Characterization of a Prostate-specific Tyrosine Phosphatase by Mutagenesis and Expression in Human Prostate Cancer Cells*

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The cellular form of human prostatic acid phosphatase (PACP) is a neutral protein-tyrosine phosphatase (PTP) and may play a key role in regulating the growth and androgen responsiveness of prostate cancer cells. The functional role of the enzyme is at least due in part to its dephosphorylation of c-ErbB-2, an in vivo substrate of the enzyme. In this study, we investigated the molecular mechanism of phosphotyrosine dephosphorylation by cellular PACP. We mutated several amino acid residues including one cysteine residue that was proposed to be involved in the PTP activity of the enzyme by serving as the phosphate acceptor. The cDNA constructs of mutant enzymes were transiently transfected into C-81 LNCaP and PC-3 human prostate cancer cells that lack the endogenous PACP expression. The phosphotyrosine level of ErbB-2 in these transfected cells was subsequently analyzed. Our results demonstrated that the phosphotyrosine level of ErbB-2 in cells expressing H12A or D258A mutant PACP is similar to that in control cells without PACP expression, suggesting that these mutants are incapable of dephosphorylating ErbB-2. In contrast, cells expressing C183A, C281A, or wild-type PACP had a decreased phosphotyrosine level of ErbB-2, compared with the control cells. Similar results were obtained from in vitro dephosphorylation of immunoprecipitated ErbB-2 by these mutant enzymes. Furthermore, transient expression of C183A, C281A, or the wild-type enzyme, but not H12A or D258A, decreased the growth rate of C-81 LNCaP cells. The data collectively indicate that His-12 and Asp-258, but not Cys-183 or Cys-281, are required for the PTP activity of PACP.

Protein tyrosine phosphorylation plays a pivotal role in controlling multiple eukaryotic cellular processes including metabolism, proliferation, differentiation, migration, and survival (1–5). Therefore, aberrant tyrosine phosphorylation can lead to abnormal outcomes such as neoplasm or cancer. Many oncogenes or protooncogenes encode protein-tyrosine kinases (PTKs) (4–6). Uncontrolled activation of these PTKs could be due to mutation, amplification, or loss of regulatory factors, which contributes to enhanced tyrosine phosphorylation. For receptor PTKs, mutation could lead to a ligand-independent activation by maintaining the receptor in a constant activated state. Amplification of these receptors can also lead to transformation through an increase in overall tyrosine kinase activity. For example, amplification of receptor PTKs has been found in several types of human cancers including breast, ovarian, lung, and colon cancers (7–9). In the case of breast cancer, overexpression of ErbB-2, due to amplification of the gene, is one of the consistent genetic alterations (7, 8). In addition, overexpression of ErbB-2 correlates with a poor patient prognosis in breast cancer as well as other cancers including ovarian (8) and gastric (10) cancers.

The homeostasis of protein tyrosine phosphorylation in the cell is maintained by PTKs and PTPs (2, 11). PTPs can directly interact with PTKs, resulting in dephosphorylation of the kinases. Alternatively, PTPs may act on the upstream or downstream signaling of PTKs. Most of known PTPs serve as negative regulators of critical signal transduction pathways (12–16) and are able to suppress transformation phenotype induced by PTKs in cultured cells (17, 18). For example, expression of PTP1B in NIH-3T3 cells leads to a reduced transformation by human NEU oncogene (19). Expression of cytoplasmic PTP-TD14 in NIH-3T3 cells is able to inhibit Ha-ras-mediated transformation (20). The wild-type PTP1B, but not the inactive mutant, inhibits transformation of rat 3Y1 fibroblasts by v-erbB, v-src, and v-ras (21). Similarly, expression of a low molecular weight bovine liver PTP in v-erbB, v-src, and v-ras-transformed NIH-3T3 fibroblast cells can inhibit anchorage-independent growth in soft agar (22). In a tumorigenic human breast carcinoma cell line that overexpresses p185 neu, expression of leukocyte common antigen-related PTP correlates with a decreased proliferation rate in vitro and a reduced tumor growth rate in athymic nude mice (23). Zander et al. (24) have demonstrated that T cell PTP can suppress transformation by murine v-fms in rat 2 cells. In the cell, the targets of PTPs include receptor PTKs (25–27) and other key signaling molecules such as adapter proteins (25), docking proteins (28, 29), Src family kinases (18, 30, 31), ERKs (32), and other PTKs (33).

