Association of Human Protein-tyrosine Phosphatase \( \kappa \) with Members of the Armadillo Family*

(Received for publication, February 15, 1996, and in revised form, April 22, 1996)

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We have identified a human receptor-like protein-tyrosine phosphatase (PTP) in the mammary carcinoma cell line SK-BR-3, which represents the human homolog of murine PTP\( \kappa \) (1, 2). These processes are thought to be counteracted by a family of proteins termed protein-tyrosine phosphatases (PTP)\(^1\) (3). Dephosphorylation by PTPs can either inactivate a signal generated by protein phosphorylation (4, 5) or, alternatively, promote signal transduction by dephosphorylation of phosphorysine residues with negative regulatory functions such as, for example, in the case of Src-like kinases (6). PTPs can be subdivided into receptor-like plasma membrane-spanning and soluble cytosolic forms. Their biological function appears to be determined not only by specific target substrates but also by their intracellular localization to distinct compartments (7, 8). The subfamily of receptor-like PTPs (RPTP) is composed of five different classes based on structural motifs in their extracellular domain (9). Identification of amino acid motifs that resemble those of cell adhesion molecules in the extracellular domain of class II and III RPTPs has led to the suggestion that these PTPs may be involved in the regulation of cell contact formation. The observation that overexpression of PTP\( \mu \) and PTP\( \kappa \) can induce cell aggregation by homophilic interaction of their respective extracellular regions supports this hypothesis (10–12). In this process the so called “MAM” domain (13) appears to determine the specificity of interaction (14).

The key molecules involved in the formation of cell-cell adhesions are members of the cadherin-catenin family (15), which connect adjacent cells via cadherin extracellular domain-mediated homophilic, Ca\(^{2+}\)-dependent interactions (16, 17). The cytoplasmic domain of E-cadherin on the other hand is responsible for the complex formation with the intracellular catenins (18–21), which, in turn, link cadherins to the actin filament network (22–26). \( \beta \)-Catenin and \( \gamma \)-catenin/plakoglobin form two distinct and mutually exclusive complexes with E-cadherin and \( \alpha \)-catenin but are also found in E-cadherin-independent pools (27, 28) and have been shown to associate with the tumor suppressor gene product adenomatous polyposis coli (29–31). The biological function of cell-cell adhesions, however, extends beyond a mere maintenance of the cellular architecture, and a direct involvement of these specialized molecules in signal transduction events has been postulated. \( \beta \)-Catenin and \( \gamma \)-catenin/plakoglobin are homologous to the Drosophila armadillo (arm) protein (32–35), and this gene product has been shown to be essential for a signal transduction pathway that involves the segmental pattern formation during Drosophila development (36). The characteristic feature of these proteins is the presence of a variable number of 42 amino acid repeats (arm motif; Ref. 32), which is the basic motif of the armadillo family, including \( \beta \)-catenin and \( \gamma \)-catenin/plakoglobin (35). The observation of tyrosine phosphorylation of the cadherin-catenin complex after growth factor stimulation or v-src transformation (37–40) as well as the association of \( \beta \)-catenin and \( \gamma \)-catenin/plakoglobin with the epidermal growth factor receptor and HER2/c-erbB-2 (41–43) further support a direct involvement of cell adhesions junctions in cell signaling. The fact that a disruption of the cadherin-catenin complex promotes the invasiveness of malignant tumors, that E-cadherin has been demonstrated to be a tumor suppressor gene (44, 45), and the notion that tyrosine phosphorylation seems to interfere with cadherin function (38, 39) makes the question as to how PTPs are involved in cell contact formation all the more compelling.

In this report, we describe the identification of a human PTP that represents the homolog of murine PTP\( \kappa \) (46). This class II transmembrane phosphatase is expressed in a cell density-dependent fashion and is recruited to areas of cell-cell contact. hPTP\( \kappa \) colocalizes with cell adhesion molecule-associated proteins at adhesions junctions and associates in vitro and in intact cells with two members of the armadillo family of proteins. Our

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\(^{*}\) This work was supported by a grant from SUGEN, Inc. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\(^{1}\) The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\(^{TM}/\)EBI Data Bank with accession number(s) Z70660.

