Identification of the Cell Cycle Regulator VCP (p97/CDC48) as a Substrate of the Band 4.1-related Protein-tyrosine Phosphatase PTPH1*

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The human band 4.1-related protein-tyrosine phosphatase PTPH1 was introduced into NIH3T3 cells under the control of a tetracycline-repressible promoter. Ectopic expression of wild type PTPH1 dramatically inhibited cell growth, whereas a catalytically impaired mutant showed no effect. To identify the direct target of PTPH1 in the cell, we generated a substrate-trapping mutant, in which an invariant aspartate residue was changed to alanine (D811A in PTPH1). The PTPH1-D811A mutant trapped primarily a 97-kDa tyrosine-phosphorylated protein, which was determined to be VCP (also named p97 or yeast CDC48), from various cell lysates in vitro. However, when expressed in mammalian cells, the D811A mutant was observed to contain high levels of phosphotyrosine and did not trap substrates. Mutation of tyrosine 676 to phenylalanine (Y676F) in the PTPH1-D811A mutant led to a marked reduction in phosphotyrosine content. Furthermore, this double mutant specifically trapped VCP in vivo and recognized the C-terminal tyrosines of VCP, whose phosphorylation is important for cell cycle progression in yeast. Like wild type PTPH1, this double mutant also inhibited cell proliferation. Moreover, induction of wild type PTPH1 resulted in specific dephosphorylation of VCP without changing the overall phosphotyrosine profile of the cells. VCP has been implicated in control of a variety of membrane functions, including membrane fusions, and is a regulator of the cell cycle. Our results suggest that PTPH1 may exert its effects on cell growth through dephosphorylation of VCP, thus implicating tyrosine phosphorylation as an important regulator of VCP function.

Reversible protein tyrosine phosphorylation, coordinated by the action of protein-tyrosine kinases and protein-tyrosine phosphatases (PTPs), is a key mechanism in regulating many cellular activities. The diversity and complexity of PTPs are beginning to rival that of protein-tyrosine kinases and protein-tyrosine kinases themselves. Band 4.1 domains are responsible for targeting proteins to the cytoskeleton-membrane interface. PDZ domains are involved in mediating protein-protein interactions, recognizing C-terminal valine residues, or binding to other PDZ domains in their targets. The PDZ domains of FAP-1, a member of the PTPH1 family, were found to interact with the cell surface receptor FAS and, as a result, it has been suggested to be involved in FAS-mediated apoptosis. We have shown that the N-terminal segment of PTPH1 exerts an inhibitory effect on its enzymatic activity in vitro. In addition, we have found that 20–50% of PTPH1 is complexed with the 14-3-3 adaptor protein in cells, in a manner that is dependent on the phosphorylation of PTPH1 and is regulated by mitogenic signals. However, the biological function and physiological substrate(s) of PTPH1 remain to be elucidated.

In this study, we have identified the physiological substrates of PTP catalytic domains, which are substrates that bind specifically to the Asp → Ala mutant enzyme. By applying this strategy in this study, we have been able to identify the first substrate for the human protein-tyrosine phosphatase, PTPH1.

We expressed the substrate-trapping mutant of PTPH1 as a GST fusion protein, GST-PTPH1-D811A, and found that it interacted primarily with a 97-kDa protein from various cell lysates in vitro. Peptide sequencing revealed that the 97-kDa protein was the valosin-containing protein (VCP), an ATPase belonging to the AAA ATPase associated with different cellular functions. Approximately 80 PTPs have been identified, with ~300 members estimated from genome sequencing efforts. However, information about the function and regulation of most PTPs in cellular contexts is limited.

PTPH1 and PTPMEG are the prototypes of a growing family of PTPs characterized by N-terminal segments containing sequence motifs with homology to band 4.1 domains and PDZ domains. Band 4.1 domains are responsible for targeting proteins to the cytoskeleton-membrane interface. PTPH1 was found to be mostly associated with membrane structures in the cells and this association appears to be mediated by the N-terminal portion of PTPH1. PDZ domains have been implicated in mediating protein-protein interactions, recognizing C-terminal valine residues, or binding to other PDZ domains in their targets. The PDZ domains of PTPBAS (also known as FAP-1), a member of the PTPH1 family, were found to interact with the cell surface receptor FAS and, as a result, it has been suggested to be involved in FAS-mediated apoptosis. We have shown that the N-terminal segment of PTPH1 exerts an inhibitory effect on its enzymatic activity in vitro. In addition, we have found that 20–50% of PTPH1 is complexed with the 14-3-3 adaptor protein in cells, in a manner that is dependent on the phosphorylation of PTPH1 and is regulated by mitogenic signals. However, the biological function and physiological substrate(s) of PTPH1 remain to be elucidated.