Human PACP is a prostate epithelium-specific enzyme with intracellular and secreted forms (34, 35). Although the activity of the enzyme in circulation has been studied extensively as a marker for tumor progression, the functional role of the cellular form of the enzyme remains to be explored further. It is known that the cellular form of this enzyme has a high level of expression in normal, well differentiated prostate epithelial cells but is diminished in prostate carcinoma cells. In human prostate cancer cells, the cellular level of the enzyme is inversely corre-

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¶ The abbreviations used are: PTK, protein-tyrosine kinase; AcP, acid phosphatase; ERK, extracellular signal-regulated kinase; PACP, prostate acid phosphatase; pNPP, p-nitrophenyl phosphate; PTP, protein-tyrosine phosphatase; MAP, mitogen-activated protein; PAGE, polyacrylamide gel electrophoresis.
lated with the cell growth rate. For instance, LNCaP cells that express cellular PAcP have a slow growth rate, compared with PC-3 and DU-145 cells that lack the endogenous PAcP expression (36, 37). Introduction of cellular PAcP into prostate cancer cells results in a decreased growth rate (36–38). It has been shown that cellular PAcP is also involved in regulating the androgen-stimulated growth of prostate cells (39). C-33 LNCaP cells that express PAcP and androgen receptor are responsive to androgen stimulation, whereas C-81 LNCaP and PC-3 prostate carcinoma cells that express functional androgen receptor but lack PAcP expression are androgen-unresponsive. Moreover, androgen responsiveness of these cell lines can be restored by reintroducing cellular PAcP expression (39). The collective data clearly indicate that cellular PAcP is critical for regulating proliferation and androgen responsiveness of human prostate cancer cells.

It has been known for nearly 2 decades that human PAcP possesses intrinsic PTP activity. First, the enzyme is copurified with a PTP activity and exhibits a high specificity toward phosphotyrosyl proteins (40, 41). Subsequently, it is shown that PAcP dephosphorylates epidermal growth factor receptor preferentially at neutral pH, resulting in a decreased PTK specific activity of the receptor protein (42). Recent studies clearly show that the phosphotyrosine level of a 185-kDa protein (pp185) is inversely correlated with the cellular PAcP activity in prostate cancer cells (39, 43, 44). The pp185 is found to be c-ErbB-2/Neu/HER-2. Several lines of evidence together suggest that c-ErbB-2 is indeed an in vivo substrate of the cellular form of PAcP (43).

Little is known so far about the molecular mechanism by which PAcP dephosphorylates phosphotyrosyl proteins. In the present study, we performed experiments to determine the specific residues that are important for the PTP catalytic activity of human PAcP. We constructed several PAcP cDNA mutants by site-directed mutagenesis and transfected them into human prostate cancer cells. Subsequently, we analyzed the AcP activity of these mutants and the effect of their expression on the tyrosine phosphorylation of cellular proteins including c-ErbB-2. The results clearly demonstrated that replacement of His-12 or Cys-281 by Ala resulted in a change in amino acid. All mutant cDNAs were completely sequenced. The sequence-confirmed cDNA was subcloned into mammalian expression vectors pCDNA 3.1 and pCEP4 for transfection and expression in prostate cancer cells.

Cell Culture and Transfection—Human prostate cancer cells LNCaP and PC-3 were obtained originally from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum, 1% glutamine, and 0.5% gentamicin as described (36, 47). Cells were passaged once a week. LNCaP cells with passage numbers less than 33 and greater than 81 are defined as C-33 and C-81, respectively (39). The liposome-mediated transfection was performed using LipofectAMINE PLUS reagent. C-81 LNCaP or PC-3 cells were plated and grown in 6-well plates or 100-mm culture dishes for 40 h. About 1 and 6 μg of plasmids carrying PAcP cDNAs were used for transfection of the cells in each well and dish, respectively. To monitor the transfection efficiency, the αV-β3-Integrin immunofluorescence control vector (Promega) was used for cotransfection. Cells were harvested 72 h after transfection.