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\(^{1}\) The abbreviations used are: PTP, protein-tyrosine phosphatase; RPTP, receptor-like PTP; PCR, polymerase chain reaction; CMV, cytomegalovirus; FCS, fetal calf serum; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonic acid.
observations strongly suggest a biological role of hPTP\(\kappa\) in the regulation of cell contact and adhesion.

**MATERIALS AND METHODS**

PCR and cDNA Cloning of hPTP\(\kappa\)-Poly(A)\(^{+}\) RNA was isolated from HTB30 cells and cDNA synthesized using avian myeloblastosis virus reverse transcriptase as described (47). PTP sequence fragments were amplified using the PCR with a pool of degenerated oligonucleotide primers based on conserved amino acid sequences in the PTP catalytic domain (48) under standard conditions. PCR products were cloned in Bluescript KS\(^{+}\) vector (Stratagene) and sequenced by the dideoxynucleotide chain termination method (49). A c ZAP II library (Stratagene) generated from HTB30 poly(A)\(^{+}\) RNA was screened with a PCR fragment probe under high stringency conditions (50). The full-length cDNA of hPTP\(\kappa\) was cloned into a cytomegalovirus promoter-based eukaryotic expression plasmid (pcMV; Ref. 51).

RNA Extraction and Northern Blot Analysis—Total RNA was isolated by the guanidinium isothiocyanate method (52) from cultured cells grown to 30, 70, and 100% confluence. Poly(A)\(^{+}\) RNA was prepared by oligo(dt)-cellulose chromatography (53) and Northern blot analysis was performed as described (47).

Cloning of \(\beta\)-Catenin and \(\gamma\)-Catenin/Plakoglobin—Human \(\beta\)-catenin (accession number Z19054) and \(\gamma\)-catenin/plakoglobin (accession number MM90543) were amplified from cDNA generated from MCF 7 cells using the PCR method. PCR products were cloned in an eukaryotic expression vector under the control of the CMV promoter and confirmed by sequence analysis.

Cell Lines and Cell Culture—All cell lines were obtained from the American Type Culture Collection (ATCC). SK-BR-3 cells (HTB30 and CRL-1655) were grown in MDM\(^{+}\)S medium supplemented with 1% glutamine and 10% FCS. NBT-II cells (CRL-1655) were grown in Eagle's minimal essential medium supplemented with 1% glutamine, 10% FCS. NB T-II cells (CRL-1655) were grown in Eagle's medium supplemented with 1% glutamine, 10% FCS.

Immunofluorescence—For immunofluorescence studies, NBT-II cells were grown on uncoated glass coverslips, fixed for 30 min with 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.4) on ice, washed repeatedly with phosphate-buffered saline (PBS) containing glycerine, and permeabilized for 10 min with 0.1% Tween in PBS. Nonspecific antibody binding was blocked for 1 h with 5% normal goat serum in PBS and incubated for 2 h after dilution in PBG, 1:100 for polyclonal antibody binding was described before (57).

RESULTS

Isolation and Analysis of hPTP\(\kappa\) cDNA Clones—To investigate PT P\(\kappa\) expression and function in cancer cells, we performed PCR experiments employing mRNA preparations from the human mammary carcinoma cell line SK-BR-3 and degenerated oligonucleotide primer pools corresponding to conserved sequences within the PTP catalytic domains (48). Sequence analysis of the cloned PCR fragments revealed the expression of several previously characterized as well as novel PTPs. One of the sequences was highly represented (18%) in the 121 clones examined and was used to screen a c ZAP II SK-BR-3 cDNA library at high stringency. Eleven overlapping clones were assembled to a full-length clone of 6.1 kb in size (not shown). Its open reading frame, coding for a 1439-amino acid sequence, displayed a high degree of homology (95.4%) with the murine type II transmembrane PTP\(\kappa\) (46). We concluded that our clone represented the human homolog of this previously identified mouse PTP and accordingly termed it hPTP\(\kappa\). The deduced amino acid sequence of hPTP\(\kappa\) is shown in comparison with mPTP\(\kappa\) in Fig. 1A. Using Northern blot analysis hPTP\(\kappa\) mRNA transcripts were found at high levels in human lung, brain, and colon, and to a lesser extent in liver, pancreas, stomach, kidney, and placenta as well as in mammary carcinoma cell lines MDA-MB-453, HBL-100, T-47D, MDA-MB-435, BT-20, BT-474, SKBR-3, MDA-MB-231 and MCF-7 (data not shown). Several structural features suggested that hPTP\(\kappa\) may be
involved in cell adhesion, including a MAM domain (13), one possible Ig-like domain, and four fibronectin type III-related domains that match the FNIII-like repeats of LAR (58), PTPβ (59), PTPσ (60), and RPTPμ (61).

