51, Abacus Concepts, Inc.).

RESULTS

Re-expression of PTPμ—The receptor tyrosine phosphatase PTPμ has been shown previously to interact with E-cadherin in a variety of tissues by immunoprecipitation (13–15). To investigate whether PTPμ plays a functional role in E-cadherin-mediated adhesion, we employed the LNCaP prostate carcinoma cell line (17). Unlike normal prostate epithelial cells (NPr), these cells do not express PTPμ (Fig. 1a, VEC). To re-express PTPμ in LNCaP cells, we generated a tetracycline-regulatable retrovirus encoding the PTPμ cDNA sequence tagged with the green fluorescence protein (PTPμ-GFP) (16). By using this retrovirus, we re-expressed wild type PTPμ (PTPμWT) in the LNCaP cells. Five days after retroviral infection, the cells were analyzed for expression of PTPμWT-GFP by immunoblot and by fluorescence microscopy. Immunoblot analysis showed that LNCaP cells infected with retrovirus containing an empty vector do not express PTPμ (Fig. 1a, VEC). Cells infected with retrovirus containing PTPμWT (Fig. 1a, WT) expressed both the full-length protein (200 kDa) as well as the proteolytically processed forms (~100 kDa) (6). Due to the GFP tag, both the full-length and the proteolytically processed form of the re-expressed PTPμWT migrated at a higher molecular weight than the PTPμ expressed in normal prostate cells (Fig. 1a, NPr). The retroviral system we used is a tet-off system, and the presence of tetracycline the gene is not expressed. The re-expression of PTPμWT was inhibited by treating the cells with tetracycline (Fig. 1a, WT+T). Fluorescence microscopy revealed that between 70 and 90% of the LNCaP cells expressed PTPμWT and that PTPμ was primarily localized to the plasma membrane as expected (Fig. 2, C and D). This expression was repressed when the cells were grown in the presence of 4 μg/ml tetracycline (Fig. 2, E and F). Control cells infected with a virus containing an empty vector did not show any fluorescence (Fig. 2, A and B).

To assess the functional role of PTPμ catalytic activity in the regulation of E-cadherin-mediated adhesion, we have generated tetracycline-repressible retrovirus encoding a mutant form of PTPμ-GFP containing a single amino acid mutation in the catalytic site (16). Mutation of the conserved cysteine residue PTPμ.C1095S (C-S) results in a catalytically inactive enzyme. Immunoblot analysis showed that the C-S mutant was expressed at a similar level to PTPμWT in LNCaP cells (Fig. 1a, C-S). Fluorescence microscopy confirmed that infection with the C-S mutant (Fig. 2, G and H) resulted in expression at the plasma membrane at a similar level as PTPμWT, demonstrating that the expression and subcellular localization are not affected by the loss of catalytic activity.

Re-expression of PTPμ—Enhanced Calcium-dependent Aggregation of LNCaP Cells—To investigate whether PTPμ plays a role in E-cadherin-mediated adhesion in LNCaP cells, we trypsinized the cells in the presence of CaCl2 to selectively preserve the cadherins (22). This assay only measures calcium-dependent aggregation predominantly mediated by the cadherins (22). The cells were then allowed to aggregate for 1 h. LNCaP cells infected with an empty vector weakly aggregated (27.4%). Re-expression of PTPμWT increased the aggregation 3-fold (72.4%), as did expression of the C-S mutant (72.5%). The increased aggregation was Ca2+-dependent, because the presence of EDTA reduced the aggregation to a level below that seen in cells infected with an empty vector (10.8%). The residual Ca2+-dependent adhesion is at least due in part to the fact that LNCaP cells express N-cadherin (data not shown). Taken together, these findings demonstrate that re-expression of PTPμ in LNCaP cells induced Ca2+-dependent aggregation that is partly because of E-cadherin-dependent cell-cell adhe-
purified PTP\textsubscript{H9262} cells do not express PTP forms of PTP shown.