PAcP Activity and Protein Concentration Determination—AcP activity was assayed at pH 5.5 using pNPP as the substrate (48), and the tartrate-sensitive AcP activity was used to represent PAcP activity (43, 48). Protein concentration was determined using the Bio-Rad protein assay reagent. Bovine serum albumin was used as the protein reference.

Immunoprecipitation—Cells were washed twice with ice-cold Heps-buffered saline, harvested by scraping, and lysed in a lysis buffer containing 20 mM Hepes, pH 7.0, 0.5% deoxycholic acid, 0.1% SDS, 1% Nonidet P-40, 150 mM NaCl, 2 mM EDTA, 10 mM NaF, 0.1 mM ZnCl2, 2 mM sodium orthovanadate, and a mixture of protease inhibitors as described previously (43). Lysates were centrifuged at 12,000 × g for 10 min. The supernatants (1–2 mg in ~1 ml) were preabsorbed by incubating with 20 μl of washed protein A-Sepharose beads for 30 min at 4 °C. The supernatant fractions were further incubated with 5–6 μg of monoclonal mouse anti-ErbB-2 antibody preconjugated with protein A-Sepharose for 3 h at 4 °C. The immunoprecipitated ErbB-2 complexes were washed four times with the lysis buffer and dissolved in SDS sample buffer.

SDS-PAGE and Western Blotting—Proteins in total cell lysates or from immunoprecipitation were separated by SDS-PAGE and then electrophoretically transferred to nitrocellulose membranes (Micro Separation, MA). To increase the transfer efficiency of ErbB-2, 0.1% SDS was included in the blotting buffer (39, 43). The nitrocellulose membranes were probed with appropriate primary and horseradish peroxidase-conjugated secondary antibodies. Specific proteins on the membrane were visualized using the SuperSignal West Pico chemiluminescent reagents from Pierce.

In Vitro Dephosphorylation of ErbB-2—ErbB-2 protein was immunoprecipitated from C-81 LNCaP and PC-3 cells as described above. After washed with the lysis buffer, the immunocomplexes were further washed three times with dephosphorylation buffer (20 mM Hepes, pH 7.0, 150 mM NaCl, 2 mM mercaptoethanol) and incubated at room temperature with secreted PAcP in the conditioned medium. The ErbB-2 immunocomplexes were then washed with the lysis buffer and suspended in SDS-sample buffer for further analysis by SDS-PAGE and Western blotting.

RESULTS

Exogenous Expression of PAcP in Prostate Cancer Cells—To analyze the functional role of cellular PAcP as a PTP, a human PAcP cDNA was cloned into two types of mammalian expression vectors, pcDNA3.1 and pCEP4. The pCEP4 vector, but not the pcDNA3.1, can replicate episomal in human cells. Both constructs were individually transfected into C-81 LNCaP cells that lack the expression of endogenous PAcP (39). Control cells
were transfected with the vector alone. To quantify the transfection efficiency, a reporter plasmid containing the β-galactosidase gene was cotransfected into the same cells (49). Activities of β-galactosidase and PAcP in cellular lysates were quantified 72 h after transfection. The PAcP protein level was analyzed by immunoblotting with a rabbit anti-PAcP antibody. As shown in Fig. 1, PAcP had a higher level of expression in the cells transfected with the pCEP4/PAcP plasmid than in the cells transfected with the pcDNA 3.1/PAcP although both plasmids had similar transfection efficiency (data not shown). As a control, β-actin level was examined by probing the same membrane with anti-β-actin antibodies. An approximately equal amount of β-actin was detected in different lysates, indicating that a similar amount of proteins was loaded for electrophoresis (Fig. 1). Both constructs were subsequently used to analyze the effect of exogenous PAcP expression on protein tyrosine phosphorylation.