To investigate the properties of the hPTPκ gene product, the complete cDNA was cloned into a cytomegalovirus early promoter-based expression vector and transfected into 293 human embryonic kidney cells. Western blot analysis with antibodies directed against a fragment within the extracellular domain (Fig. 1B, left panel) or against the carboxyl terminus (Fig. 1B, right panel) of hPTPκ resulted in the detection of three bands of 185 kDa, approximately 115 kDa, and 97 kDa, respectively. Since the calculated mass of hPTPκ was determined to be 163 kDa, it was likely that the 185-kDa band represented the

FIG. 1. A, comparison of the deduced amino acid sequence of hPTPκ with murine PTPκ. The predicted amino acid sequence of hPTPκ is presented in comparison with the murine homolog. Identical amino acids are shown only for hPTPκ. The putative signal sequence is overlined. The MAM domain is boxed, the Ig domain is indicated by a hatched overline, and the four fibronectin type III-like domains are indicated by overline brackets. Both PTP domains are indicated by shaded boxes. B, expression of hPTPκ cDNA. hPTPκ was transiently overexpressed by transfection of human embryonic kidney cells with a hPTPκ expression vector (CMV-hPTPκ) or an empty vector as control (CMV). Lysates of CMV-hPTPκ (lanes 1 and 3) or CMV (lanes 2 and 4) transfected cells were resolved by SDS-PAGE and Western blot analysis performed using the anti-NH2-terminal antiserum 116 (lanes 1 and 2) or the COOH-terminal antiserum CT-1 (lanes 3 and 4). Proteins recognized by the antibody were visualized by ECL detection (Amersham). Molecular mass standards in kilodaltons are indicated on the right.
lysates from SK-BR-3 cells (50% confluence) were prepared from SK-BR-3 cells at different states of confluence (lane 1: 30%; lane 2: 70%; lane 3: 100%) were probed with a 32P-labeled DNA probe corresponding to the extracellular domain of hPTP<sub>k</sub>. Western blot analysis with a probe corresponding to the extracellular protein level with increasing cell density (Fig. 2A, top) and with a fragment coding for GAPDH (A, bottom). B, lysates from SK-BR-3 cells (50 μg) were separated by SDS-PAGE and Western blot analysis using the anti-hPTP<sub>k</sub>-specific antibody D2-1 or an anti-GAPDH-specific antibody was performed (B, top). Proteins recognized by the antibody were visualized by ECL detection (Amersham). Molecular mass standards in kilodaltons are shown at the left.

Fig. 2. Cell density-dependent induction of hPTP<sub>k</sub>.A, poly(A)<sup>+</sup> RNA (4 μg/lane) prepared from SK-BR-3 cells at different states of confluence (lane 1: 30%; lane 2: 70%; lane 3: 100%) were probed with a 32P-labeled DNA probe corresponding to the extracellular domain of hPTP<sub>k</sub> (A, top) and with a fragment coding for GAPDH (A, bottom). B, lysates from SK-BR-3 cells (50 μg) were separated by SDS-PAGE and Western blot analysis using the anti-hPTP<sub>k</sub>-specific antibody D2-1 or an anti-GAPDH-specific antibody was performed (B, top). Proteins recognized by the antibody were visualized by ECL detection (Amersham). Molecular mass standards in kilodaltons are shown at the left.