C-S point mutation in the catalytic site (assays (Fig. 3)).

Following infection, the expression of GFP-tagged proteins was visualized by fluorescence microscopy (×128 magnification). Representative phase contrast (A, C, E, and G) and fluorescent (B, D, F, and H) images are shown.

Re-expression of PTP\textsubscript{H9262}—To study the specific interactions between cell-cell adhesion molecules in LNCaP cells, we developed an in vitro adhesion assay where purified, recombinant proteins were immobilized on nitrocellulose-coated coverslips. Basically, three spots of protein (laminin, E-cadherin, and PTP\textsubscript{H9262}) were added to each nitrocellulose-coated coverslip. The field shown in each panel represents virtually the entire spot for a given adhesion molecule. PTP\textsubscript{H9262} has been shown to mediate cell-cell adhesion via homophilic binding (4, 5). To verify that the re-expressed forms of PTP\textsubscript{H9262} were able to mediate homophilic binding in LNCaP cells, we investigated the adhesion of LNCaP cells to purified recombinant PTP\textsubscript{H9262} that was immobilized on nitrocellulose-coated coverslips. As expected, cells infected with an empty vector did not adhere to PTP\textsubscript{H9262} (Fig. 3A) because these cells do not express PTP\textsubscript{H9262}. Re-expression of PTP\textsubscript{H9262}WT induced LNCaP adhesion to purified PTP\textsubscript{H9262} (Fig. 3D), as did re-expression of the C-S mutant (Fig. 3G). Quantitation of the adhesion assays (n = 6) showed that the number of cells that adhered to purified PTP\textsubscript{H9262} was significantly higher for cells infected with both the WT and the C-S mutant form of PTP\textsubscript{H9262} as compared with cells infected with vector only (Table I). However, there was no difference between cells expressing PTP\textsubscript{H9262}WT compared with the C-S mutant in their ability to adhere to purified PTP\textsubscript{H9262} (Table I). To ensure the specificity of the adhesion assay, we repeated the experiments in the presence of function-blocking antibodies to either PTP\textsubscript{H9262} or E-cadherin. The presence of an antibody to the extracellular domains of PTP\textsubscript{H9262} specifically inhibited the adhesion to recombinant PTP\textsubscript{H9262} of LNCaP cells re-expressing PTP\textsubscript{H9262}WT (Fig. 4A, WT+PTP\textsubscript{H9262} Ab) or cells expressing the C-S mutant (Fig. 4A, C-S+PTP\textsubscript{H9262} Ab). As expected, the presence of the E-cadherin antibody had no effect on adhesion to PTP\textsubscript{H9262} (Fig. 4A, WT+E-ca. Ab, C-S+E-cad Ab). Taken together, these data confirm that the re-expressed PTP\textsubscript{H9262} is present at the cell surface and capable of mediating homophilic binding. In addition, PTP\textsubscript{H9262} phosphatase activity is not necessary for PTP\textsubscript{H9262}-dependent adhesion to occur as demonstrated previously (4).

As an internal control in each experiment, cells were allowed to adhere to laminin. Adhesion to extracellular matrix proteins such as laminin is mediated through integrin receptors. Because there is no evidence indicating that PTP\textsubscript{H9262} regulates integrin function, LNCaP adhesion to laminin should not be affected by the re-expression of PTP\textsubscript{H9262}. As expected, LNCaP cells infected with an empty vector adhered to laminin (Fig. 3B), and this adhesion was not significantly affected by re-expression of either WT (Fig. 3E) or C-S mutant forms of PTP\textsubscript{H9262} (Fig. 3H and Table I). None of the retrovirally infected cells adhered to nitrocellulose coated with BSA only (data not shown).