Effect of Mutation on PAcP Activity—To investigate the amino acid residues that are potentially involved in the PTP activity of PAcP, we performed site-directed mutagenesis of the PAcP molecule. Mutation of individual amino acid residues was carried out as described under “Experimental Procedures.” Based on the results from previous studies (50–53), four amino acid residues, i.e. His-12, Cys-183, Asp-258 and Cys-281, were chosen for mutation by substituting the corresponding residue with Ala. The mutated cDNA was sequenced, subcloned into the expression vectors, and subsequently transiently transfected into human prostate cancer cells for functional analysis.

As shown in Fig. 2A, PAcP proteins including the wild-type and mutants were expressed in C-81 LNCaP cells as demonstrated by Western blotting analyses. Using pNPP as the substrate specificity of PAcP, we performed site-directed mutagenesis of the PAcP molecule. Mutation of individual amino acid residues was carried out as described under “Experimental Procedures.” The activity was normalized to the PAcP protein level and shown as a percentage relative to that of the wild-type enzyme. The standard deviations are indicated by error bars. The data are the average of three sets of experiments.

from immunodepletion studies revealed that ErbB-2 is the major pp185 and contributes more than 80% of the phosphotyrosine at the position (43). Immunoblotting with anti-phosphotyrosine antibodies (Fig. 3A, top panel) demonstrated that expression of the H12A or D258A PAcP mutant did not have a significant effect on the tyrosine phosphorylation of ErbB-2, indicating a lack of PTP activity for these mutants. The loss of activity is not due to a global structure change of the enzyme since both mutants and the wild-type PAcP have a very similar overall structure (50). Expression of the C183A or C281A mutant decreased the tyrosine phosphorylation level of ErbB-2, suggesting that these mutant enzymes retain their PTP activity. A similar level of ErbB-2 protein (Fig. 3A, middle panel) was detected in all cell lysates by stripping and reprobing the same membrane with anti-ErbB-2 antibodies. Exogenous expression of the wild-type PAcP and its mutants was also clearly shown by Western blotting (Fig. 3A, bottom panel). To analyze further the specificity of dephosphorylation by PAcP, we semi-quantified the phosphorylation level of two other unknown phosphotyrosyl proteins, pp160 and pp50, in LNCaP cell lysates by densitometric analyses (Fig. 3A). Although the phosphotyrosine level of pp160 inversely correlated with the PTP activity of PAcP, the phosphotyrosine level of pp50 was not significantly affected by PAcP expression. The pp160 might be directly or indirectly regulated by ErbB-2 as a downstream or interacting molecule. Further studies are required to determine the actual relationship. Thus, these results suggest the substrate specificity of PAcP.

The effect of PAcP expression on protein tyrosine phosphorylation was also examined in PC-3 cells, another PAcP-defi-
cient human prostate cancer cell line. Similarly, both H12A and D258A mutants lost their PTP activity (Fig. 3B). Although the overall tyrosine phosphorylation pattern of PC-3 cells was somewhat different from that of LNCaP cells, the tyrosine phosphorylation level of the pp185 inversely correlated with the functional PAcP activity as in LNCaP cells (Fig. 3). To exclude the possible interfering effect by pCEP4 vector, both C-81 LNCaP and PC-3 cells were transfected with PAcP cDNA constructs cloned into another expression vector, pcDNA3.1. The same results were obtained as described above, i.e. the H12A and D258A PAcP mutants lost their PTP activity, whereas the C183A and C281A mutants retained the activity (data not shown). Again, the phosphotyrosine level of ErbB-2 inversely correlated with the cellular PAcP activity as shown in Fig. 3.

Dephosphorylation of ErbB-2 by PAcP Mutants—The PTP activity of PAcP mutants toward ErbB-2 was further analyzed. ErbB-2 protein was immunoprecipitated from C-81 LNCaP cells transiently transfected with the mutant and wild-type PAcP cDNAs. The phosphotyrosine level of the immunoprecipitated ErbB-2 was detected by Western blotting using the anti-phosphotyrosine antibody. As shown in Fig. 4A, the tyrosine phosphorylation level of ErbB-2 in C-81 LNCaP cells which expressed the H12A or D258A PAcP was very similar to that in control cells without PAcP expression. In contrast, the phosphotyrosine level of ErbB-2 in the cells expressing the C183A or C281A PAcP was decreased by ~40% compared with that in the control cells, while comparable to that in the cells expressing the wild-type PAcP. These data further indicated that the C183A and C281A PAcP mutant proteins retain the PTP activity in prostate cancer cells. In contrast, the H12A and D258A PAcP mutants lost their PTP activities.