To understand the function of PTPH1, it is critical to identify its physiological substrates. PTP catalytic domains consist of ~240 residues containing a number of invariant amino acids, including those in the PTP signature motif. Of particular interest are the invariant cysteine residue, which functions in nucleophilic attack upon the phosphate group of the substrate, and the invariant aspartate, which acts both as a general acid to facilitate the protonation of the tyrosyl leaving group and as a general base to facilitate hydrolysis of the cysteinyl-phosphate intermediate. Mutation of the aspartate residue to alanine dramatically slows catalysis, with minimal effect on substrate affinity, thus stabilizing the enzyme-substrate complex. This observation led us to develop a “substrate trapping” approach for identifying physiological substrates of various PTPs. In this approach, substrates are isolated as proteins that bind specifically to the Asp → Ala mutant enzyme. By applying this strategy in this study, we have been able to identify the first substrate for the human protein-tyrosine phosphatase, PTPH1.
lar activities) family (14). However, upon expression in mammalian cells, we observed a high phosphotyrosine content in the PTPH1-D811A mutant which appeared to curtail its trapping ability in a cellular context. By mutating tyrosine 676, which defines one side of the catalytic cleft, to phenylalanine in PTPH1-D811A, we created a mutant in which phosphotyrosine content was reduced and which displayed the ability to trap VCP specifically in mammalian cells with concomitant inhibition of cell proliferation. Importantly, the induction of wild type PTPH1 in stable cell lines also inhibited cell proliferation and led to specific dephosphorylation of VCP, without changes in the overall level and pattern of tyrosine-phosphorylated proteins. These results suggest that PTPH1 may exert its effect on cell growth through dephosphorylation of VCP.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Monoclonal antibodies against phosphotyrosine were either developed in our laboratory (G98) (13) or purchased from Upstate Biotechnology (4G10) and Transduction Labs (PY20). Polyclonal antibodies against FAK (SC-558) and cyclin D1 (SC-753) were from Santa Cruz Biotechnology. Agarose-coupled anti-phosphotyrosine antibodies PTP6 was from Sigma. Asxcs containing monoclonal antibodies against epitopes HA (12CA5) and Myc (9E10) were produced in our laboratory. Polyclonal antisera CS531 against VCP was raised in rabbits using a peptide (GGSVSYTEDDNLLYG), corresponding to the last 15 residues of murine VCP, conjugated to keyhole limpet hemocyanin (Pierce).

Cell lysates were clarified by centrifugation for 10 min at 10,000 g. Lysates, Immunoprecipitation, and Immunoblotting—After washing twice with phosphate-buffered saline, cells were lysed in one of the following three buffers: (i) Nonidet P-40 buffer, which consists of 10 mM sodium phosphate, pH 7.0, 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 50 mM sodium fluoride, 1 mM Na3VO4, and 1 μM protease inhibitor mixture (aprotinin, 5 μg/ml, aprotinin, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride); (ii) RIPA buffer, which is Nonidet P-40 buffer supplemented with 1% sodium deoxycholate and 0.1% SDS; or (iii) hypotonic buffer, which contains 20 mM HEPES, pH 7.5, 250 mM sucrose, 20 mM sodium fluoride, 1 mM EDTA, 1 mM dithiothreitol, 1 mM Na3VO4, 50 mM sodium fluoride, and 1 μM protease inhibitor mixture. Cell lysates were clarified by centrifugation for 10 min at 10,000 × g.

Immunoprecipitations were performed with various antibodies bound to protein A-Sepharose CL-4B (Pharmacia), as described previously (4). Immunoprecipitations, or cell lysates, were solubilized with SDS sample buffer, resolved by SDS-PAGE on 8% gels and transferred onto Imobilon-P membranes (Millipore). Immunoblot analysis was performed in a buffer containing 5% nonfat dry milk, 150 mM NaCl, 0.05% Tween 20, and 0.3 M Tris, pH 7.5, using enhanced chemiluminescence (ECL) detection.