Re-expression of PTP\textsubscript{H9262} Restores E-cadherin-mediated Adhesion—To study the role of PTP\textsubscript{H9262} in the regulation of E-cadherin-mediated adhesion in LNCaP cells, we immobilized purified recombinant E-cadherin on the nitrocellulose-coated coverslips. Despite the fact that these cells express E-cadherin as well as α\textsubscript{v}, β\textsubscript{3}, and γ\textsubscript{catenin} and p120 (Fig. 5), LNCaP cells infected with an empty vector did not adhere to E-cadherin (Fig. 3C). Re-expression of PTP\textsubscript{H9262}WT restored the ability of LNCaP cells to adhere to E-cadherin (Fig. 3F). Quantitation of the adhesion assays show that the number of cells infected with PTP\textsubscript{H9262}WT that adhered to E-cadherin was significantly higher than the number of cells infected with vector only (Table I). These data show that expression of PTP\textsubscript{H9262} is necessary for E-cadherin-mediated adhesion in LNCaP cells.
PTP\textsubscript{\mu} Restores E-cadherin-dependent Adhesion

The data shown in Figs. 4 and 7a are presented as mean number of adherent cells, (± S.E.) Six independent adhesion assays (from six different experiments) were averaged to produce the mean number of adherent cells. For each substrate, Student’s \textit{t} test was used to compare the number of cells infected with PTP\textsubscript{\mu} with the number of adherent cells infected with an empty vector.

| Virus type | PTP | Laminin | E-cadherin |
|------------|-----|---------|------------|
| Vector     |     |         |            |
| PTP WTGFP  | 95.5–45.7 | 1483.4–368.2 | 132.7–62.8 |
| C1095S     | 1022.5–192.0 | 1391.8–192.7 | 1561.5–350.0 |
| PTP-extra  | 1102.0–240.1 | 1736.2–463.0 | 1117.8–210.9 |

\( p \) values were obtained by Student’s \textit{t} test, 99% confidence interval.

![Image](340x352 to 522x543)

**Fig. 4. Specificity of the adhesion assay.** LNCaP cells were infected with retrovirus containing an empty vector (VEC), PTP\textsubscript{\mu} WT (WT), a catalytically inactive mutant form of PTP\textsubscript{\mu} (C-S), or PTP\textsubscript{\mu}-extra (Extra). Five days after infection, the cells were incubated with coverslips spotted with purified recombinant PTP\textsubscript{\mu} (A) or E-cadherin (B) in the presence of either a PTP\textsubscript{\mu} antibody (BK2), an E-cadherin antibody (DECMA), 5 mM EDTA, or were left untreated. Adherent cells were fixed after an overnight incubation and were visualized by darkfield microscopy and photographed using a 35-mm camera. The 35-mm negatives from each experiment were scanned, and the digitized images were analyzed using the Metamorph image analysis program. To measure the number of adherent cells per image, the cells were highlighted using the threshold function, and the total number of adherent cells per image was calculated. The data is presented as mean ± S.E.

Because tyrosine phosphorylation has been reported to negatively regulate cadherin-mediated adhesion, we investigated whether PTP\textsubscript{\mu} restored E-cadherin-mediated adhesion by dephosphorylating key components of the cadherin-catenin complex. To do this, we repeated the adhesion assays with cells expressing the C-S mutant form of PTP\textsubscript{\mu}. Expression of the C-S mutant restored E-cadherin-mediated adhesion (Fig. 3b). As shown in Table I, re-expression of WT or the C-S mutant form of PTP\textsubscript{\mu} induced a significant increase in adhesion to E-cadherin as compared with LNCaP cells infected with an empty vector. In contrast, there was no difference in adhesion between cells infected with PTP\textsubscript{\mu} WT compared with cells infected with the C-S mutant. The statistical analysis for LNCaP cell adhesion to E-cadherin is summarized in Table I. In control experiments, the adhesion to E-cadherin was totally blocked by the function-blocking antibody to E-cadherin (Fig. 4b, WT+E-cad Ab and C-S+E-cad Ab, respectively). In contrast, the PTP\textsubscript{\mu} antibody did not affect the adhesion to E-cadherin induced by the re-expression of PTP\textsubscript{\mu} WT (Fig. 4b, WT+PTP\textsubscript{\mu} Ab) or by the expression of the C-S mutant (Fig. 4b, C-S+PTP\textsubscript{\mu} Ab) as expected. E-cadherin-mediated adhesion in this assay is Ca\textsuperscript{2+}-dependent, and addition of 5 mM EDTA abolished adhesion to E-cadherin (Fig. 4b, WT+EDTA and C-S+EDTA, respectively). However, the presence of EDTA did not affect the adhesion to PTP\textsubscript{\mu}, which is calcium-independent (4), of cells either re-expressing PTP\textsubscript{\mu}-WT (Fig. 4a, WT+EDTA) or expressing the C-S mutant (Fig. 4a, C-S+EDTA). Taken together, these data
indicate that although the presence of the PTP\(\mu\) protein is required for E-cadherin-mediated adhesion in LNCaP cells, it does not require PTP\(\mu\) catalytic activity.