The PTP activity of the wild-type and mutant PAcP enzymes toward ErbB-2 was also studied by in vitro dephosphorylation. ErbB-2 protein was immunoprecipitated from C-81 LNCaP cell lysates and subsequently dephosphorylated by PAcP in the conditioned medium from cells expressing the wild-type or mutant PAcP. As shown in Fig. 4B, the wild-type, C183A, and C281A mutants dephosphorylated ErbB-2 at pH 7. The extent of dephosphorylation by these enzymes was ~40% in comparison with the control and did not change significantly by extending the incubation time from 20–60 min or by increasing the amount of PAcP (data not shown). This observation suggests that PAcP may specifically target one or some of the multiple phosphotyrosyl residues on the ErbB-2 protein. The phosphotyrosine level of ErbB-2 protein was not significantly changed after incubation with the H12A or D258A mutant enzyme, indicating a lack of PTP activity for these mutants. Taken together, the data indicated that His-12 and Asp-258 of PAcP are involved in the phosphotyrosine dephosphorylation of ErbB-2.

Effect of PAcP Expression on ERK Phosphorylation and Prostate Cancer Cell Growth—To address whether dephosphoryl-
ation of ErbB-2 by PAcP has any impact on its signaling ca-

pacity, we examined the phosphorylation status of the
downstream ERK/MAP kinases. PC-3 cells were transiently
transfected with the wild-type PAcP cDNA or vector alone since
these cells exhibit a higher transfection efficiency and basal
phosphorylation level of ERK/MAP kinase than C-81 LNCaP
cells. The phosphorylation of ERK was then detected by West-
ern blotting using the antibodies specific to the activated MAP
kinase protein that is phosphorylated at both Thr-202 and
Tyr-204. The phosphorylation of ErbB-2 was also examined by
using the antibody against an activated ErbB-2 protein that is
phosphorylated at Tyr-1248. The data in Fig. 5A showed that
the expression of exogenous cellular PAcP correlates not only
with a decreased ErbB-2 phosphorylation at Tyr-1248 but also
with a reduced phosphorylation level of the p42 ERK/MAP
kinase in the cells, suggesting a down-regulation of the MAP
kinase activity. The phosphorylation level of p44 ERK/MAP
kinase is below the detection limit. These results thus indicate
that dephosphorylation of ErbB-2 by PAcP can suppress its
mitogenic signaling abilities.

We further analyzed the effect of PAcP expression on the
proliferation of prostate cancer cells. C-81 LNCaP cells were
transiently transfected with various PAcP cDNAs or the vector
alone. After 3 days, the cell number was counted. As shown in
Fig. 5B, the growth rate of the cells transfected with the wild-
type, C183A, or C281A mutant PAcP decreased significantly,
~20% lower than the control cells transfected with the vector
alone. In contrast, the H12A or D258A mutant PAcP had no
effect on the growth of LNCaP cells. To clarify further the
significance of PAcP effect on cell growth, stable subclone cell
lines were established from LNCaP and PC-3 cells transfected
with the wild-type PAcP. As shown in Fig. 5, C and D, the
expression of exogenous PAcP correlated with a decreased cell-
ular proliferation. The data together indicated that dephos-
phorylation of ErbB-2 by PAcP results in a reduced mitogenic
signaling, leading to a diminished growth rate of prostate can-
cer cells.

**DISCUSSION**

PAcP has been extensively studied as a differentiation anti-
gen of prostate epithelia and as a marker for prostate cancer
(54). Several lines of evidence indicate that cellular PAcP func-
tions as a neutral PTP (40–42) and is involved in the regula-
tion of proliferation and androgen responsiveness of human
prostate cancer cells (36, 37, 39). The intrinsic PTP activity of
the enzyme is apparently critical for its functional role in the
cancer cells (43, 44, 55).