hPTP<sub>k</sub> Associates with β-Catenin and γ-Catenin/Plakoglobin—The colocalization of hPTP<sub>k</sub> with β-catenin and γ-catenin/plakoglobin prompted us to investigate whether these proteins associate with hPTP<sub>k</sub> in vitro. We constructed GST fusion proteins containing either the whole cytoplasmic part of hPTP<sub>k</sub> (GST-hPTP<sub>k</sub>) or only the juxtamembrane region (GST-hPTP<sub>k</sub>-JM), which displays limited homology to the intracellular domain of E-cadherin (65). β-Catenin and γ-catenin/plakoglobin were transiently overexpressed in human 293 embryonic kidney fibroblasts and lysates of these cells incubated with GST-hPTP<sub>k</sub>, or GST-hPTP<sub>k</sub>-JM immobilized on glutathione-Sepharose. Matrix bound protein was subjected to Western blot analysis with either anti-β-catenin or anti-γ-catenin/plakoglobin antibody and led to the detection of both proteins bound to GST-hPTP<sub>k</sub> (Fig. 4A, lane 4–6) as well as to the GST-hPTP<sub>k</sub>-JM fusion protein (Fig. 4A, lane 7–9). No binding was detected to GST protein alone (Fig. 4A, lane 1–3). This interaction appeared to be specific since α-catenin, another member of the catenin family that does not contain an arm motif, and E-cadherin did not associate with hPTP<sub>k</sub> under the same conditions (data not shown). We therefore concluded that hPTP<sub>k</sub> binds specifically to the arm motif containing adhesion protein-associated β-catenin and γ-catenin/plakoglobin. The presence of the juxtamembrane region of hPTP<sub>k</sub> appeared to be sufficient for this association (Fig. 4A).

Next, we determined whether hPTP<sub>k</sub> and “arm proteins” could be shown to associate in intact cells. To this end, human HT29 cells were serum-starved or stimulated with the tyrosine phosphatase inhibitor pervanadate, lysed, and immunoprecipitated with an anti-hPTP<sub>k</sub>-specific antibody. Western blot analysis using a monoclonal anti-β-catenin or anti-γ-catenin/plakoglobin antibody detected both proteins in anti-hPTP<sub>k</sub> immunoprecipitates (Fig. 4B, lane 3 and 4; top and middle panel, respectively) and therefore confirmed the specificity of this association. No co-immunoprecipitation was detected with the antibody JM-1 prior to preincubation with the specific antigen (Fig. 4B, lanes 5 and 6) or nonimmunserum (Fig. 4B, lane 1 and 2). The presence of hPTP<sub>k</sub> was confirmed by reblotting with anti-hPTP<sub>k</sub> antibody D2-1. We found the association to be independent of the phosphorylation state of β-catenin or γ-catenin/plakoglobin, which are both phosphorylated after treatment with pervanadate (data not shown). The precipitated proteins, however, were phosphorylated after pervanadate treatment, as indicated by the shift in the apparent protein size (Fig. 4B, lane 4).

The in vivo and in vitro association of β-catenin and γ-catenin/plakoglobin with hPTP<sub>k</sub> led us to investigate whether these proteins could serve as potential substrates for hPTP<sub>k</sub>.
To examine this possibility we performed an in vitro assay using GST fusion proteins of hPTP$_\kappa$ (GST-hPTP$_\kappa$) as well as a catalytically inactive mutant of hPTP$_\kappa$ (GST-hPTP$_\kappa$-C/A). Prior to the experiment, we determined the enzymatic activity of GST-hPTP$_\kappa$ or GST-hPTP$_\kappa$-C/A, respectively, using pNPP as a substrate (data not shown). Next, $\beta$-catenin was transiently overexpressed in human 293 embryonic kidney fibroblasts, which were treated with pervanadate to induce tyrosine phosphorylation of substrates. Immunoprecipitated phosphotyrosine containing $\beta$-catenin was then incubated with GST-hPTP$_\kappa$ or GST-hPTP$_\kappa$-C/A. The reactions were terminated after different time intervals and samples were separated by SDS-PAGE. Western blot analysis with an anti-phosphotyrosine-specific antibody revealed a strong reduction in the tyrosine phosphorylation signal within the first 15 min when phosphorylated $\beta$-catenin was incubated with GST-hPTP$_\kappa$ (Fig. 5A, lanes 1-4). No detectable change in phosphorylation levels, however, was observed after treatment with GST-hPTP$_\kappa$-C/A (Fig. 5A, lanes 5 and 8). Blots were reprobed with an anti-$\beta$-catenin-specific antibody to confirm that identical amounts of protein were loaded (Fig. 5B). We therefore concluded that $\beta$-catenin may represent a substrate of hPTP$_\kappa$.