It is possible that the PTP\(\mu\) intracellular domain may recruit other proteins that aid in restoring E-cadherin-mediated adhesion. To determine whether the intracellular PTP domains of PTP\(\mu\) were required to affect E-cadherin-dependent adhesion, we constructed a retrovirus encoding the extracellular, transmembrane, and 55 amino acids of the intracellular domains of PTP\(\mu\) (PTP\(\mu\)-extra) (4). Western blot analysis confirmed that this construct was expressed in LNCaP cells (Fig. 1b, Extra). The cytoplasmic domain of PTP\(\mu\) is known to bind to E-cadherin (13). Immunoprecipitation experiments confirmed that PTP\(\mu\)-extra does not associate with E-cadherin (data not shown). Expression of PTP\(\mu\)-extra induced LNCaP adhesion to purified recombinant PTP\(\mu\) (Fig. 3f, Table I), confirming that the intracellular domains are not required for PTP\(\mu\) to mediate homophilic binding (4). In addition, adhesion to PTP\(\mu\) was blocked by an antibody to PTP\(\mu\) (Fig. 4a, Extra + PTP\(\mu\)Ab). The antibody to E-cadherin and 5 mM EDTA had no major effect on the adhesion to PTP\(\mu\) (Fig. 4a, Extra + E-cad Ab and Extra + EDTA, respectively) as expected. However, PTP\(\mu\)-extra did not restore LNCaP adhesion to recombinant E-cadherin (Fig. 3L), demonstrating that the intracellular domains of PTP\(\mu\) are necessary for restoring E-cadherin-mediated adhesion. Because LNCaP cells expressing PTP\(\mu\)-extra did not adhere to E-cadherin (Fig. 3L, 4b, Extra), the presence of either the PTP\(\mu\) antibody, the E-cadherin antibody, or 5 mM EDTA had no effect on adhesion to E-cadherin (Fig. 4b, Extra + PTP\(\mu\)Ab, Extra + E-cad Ab, and Extra + EDTA, respectively). As expected, the expression of PTP\(\mu\)-extra did not affect LNCaP adhesion to laminin (Fig. 3K). Together, these results suggest that PTP\(\mu\)-extra is expressed at the cell surface and capable of inducing adhesion to PTP\(\mu\) but not restoring E-cadherin-dependent adhesion.

Similar to the results shown in Fig. 3, LNCaP cells expressing an empty vector did not adhere to either PTP\(\mu\) or E-cadherin (Fig. 4a, VEC, and Fig. 4b, VEC, respectively), and this was not altered by the presence of either the PTP\(\mu\) antibody, the E-cadherin antibody, or 5 mM EDTA (data not shown). Taken together, these experiments demonstrate that this in vitro adhesion assay can be used to study specific binding to cell-cell adhesion molecules.