PTPs are a large family of enzymes with diverse structure
and complexity (3, 12, 15, 56). Most of them contain an ~240-
residue conserved PTP domain with the exception of some
members such as the low molecular weight PTPs (13, 16, 57).
They all utilize an invariant Cys residue located in the signa-
alone. The phosphorylation level of ErbB-2 and ERKs in the cells was
respectively analyzed with antibodies specific to the phospho-ErbB-2
(Tyr-1248) and the phospho-ERK (Thr-202 and Tyr-204) by Western
blotting. The protein levels of ErbB-2, p44/p42 ERKs and PAcP were
examined with the corresponding antibodies after the membranes were
stripped. B, C-81 LNCaP cells were transiently transfected with the
vector alone, wild-type, or mutant PAcP cDNAs. After 3 days, the cell
number was determined. The significance of difference in cell number
between control and experimental cells was analyzed by Student’s t
test. * p < 0.05 (n = 3) and ** p < 0.01 (n = 3). C and D, PAcP-
expressing stable subclone cells, i.e. LNCaP-28 and LNCaP-40, or PC-
411, PC-412, and PC-416, were established from C-81 LNCaP (C) or
PC-3 (D) cells transfected with the wild-type PAcP cDNA. The cell
number was counted on the time points as indicated in the figure.
Similar results were obtained from two sets of independent experi-
ments in triplicates.
ture motif (CXXXXXR(S/T)) as the phosphate mediator. Based on the structural studies, enzymes in the PTP family have a very similar active site and catalytic mechanism (3, 16, 57–59).

Since PAcP shares little sequence homology with other PTPs, it is not known about the catalytic mechanism whereby this enzyme uses to dephosphorylate phosphotyrosyl proteins. Results from titration experiments indicate that each of the two subunits contains three disulfide bonds (51). Nevertheless, analyses on the crystal structure of rat PAcP reveal that only four of the six Cys residues participate in the formation of disulfide bridges for each monomer (52). It has also been shown that there is no covalent linkage between the two subunits. Given the high homology in sequence and similarity in three-dimensional structure between rat and human PAcP (52, 53, 61), it is conceivable that each subunit of human PAcP also possesses two Cys residues with free sulfhydryls. The existence of two free sulfhydryl groups in human PAcP is further demonstrated by biochemical studies (50). Thus, it is proposed that one of the two Cys residues, Cys-183 and Cys-281, is indeed involved in the phosphotyrosine dephosphorylation (50, 52). In fact, based on the three-dimensional structure of rat PAcP, Schneider et al. (52) have proposed that Cys-183 is located in the cleft near the active site and could participate in the substrate binding and/or catalysis. They further propose that the conformational change triggered by substrate binding can bring residues including Cys-183 closer to the catalytic center. The notion that the free “Cys” can function as the phosphate acceptor is further indicated by titration experiments (50). Thus, PAcP may belong to the family of “cysteine” protein-tyrosine phosphatases.

In this report, our data clearly show that mutation of either Cys-183 or Cys-281 to Ala has little effect on the AcP activity toward pNPP or the PTP activity as indicated by the in vitro phosphotyrosine level of c-ErbB-2 (Figs. 2B, 3, and 4). Furthermore, over a period of 72 h, C-81 LNCaP cells that were transiently transfected with the C183A or C281A mutant PAcP cDNA had a growth rate about 20% lower than the cells transfected with the vector alone (Fig. 5B). The extent of the down-regulation on cell growth by these mutants is similar to that caused by transient expression of the wild-type PAcP. Compared with LNCaP or PC-3 subclone cells stably expressing wild-type PAcP, cells transiently transfected with the wild-type, C183A, or C281A cDNA exhibited a higher growth rate (Fig. 5). It should be noted that the relatively low level of growth suppression is in part due to a low transfection efficiency of those cells (49). In addition, those cells have a slow growth rate (39). It appears that the suppression of prostate cancer cell growth by PAcP is due to down-regulation of the mitogenic MAP kinase pathway since the phosphorylation of ERK2/MAP kinase is down-regulated in PAcP-expressing cells (Fig. 5). Furthermore, our results show that phospho-Tyr-1248 of ErbB-2, one of the major autophosphorylation sites, is targeted by PAcP (Fig. 5A). Preliminary data indicate that the tyrosine phosphorylation level of p52Sca is also decreased in the cells expressing PAcP. Therefore, the data collectively indicate that a Cys residue is not required for the PTP catalytic activity of PAcP. To the best of our knowledge, this is the first study that clearly demonstrates a novel mechanism of dephosphorylation by a PTP in the cell.