**DNA Constructs**—The C842S, D811A, and Y676F/D811A mutations in PTPH1 cDNA were made by site-directed mutagenesis using the Muta-Gen kit (Bio-Rad) and confirmed by double strand DNA sequencing. The catalytic domain (residues 634 to 913) of PTPH1 was fused to the C terminus with the HA epitope (SYPYDVPDYA). After confirmation by sequencing, these constructs were cloned into vector pCDNA3 (Invitrogen) and retroviral vector pBSTR1 (S. Reeves, Massachusetts General Hospital). Myc-tagged murine VCP constructs, wild type (VCPmyc), and mutant with a Y796F/Y805F double mutation (VCPmyc-F10) were generous gifts of Dr. L. Samelson (National Institutes of Health).

**Cell Culture, Stable Cell Lines, and Cell Treatments**—Mammalian cell lines, including 293, A431, COS-7, HepG2, MDCK, NIH3T3, REF-52, Saos-2 and Vero, were all maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Life Technologies, Inc.). Stable NIH3T3 cell lines expressing full-length PTPH1 under the control of a tetracycline-repressible promoter were made using a retroviral gene delivery system (5, 15). After confirmation by sequencing, these constructs were cloned into vector pCDNA3 (Invitrogen) and retroviral vector pBSTR1 (S. Reeves, Massachusetts General Hospital), Myc-tagged murine VCP constructs, wild type (VCPmyc), and mutant with a Y796F/Y805F double mutation (VCPmyc-F10) were generous gifts of Dr. L. Samelson (National Institutes of Health).

**RESULTS**

**Ectopic Expression of PTPH1 Inhibited Cell Growth**—In order to gain insight into the biological function of PTPH1, we made stable NIH3T3 cell lines expressing the phosphatase under the control of a tetracycline-repressible promoter. As shown in Fig. 1, when expression of wild type PTPH1 was induced by removal of tetracycline from the media, cell growth was strongly inhibited. The stable cell line, containing the wild type PTPH1 expression construct, accumulated 7-fold fewer cells upon induction of PTPH1 (Fig. 1B). Approximately 10% of the cells gradually detached and floated off the dish during the induction of wild type PTPH1. These floating cells were stained by trypan blue, indicating that they were no longer viable. In contrast, overexpression of the catalytically impaired D811A mutant of PTPH1 had no effect on either cell growth or viability, suggesting that the catalytic activity of PTPH1 is required for growth inhibition. Similar results were obtained in 3 individual cell lines for each PTPH1 construct, indicating that the results presented do not arise from cloning variation.

In light of the fact that ~10% of cells induced to express wild type PTPH1 died, we considered that one explanation for the growth inhibition imposed by PTPH1 could be the induction of apoptosis. However, using a DNA fragmentation assay, we did not detect any apoptotic traits in cells induced to express PTPH1 (data not shown). Since there was no apparent effect on apoptosis, we investigated whether the growth inhibition may arise from interference with the cell cycle machinery. Using flow cytometric assays of DNA content, we observed that upon induction of PTPH1 expression the distribution of cells throughout the cell cycle was not altered compared with control cells, indicating that the arrest did not occur in the S phase of the cell cycle (data not shown). In addition, we investigated the effects of PTPH1 expression on reentry into the cell cycle during the recovery of cells from G1/S arrest by hydroxyurea. As shown in Fig. 2, the elevation of cyclin D1 during cell cycle reinitiation and progression was totally abolished by the induction of PTPH1. These data indicate that expression of PTPH1...
disrupts cell cycle progression, thus slowing down or arresting cell growth.

Identification of VCP as a Substrate of PTPH1 in Vitro—In order to understand the molecular mechanism of PTPH1-induced growth inhibition, we generated a substrate-trapping mutant form of the PTP (PTPH1-D811A) with which to identify critical substrates. Pervanadate-treated cell lysates were incubated with GST-PTPH1 catalytic domain fusion proteins bound on beads. Bound proteins were eluted with sample buffer, fractionated by SDS-PAGE, and immunoblotted with anti-phosphotyrosine antibodies. We observed that a prominent tyrosine-phosphorylated protein of 97 kDa (pp97) was isolated specifically by the PTPH1-D811A mutant from 293 cell lysates, but not by either the wild type or the PTPH1-C842S mutant (Fig. 3). Furthermore, pp97 was also consistently recovered as the major tyrosine-phosphorylated protein from other cell lines tested including A431, COS-7, HepG2, MDCK, REF-52, Saos-2, and Vero (data not shown). Other proteins were occasionally isolated at lesser intensities, but due to their variable recovery they were not considered further. However, it is important to note that in any starting lysate, which contains hundreds of tyrosine-phosphorylated proteins, pp97 is not a major component, thus indicating the selectivity of the interaction between the isolated catalytic domain of PTPH1 and this substrate.