**Expression of Cadherins and Catenins**—Cadherin-mediated cell-cell adhesion is dependent on the expression of both cadherins and catenins. Immunoblot analysis demonstrated that LNCaP cells expressed E-cadherin as well as \(\alpha\), \(\beta\), and \(\gamma\)-catenin and p120 (Fig. 5, lysate). This is in accordance with normal prostate epithelial cells, which were found to express similar amounts of E-cadherin as well as \(\alpha\)-, \(\beta\)-, and \(\gamma\)-catenin (data not shown). Infection of LNCaP cells with an empty vector, PTP\(\mu\)WT, or the C-S mutant form of PTP\(\mu\) did not alter the expression of any of the proteins in the cadherin-catenin complex (Fig. 5). It is possible that re-expression of wild type or mutant forms of PTP\(\mu\) may alter the subcellular localization of the proteins in the cadherin-catenin complex, thereby altering the function of the complex. To address this question, we performed immunocytochemical analysis on LNCaP cells using antibodies to E-cadherin as well as \(\alpha\)-, \(\beta\)-, and \(\gamma\)-catenin and p120. However, re-expression of either PTP\(\mu\)WT or the C-S mutant did not significantly alter the subcellular localization of any of the proteins examined (data not shown).

**PTP\(\mu\) Does Not Alter the Association of \(\alpha\)-, \(\beta\)-, \(\gamma\)-Catenin or p120 to E-cadherin**—To examine the possibility that the presence of PTP\(\mu\) affects the binding of \(\alpha\)-, \(\beta\)-, and \(\gamma\)-catenin or p120 to E-cadherin, we immunoprecipitated E-cadherin from cells infected with an empty vector (VEC), PTP\(\mu\)WT (WT), a catalytically inactive mutant form of PTP\(\mu\) (C-S), or PTP\(\mu\)-extra (Extra). As shown in Fig. 5, the immunoprecipitates from cells infected with an empty vector, PTP\(\mu\)WT, as well as the C-S and PTP\(\mu\)-extra contained equal amounts of E-cadherin. The immunoblot was stripped and reprobed with antibodies to the catenins. Immunoprecipitates from cells infected with an empty vector contained \(\alpha\)-, \(\beta\)-, and \(\gamma\)-catenin as well as p120. Infection of cells with various forms of PTP\(\mu\) did not significantly alter the amounts of the catenins that co-immunoprecipitated with E-cadherin. As a control, a monoclonal antibody to cluck L1 (SD9) was used. This antibody did not immunoprecipitate either E-cadherin or any of the catenins.

**The PTP\(\mu\) Cytoplasmic Domain, Regardless of Catalytic Activity, Is Required for the Interaction with RACK1**—Even though the presence of PTP\(\mu\) does not alter the composition of the E-cadherin-catenin complex, it is possible that full-length PTP\(\mu\) regulates E-cadherin-dependent adhesion by recruiting other signaling molecules to the cadherin-catenin complex. In a recent paper (18), we demonstrated an interaction between the membrane-proximal phosphatase domain of PTP\(\mu\) and RACK1, a receptor for activated PKC (19). Because RACK1 binds to the catalytic domain of PTP\(\mu\), we tested whether catalytic activity of PTP\(\mu\) was required to interact with RACK1. We performed immunoprecipitation with antibodies directed against PTP\(\mu\) or RACK1 and subjected the immunoprecipitates to SDS-PAGE and immunoblotted the gels with anti-RACK1 antibodies. Immunoprecipitation of RACK1 showed that LNCaP cells infected with an empty vector expressed RACK1 (Fig. 6a, VEC) and that infection of cells with various forms of PTP\(\mu\) did not alter the expression of RACK1 (Fig. 6a, WT, C-S, and E, respectively). To investigate whether PTP\(\mu\)WT and the C-S mutant associate with RACK1 in LNCaP cells, we immunoprecipitated PTP\(\mu\) using an antibody to the extracellular domain of PTP\(\mu\) (BK2). RACK1 was found to associate with both PTP\(\mu\)WT (Fig. 6b, WT) and the C-S mutant (Fig. 6b, C-S) but not with PTP\(\mu\)-extra (Fig. 6b, E). As expected, PTP\(\mu\) antibody did not immunoprecipitate RACK1 from cells infected with an empty vector (Fig. 6b, VEC). This experiment was repeated with an antibody to the intracellular do-
PKC with RACK1. PMA on LNCaP adhesion are shown in Table I.