In vitro studies indicated that a group of residues, including His-12 and Asp-258, are essential for the catalytic activity of human PAcP to hydrolyze pNPP or phenyl phosphotyrosine (50). His-12 is involved in the formation of the phosphate-binding site and is phosphorylated during catalysis as an acceptor of the phosphate group. Asp-258 may function as a general acid to donate a proton for the substrate leaving group during phosphoester hydrolysis (50, 52). Similarly, other PTPs also require an Asp residue (equivalent to Asp-181 in PTP1B) to protonize the phenolic oxygen of the tyrosyl leaving group during catalysis (59). Consistent with previous observations (50), the H12A and D258A mutants are inactive to pNPP (Fig. 2B). More importantly, our results demonstrate that mutation of His-12 or Asp-258 to Ala has resulted in a loss of the in vivo PTP activity toward ErbB-2 in human prostate cancer cells (Figs. 3 and 4A). The lack of PTP activity for these PAcP mutants is further confirmed by in vitro dephosphorylation of ErbB-2 (Fig. 4B) and the cell growth study (Fig. 5). It should be noted that mutation of His-12 or Asp-258 to Ala does not cause a global structural change of PAcP (50). Thus, our results from in vivo and in vitro studies indicate that both His-12 and Asp-258 residues are essential for the PTP activity of PAcP in human prostate cancer cells.

Human PAcP may represent a distinct subgroup of PTPs. Although sequence analyses reveal some homologous segments (data not shown, see Refs. 51 and 60), there is no overall sequence similarity between PAcP and other members of the PTP family. In addition, PAcP protein does not contain the signature motif (CXXXXXR(S/T)) despite the existence of two free sulfhydryls in each subunit. Previous studies (50, 52) suggest that one Cys residue could be involved in dephosphorylating phosphotyrosyl proteins, raising the possibility that the enzyme might use different molecular mechanisms to dephosphorylate pNPP in vitro and phosphoproteins in vivo. Studies in this report clearly demonstrate that neither of the two Cys residues, Cys-183 and Cys-281, is required for the PTP activity of human PAcP. Conversely, both His-12 and Asp-258 are required for the PTP and AcP activities. These data therefore indicate that both AcP and PTP activities of human PAcP share the same active site and apparently use the same amino acid residues for catalysis. This notion is consistent with the competitive inhibition phenomenon between pNPP and phospho-angiotensin (41). However, the enzyme possesses the specificity to different substrates. For example, kinetic studies indicate that the K_m value for PAcP toward phosphotyrosyl proteins is more than 50-fold lower than toward phosphoserine or phosphothreonine proteins (41). In this study, the in vivo preferential dephosphorylation of ErbB-2 by PAcP in both C-81 LNCaP and PC-3 cells also suggests that PAcP exhibits a substrate specificity toward different phosphotyrosyl proteins (Fig. 3). Residues such as Tyr-123 and Arg-127 could be involved in determining the substrate specificity (52). Interestingly, it is reported that a tartrate-resistant AcP from osteoclasts and macrophages is also an active PTP (62). Based on the competitive nature of the substrates, the enzyme may catalyze pNPP hydrolysis and phosphotyrosyl protein dephosphorylation in the same active site. However, further studies on this tartrate-resistant AcP are required to identify its active site and to delineate the catalytic mechanism.

To the best of our knowledge, this study represents the first effort to determine the mechanism and residues used for the PTP catalytic activity of human PAcP. Our collective data from both in vivo and in vitro studies clearly demonstrate that His-12 and Asp-258 are required for the PTP activity of the enzyme. In contrast, Cys-183 and Cys-281 are not essential for this activity. The results thus indicate a novel PTP catalytic mechanism underlying the role of PAcP in regulating the proliferation and androgen responsiveness of human prostate cancer cells.

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