**DISCUSSION**

Little is known about interacting proteins or in vivo substrates of type II and type III phosphatases. Type II PTP LAR was recently described to interact and colocalize with a novel protein termed LIP-1 (LAR interacting protein-1) at focal adhesions. However, LIP-1 does not appear to be a substrate for LAR PTP activity (66). Interestingly, a novel adhesion molecule-like protein was found to interact with and serve as a substrate of the *Drosophila* phosphatase DPTP10D (67). Re-
cent findings by Brady-Kalnay et al. (68) and our observations now provide evidence for the association between proteins of cadherin/catenin complex with the receptor tyrosine phosphatases PTP\(\mu\) and hPTP\(\kappa\), respectively.

A role of PTPs in the regulation of cell-cell contacts was originally proposed by Klarlund (69), who found that or-thovanadate, a potent inhibitor of phosphatase activity, diminished normal contact inhibition between NRK-1 cells. Recently, overexpression of PTP\(\kappa\) and PTP\(\mu\) was shown to induce cell aggregation due to homophilic interactions of their extracellular domains, assigning to these PTPases a physiological role at cell adherens sites (10–12). Moreover, the receptor-like type III phosphatase DEP-1 was found to be expressed at elevated protein levels with increased cell confluence (70). Recently, Gebbink et al. (74) reported an increase in the expression of PTP\(\mu\) protein when cells were grown to high density. Similarly, we found an approximately threefold elevation of hPTP\(\kappa\) in the mRNA transcript and a more drastic elevation in PTP\(\kappa\) protein level with increased cell density. Because the increase in protein PTP\(\kappa\) level seems to be more pronounced than the up-regulation of PTP\(\kappa\) mRNA transcripts we conclude that PTP\(\kappa\) expression is transcriptionally and in addition similar to PTP\(\mu\) (74) also posttranscriptionally regulated in a cell density dependent manner.

Our observations are supported by earlier evidence suggesting an involvement of protein tyrosine phosphorylation in processes that regulate cadherin/catenin-mediated cell adhesion phenomena. Initially, a correlation was observed between v-src transformation of cells leading to a loss of adhesive properties and in vitro invasiveness of cells and tyrosine phosphorylation of the cadherin-catenin complex (38, 39, 71). Furthermore, epidermal growth factor can stimulate tyrosine phosphorylation of \(\beta\)-catenin and \(\gamma\)-catenin/plakoglobin, and both proteins were shown to associate with HER2/c-erb-2 and the epidermal growth factor receptor (41–43). In addition, growth factors such as epidermal growth factor, acidic fibroblast growth factor, and hepatocyte growth/scatter factor have been shown to induce migration of epithelial cells, and a correlation has been established between migration, phosphorylation of \(\beta\)-catenin, and redistribution of the proteins of the cadherin-catenin complex (40, 72, 73).

We demonstrate here that hPTP\(\kappa\) colocalizes with and asso-
hPTPκ Receptor Tyrosine Phosphatase

The interaction of hPTPκ with cell adhesion molecules such as cadherins and the catenins plays a biological role in the regulation of cell contact and adhesion control events such as cell proliferation, tumor invasion, and metastatic spread.

Acknowledgments—We thank Jeanne Arch for preparation of this manuscript, Wolfgang Vogel and Irmi Sures for initial support, and Rainer Lammers for generation of hPTPκ-specific antisera 116. We further want to thank Oliver Hobert, Karen Martel, and Oliver Naylor for their critical reading of the manuscript and John Murphy and Richard Albrecht for expert assistance with confocal microscopy.

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