from E-cadherin but not from PTP

the cells were added to coverslips spotted with purified recombinant

LNCaP cells to adhere to E-cadherin. Uninfected LNCaP cells

involved in restoring E-cadherin-mediated adhesion, we stud-

co-immunoprecipitated RACK1 from cells infected with

infected LNCaP cells were incubated overnight with coverslips spotted

infected with retrovirus containing PTP

E-cadherin-mediated adhesion. LNCaP cells infected with ret-

co-immunoprecipitated RACK1 from cells infected with

an empty vector. Taken together, these data demonstrate that

regulates N-cadherin-mediated neurite out-

DISCUSSION

Alterations in the function of the E-cadherin/catenin adhe-

membrane of PTP\(\mu\) (SK7). As seen in Fig. 6c, the SK7 antibody also

cell surface at a level that could mediate homophilic binding. These results also

show that perturbation of the phosphatase activity did not alter the subcellular localization or the ability of PTP\(\mu\) to

mediate homophilic binding as expected (4).

Although LNCaP cells express E-cadherin, as well as \(\alpha\), \(\beta\),

and \(\gamma\)-catenin and p120, they were unable to mediate E-cadherin-

dependent adhesion. Re-expression of PTP\(\mu\)WT restored this adhesion, demonstrating a functional role for PTP\(\mu\) in E-cadherin-

mediated adhesion. The fact that the re-expression of the cata-

lytically inactive mutant did not restore E-cadherin-mediat-
ated adhesion indicates that PTPµ exerts an effect on E-cadherin-dependent adhesion that is independent of its catalytic activity. This process requires the presence of the intracellular domain, because expression of PTPµ-extra failed to restore E-cadherin-mediated adhesion. It is possible that PTPµ alters the cadherin function by recruiting some signaling protein(s) to the cadherin complex through protein-protein interactions involving the PTPµ intracellular domain.

The interaction between RPTPs and various proteins may serve to regulate either the subcellular localization of RPTPs or to recruit other signaling molecules to form a larger signaling complex. The fact that PTPµ, regardless of its catalytic activity, could restore E-cadherin-mediated adhesion suggests that part of its role in the cadherin complex is to recruit other signaling molecules that may be needed for functional E-cadherin-dependent adhesion. The importance of the intracellular domain of PTPµ is clearly demonstrated by the finding that LNCaP adhesion to E-cadherin was not restored by the expression of a construct where the majority of the intracellular domain of PTPµ had been deleted (PTPµ-extra). In this regard, we isolated RACK1 as a protein that binds to the first phosphatase domain of PTPµ in a two-hybrid screen (18). RACK1 is a homologue of the G68 subunit of heterotrimeric G-proteins (19) and consists of seven WD repeats that are believed to form a propeller-like structure (28). RACK1 is thought to be a scaffolding molecule because each of the seven WD repeats could potentially mediate protein-protein interactions. RACK-1 was originally described as a receptor for activated PKC (19), but more recent studies have described its interaction with a variety of signaling proteins, such as Src (29), and with select pleckstrin homology domains in vitro (30). In this study, we found that full-length PTPµ interacts with RACK1 and that this interaction is not dependent upon the catalytic activity of PTPµ. The interaction between PTPµ and RACK1 suggests that PTPµ may regulate E-cadherin-mediated adhesion by recruiting RACK1 and other signaling molecules to the PTPµ adhesion complex.

Despite numerous attempts to clarify the regulation of cadherin function by tyrosine phosphorylation, it is not fully understood. Tyrosine phosphorylation has been correlated with loss of cadherin-mediated adhesion and destabilization of adherens junctions (reviewed in Ref. 2). Therefore, adhesive function may be controlled by reversible tyrosine phosphorylation. Components of the cadherin-catenin complex are phosphorylated by a number of cytoplasmic and receptor protein tyrosine kinases including Src, EGF receptor, and Met (the scatter factor receptor) (2). In addition, PTPµ and a few other PTPs interact with cadherins and catenins (2). The association of the cadherins with both kinases and phosphatases indicates a critical role for dynamic tyrosine phosphorylation in cadherin function.