We subjected pp97 to a large scale purification and obtained protein sequence by Edman degradation of K-endopeptidase-digested peptides. Sequences of 7 individual peptides were obtained and were all found to match sequences in VCP, a membrane-associated ATPase (18). VCP, also named p97, and its yeast ortholog CDC48, has been demonstrated to play an essential role in regulating many membrane activities and is a well established cell cycle regulator (14). This function of VCP as a regulator of cell cycle progression is interesting in light of the suppression of cell proliferation induced by ectopic expression of PTPH1.

The PTPH1-Y676F/D811A Double Mutant Trapped VCP in Vivo—Although the PTPH1-D811A mutant of PTPH1 trapped VCP in the above assay in vitro, it was difficult to detect trapping directly in mammalian cells. We noted that the PTPH1-D811A mutant incorporated significant levels of phosphotyrosine when expressed in mammalian cells (Fig. 4A), whereas the GST-PTPH1-D811A fusion protein expressed in E. coli bore no phosphotyrosine (Fig. 3). We reasoned that the presence of phosphate on the PTPH1-D811A mutant may impede access of other tyrosine-phosphorylated substrates to its active site and thus compromise its trapping ability. A con-

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**FIG. 1.** Growth inhibition of stable NIH3T3 cell lines overexpressing PTPH1. Cells were plated at a density of $4 \times 10^5$ per 10-cm dish and PTPH1 was either induced (+) or left uninduced (−). At the indicated time (days) after plating, cells were either photographed with a CCD camera or counted after removal from the dish by trypsin treatment. A, photographs of the cells at days 1 and 4 after replating. B, total cell numbers (the mean from triplicate plating). □, wild type; □, wild type; △, PTPH1 D811A mutant; ▲, +PTPH1 D811A mutant.

**FIG. 2.** Overexpression of PTPH1 inhibits cell cycle progression. Stable NIH3T3 cells were synchronized by treatment with hydroxyurea, with (+) or without (−) induction of PTPH1. Cells were collected at different times (h) after release from the hydroxyurea block and subjected to immunoblot analysis with antibodies against the HA epitope (for PTPH1) and cyclin D1.
served tyrosine residue is located in the active site and provides a hydrophobic interaction with the substrate phosphotyrosine, serving a role in orienting the substrate (10). We thought it was possible that this residue may be a receptor for a phosphate from the highly active thiophosphate intermediate formed between the cysteine residue of the Asp → Ala mutant and the phosphate group of its substrates. When we mutated this tyrosine residue (Tyr776) in PTPH1-D811A to phenylalanine to make a Y676F/D811A double mutant, we observed that the level of phosphotyrosine was dramatically reduced compared with the PTPH1-D811A single mutant (Fig. 4B). More importantly, the PTPH1-Y676F/D811A double mutant specifically trapped VCP in vivo. These results indicate that mutation of this conserved tyrosine residue may improve the efficiency of substrate trapping, particularly for those PTPs in which the Asp → Ala mutants are susceptible to modification of the tyrosine residue that defines the active site.

**PTPH1 Dephosphorylates the C-terminal Tyrosines of VCP**—The tyrosines (Tyr796 and Tyr805) at the C terminus of VCP have been reported to be the major sites of phosphorylation, with Tyr805 accounting for more than 90% of the tyrosine phosphorylation on the protein (19). Mutation of the C-terminal tyrosine phosphorylation site in yeast CDC48, equivalent to Tyr805 in VCP, abolished translocation of the protein to the nucleus during mitosis and led to elongation of the cell cycle and a growth defect (20). We tested whether PTPH1 recognized these residues in VCP in mammalian cells. Human 293 cells were co-transfected with individual PTPH1 constructs and wild type VCP, or VCP containing a Y796F/Y805F double mutation. Immunoprecipitates of PTPH1 from lysates of these co-transfected 293 cells were monitored for the presence of VCP (Fig. 5). We found that only wild type VCP was trapped by the PTPH1-Y676F/D811A mutant and that the wild type or the D811A single mutant of PTPH1 were ineffective substrate traps. The Y796F/Y805F VCP mutant was not associated with any of the PTPH1 constructs. It is important to note that under the conditions of these experiments, the wild type and mutant PTPH1 were expressed to similar levels, as were both forms of VCP (data not shown). Therefore, these results indicate that phosphorylation of the C-terminal tyrosines of VCP is required for its recognition as substrate by PTPH1.