We performed studies on the role of tyrosine phosphorylation in regulating the association between PTPµ and E-cadherin in cells transformed with a temperature-sensitive form of the Rous sarcoma virus (14). The mutant Rous sarcoma virus is temperature-sensitive for pp60<sup>src</sup> tyrosine kinase activity. When grown at the permissive temperature, increased tyrosine phosphorylation induced by Src resulted in an increased tyrosine phosphorylation of E-cadherin, which correlated with a decreased association between PTPµ and E-cadherin. However, in this study we show that PTPµ regulates the cadherin function independently of its phosphatase activity, indicating that the cadherin-catenin complex may not be the primary substrates for PTPµ. We have shown previously that PTPµ catalytic activity is required for N-cadherin-mediated neurite outgrowth (16). These data indicate that PTPµ catalytic activity may be required for signaling events that regulate the cytoskeleton and thus other cadherin-dependent functions downstream of adhesion.

An alternative hypothesis is that the C-S mutant form of PTPµ may indirectly alter the tyrosine phosphorylation of the cadherin-catenin complex. In a recent paper (18), we found that PTPµ and Src compete for binding to RACK1. RACK1 binds to the SH2 domain of Src, an interaction that inhibits Src kinase activity (29). The interaction between RACK1 and PTPµ may regulate the presence of the Src protein tyrosine kinase in the cadherin-catenin complex. The presence of the PTPµ protein could recruit RACK1 to the plasma membrane where it could dissociate from PTPµ and possibly bind to and inactivate Src. Inactivation of Src could indirectly regulate the tyrosine phosphorylation of either E-cadherin or the catenins, thereby restoring E-cadherin-mediated adhesion.

Several studies have indicated that PKC is involved in the regulation of E-cadherin-mediated adhesion and the formation of adherens junctions. The molecular mechanisms whereby PKC regulates E-cadherin function are unknown. Additionally, the activation of PKC has been reported to have the opposite effects on E-cadherin function in different cell types. For example, the calcium-induced formation of adherens junctions in keratinocytes is dependent on the activation of PKC (24). On the other hand, activation of PKC has been shown to induce the dissociation of E-cadherin from the cytoskeleton (25), followed by cell scattering in the HT29 intestinal cell line (26). In this study, we show that the inhibition of PKC<sup>δ</sup> restored E-cadherin function in LNCaP cells. In addition, PTPµ restored E-cadherin-dependent adhesion, which could be reversed by PMA stimulation of PKCs. Together, these data suggest that PTPµ may negatively regulate PKC activity in LNCaP prostate carcinoma cells. Although the precise mechanism is unclear, it is likely to involve the PTPµ-RACK1 complex.

Others have shown (31, 32) that serine/threonine phosphorylation of p120 negatively regulates E-cadherin-mediated adhesion. It is therefore possible that the inactivation of PKC<sup>δ</sup> leads to decreased phosphorylation of p120 and thereby increased E-cadherin-mediated adhesion. However, we could not detect any alteration in the phosphorylation of p120 either after re-expression of PTPµ or after treatment of uninfected LNCaP cells with the PKC<sup>δ</sup> inhibitor rottlerin (data not shown). In addition, activation of PKC caused cells expressing PTPµ<sup>WT</sup> to dissociate from an E-cadherin substrate. The fact that this dissociation occurred within 15 min after the addition of PMA argues that PKC directly affects the E-cadherin complex, rather than down-regulating the expression of either E-cadherin or the catenins. Therefore, the role of PTPµ in regulating E-cadherin-mediated adhesion could be to recruit RACK1 to the plasma membrane, thereby regulating the PKC pathway.

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