**PTPH1 Specifically Dephosphorylated VCP while Retaining the Overall Phosphotyrosine Profile in Cells**—Our trapping studies have identified the cell cycle regulator VCP as a substrate of PTPH1. Therefore, it was important to study the phosphorylation status of VCP in stable cell lines expressing wild type PTPH1. However, tyrosine phosphorylation of VCP has been found to be very transient in vivo and difficult to measure (19, 21). We addressed this issue in two ways. First, we examined the level of phosphotyrosine in VCP immunoprecipitated from cells that were pretreated with 1 mM vanadate before lysis. Vanadate is a PTP inhibitor which preserves phosphotyrosine residues in cellular proteins and will minimize nonspecific dephosphorylation events during the experimental manipulations. We observed a 3–5-fold decrease in the phosphotyrosine level of VCP following ectopic expression of PTPH1 (Fig. 6a). Second, we determined the distribution of VCP in the total population of phosphotyrosine-containing proteins immunoprecipitated from randomly growing cells. In pools containing similar amounts of tyrosine-phosphorylated proteins, the population of tyrosine-phosphorylated VCP was dramatically reduced following the induction of PTPH1 (Fig. 6B). In contrast, the tyrosine phosphorylation of FAK, a well known tyrosine-phosphorylated protein in NIH3T3 cells, was unchanged when analyzed under the same conditions. Furthermore, expression of PTPH1 did not alter the global pattern of tyrosine phosphorylation in starved, randomly growing or insulin-stimulated cells (Fig. 6C). This indicates that the overexpression of PTPH1 results in selective, rather than random, dephosphorylation of tyrosine-phosphorylated proteins and highlights the importance of VCP as a target in the cell growth arrest induced by PTPH1 expression.

**DISCUSSION**

An important step in understanding the function of PTPs is to identify their physiological substrates. Use of catalytically impaired, substrate-trapping mutants, in which the invariant general acid aspartate residue is changed to alanine, has proved to be a potent strategy for identifying substrates of a number of PTPs (11–13). In our pursuit of the substrate(s) of PTPH1, we have refined the approach by combining the Asp → Ala mutation with a second mutation, which changes a conserved tyrosine residue at the active site to phenylalanine, to produce an effective substrate trap in a cellular context. As a result, we have identified the cell cycle regulator VCP/CDC48 as a substrate of PTPH1.

**A Refinement of the Method for Producing Substrate-trapping Mutant PTPs**—In the initial substrate trapping experiments performed in vitro, we utilized a GST-PTPH1-D811A mutant fusion protein, which was produced in bacteria, to identify VCP as a potential substrate of PTPH1. However, no tyrosine-phosphorylated proteins co-immunoprecipitated with the PTPH1-D811A mutant following expression in mammalian cells. We found that, unlike the bacterially expressed D811A mutant, the PTPH1-D811A expressed in mammalian cells was itself recovered in a tyrosine-phosphorylated form and could not trap substrates. We have also observed this problem with several other members of the PTP family.3 Interestingly, the inactive Cys → Ser mutants of these PTPs, in which the essential nucleophilic cysteine was changed to serine, were not tyrosine-phosphorylated, suggesting that the catalytic activity of these PTPs was required for the accumulation of phosphotyrosine. Thus the presence of phosphotyrosine may be a conse-

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3 S.-H. Zhang, and N. K. Tonks, unpublished data.
Expression of PTPH1 Leads to Suppression of Cell Growth and Specific Dephosphorylation of VCP—VCPp97/CDC48 and other members of the AAA family of ATPases are involved in cell cycle regulation, protein degradation, organelle biogenesis, and vesicle-mediated protein transport (14). The importance of VCP for normal cellular function can also be implied by the fact that it is highly conserved in protein sequence from yeast, Caenorhabditis elegans, plants to animals. Mutants of CDC48, the yeast ortholog of mammalian VCP, are arrested late in mitosis and accumulate elongated nuclei that appear unable to undergo fission, a process that involves membrane fusion (22). A homoyzygous insertion mutation in smallmind, a Drosophila gene encoding a protein in the subfamily of VCP/p97/CDC48, arrests development at the larval stage with neuronal defects (23). VCP/p97/CDC48 was found to be a critically required component in organelle membrane fusions, such as the fusion of endoplasmic reticulum membrane and the rebuilding of Golgi cisternae from mitotic Golgi fragments, that occur at the end of mitosis in both yeast and animal cells (24–26). VCP/CDC48 is mainly attached to the endoplasmic reticulum in quiescent cells and relocalizes in a cell cycle-dependent manner: CDC48 enters the nucleus during late G1 in yeast and VCP aggregates at the centrosomes during mitosis in mammalian cells (20).

Tyrosine phosphorylation of VCP was stimulated upon activation of T cell antigen receptors and tyrosine 805 of VCP accounted for more than ~90% of the total phosphorylation (18, 19, 21). Rather than directly effecting the ATPase activity of VCP (19), tyrosine phosphorylation of VCP and CDC48 appears to play a role in regulating subcellular distribution (20). Failure of CDC48/VCP to migrate into the nucleus in yeast, or to centrosomes in mammalian cells, would be expected to result in disturbance of cell division. Mutation of tyrosine 834 of CDC48, which is equivalent to the major phosphorylation site Tyr805 in VCP, to phenylalanine abolishes this translocation and causes an elongated cell cycle and growth retardation, whereas mutation of the same residue to glutamate, to mimic phosphorylation, allows translocation to the nucleus and results in normal cell growth (20). The growth inhibition mediated by ectopic expression of PTPH1 is reminiscent of the yeast CDC48 mutant.
that harbors this tyrosine to phenylalanine mutation. We demonstrated that ectopic expression of wild type PTPH1 inhibited cell growth in the stable NIH3T3 cell lines and that the phosphatase activity of PTPH1 is required for this growth arrest. In cells in which PTPH1 expression had been induced, tyrosine phosphorylation of VCP was dramatically and selectively reduced, with no change detected in the phosphotyrosine levels on FAK or the total profile of tyrosine-phosphorylated proteins in the cell. These results suggest that a selective interaction between VCP and PTPH1 was not compromised by overexpression of the phosphatase and that the growth inhibition by PTPH1 may be due to the specific dephosphorylation of VCP. Moreover, the PTPH1-Y676F/D811A double mutant recognizes tyrosine-phosphorylated VCP specifically and engagement of VCP in a complex with this trapping mutant, presumably interfering with the interaction between VCP and its appropriate targets in the membrane, yields the same phenotype as expression of the wild type enzyme. These results argue that PTPH1 specifically dephosphorylates the C-terminal tyrosines of VCP, thus interfering with the phosphotyrosine-dependent regulation of VCP in the cell cycle, resulting in growth inhibition.

In summary, using a modification of the substrate-trapping strategy developed in the laboratory, we have identified the cell cycle regulator VCP as a major cellular substrate of PTPH1. Ectopic expression of PTPH1 led to selective dephosphorylation of VCP in stable NIH3T3 cell lines and inhibited cell cycle progression and growth. In light of reports that disruption of VCP/CDC48 function, and in particular the disruption of tyrosine phosphorylation site in VCP/CDC48, also inhibits cell proliferation, our data are consistent with the interpretation that the effects of ectopic expression of PTPH1 are manifested primarily through its dephosphorylation of VCP. These results further demonstrate the importance of tyrosine phosphorylation in regulating the function of VCP in cell cycle progression.

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Fig. 6. VCP was specifically dephosphorylated in stable NIH3T3 cell lines expressing wild type PTPH1. A, VCP was isolated by immunoprecipitation from cells treated with vanadate. The immunoprecipitates were analyzed by immunoblotting with a mixture of two horseradish peroxidase-coupled anti-phosphotyrosine antibodies (PY20 and 4G10) (upper panel) or anti-VCP (lower panel). B, phosphotyrosine-containing proteins were isolated from cells by agarose beads coupled with anti-phosphotyrosine antibody PT66. Then, the bound materials were analyzed by immunoblotting with a mixture of two horseradish peroxidase-coupled anti-phosphotyrosine antibodies (PY20 and 4G10). The same blot was also analyzed with anti-FAK antibodies. C, the overall profile of tyrosine-phosphorylated proteins was not altered by the overexpression of PTPH1. Stable NIH3T3 cells, with (+) or without (−) the induction of PTPH1, were treated as indicated. Cell lysates were analyzed by immunoblotting with a mixture of two horseradish peroxidase-coupled anti-phosphotyrosine antibodies (PY20 and 4G10).
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