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PTEN Associates with the Vault Particles in HeLa Cells*

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PTEN is a tumor suppressor that primarily dephosphorylates phosphatidylinositol 3,4,5-trisphosphate to down-regulate the phosphoinositide 3-kinase/Akt signaling pathway. Although the cellular functions of PTEN as a tumor suppressor have been well characterized, the mechanism by which PTEN activity is modulated by other signal molecules *in vivo* remains poorly understood. In searching for potential PTEN modulators through protein-protein interaction, we identified the major vault protein (MVP) as a dominant PTEN-binding protein in a yeast two-hybrid screen. MVP is the major structural component of vault, the largest intracellular ribonucleoprotein particle. Co-immunoprecipitation confirmed the interaction between PTEN and MVP in transfected mammalian cells. More importantly, we found that a significant portion of endogenous PTEN associates with vault particles in human HeLa cells. Deletion mutation analysis demonstrated that MVP binds to the C2 domain of PTEN and that PTEN interacts with MVP through its EF hand-like motif. Furthermore, the *in vitro* binding experiments revealed that the interaction of PTEN with MVP is Ca^{2+}-dependent.

PTEN was originally identified as a tumor suppressor gene based on its high frequency of mutation in a variety of tumors (1–3). Germ-line mutations of PTEN are the cause of Cowden disease, an autosomal-dominant hamartoma syndrome that results in an increased risk for development of tumors in a variety of tissues (4–7). The genetic evidence that PTEN is an important tumor suppressor is supported by the fact that heterozygous disruption of the PTEN gene in knockout mice results in the spontaneous development of tumors (8–10). Although PTEN as a protein phosphatase is capable of dephosphorylating tyrosine and threonine/serine residues (11, 12), the primary substrates of PTEN are 3′-phosphoinositides, PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 (13). Genetic and biochemical studies have demonstrated that the tumor suppressor functions of PTEN are linked primarily with the lipid phosphatase activity and its association with the well defined phosphoinositide 3-kinase pathway (reviewed in Refs. 14–20). Substantial progress has been made in the characterization of PTEN as a tumor suppressor as well as in the regulation of many cellular processes including growth, adhesion, migration, invasion, and apoptosis. Nevertheless, the mechanism by which PTEN activity is modulated in various cellular signaling complexes remains elusive. It is assumed that the activity and the cellular function of PTEN may be regulated through *in vivo* protein-protein interactions. PTEN contains a number of putative regulatory modules, including the N-terminal phosphoinositide binding motif, a C2 domain, a PDZ-binding site, and two proline-, glutamic acid-, serine-, and threonine-rich segments (21). The C2 domain of PTEN has been implicated in mediating membrane association (22). The C-terminal tail of PTEN interacts with several PDZ domain-containing proteins such as hDLG, hMAST205, MAGI-2, and MAGI-3 (23–25). The interaction of PTEN with these proteins may be important for its biological function, as it has been reported that MAGI-2 and MAGI-3 can enhance the activity of PTEN (23, 24). In contrast, several groups found that the PDZ-binding site of PTEN is not required for tumor suppression or other biological activities (21, 25–28). Therefore, the complete spectrum of PTEN-interacting proteins and the effects of the interactions on PTEN function are not yet defined.

To date, the vault complex with a molecular mass of 13 MDa is the largest intracellular ribonucleoprotein particle to be described. Vaults were first observed in preparations of clathrin-coated vesicles (29) and were so-named because of their arched morphology, reminiscent of the vaulted ceilings of a cathedral (29). Vaults are conserved in phylogenetic groups as diverse as mammals, avians, amphibians, sea urchins, and slime molds. The mammalian vaults comprise three proteins, the major vault protein (MVP)[1] (30), the vault poly(ADP-ribose) polymerase (VPARP) (31), and the telomerase-associated protein 1 (32), as well as one or more small untranslated RNA molecules (33). MVP constitutes >70% of the total mass and is the major vault structural component.

The precise cellular function(s) of the vaults are not yet completely understood. However, several studies have implicated that the vaults are involved in nucleocytoplasmic transport. The vaults apparently reside in the cytoplasm, but ~5% of the vaults are occasionally localized to the cytoplasmic face of the nuclear membrane at or near nuclear pore complexes (34). Moreover, the 31-Å resolution structure of vault determined by cryoelectron microscopy reveals a hollow interior that is big enough to enclose a complex as large as intact ribosome (35). The hollow structure may indicate an important role for vault in the transport/sequestration of cellular molecules. In addition, MVP has been identified as the lung resistance-related protein (36). Many multidrug-resistant cancer cells frequently

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[1] The abbreviations used are: MVP, major vault protein; VPARP, vault poly(ADP-ribose) polymerase; HA, hemagglutinin; GST, glutathione *S*-transferase; PLC, phospholipase C.

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overexpress MVP/lung resistance-related protein and intact vault particles (reviewed in Ref. 37). How vault functions in drug resistance is unknown. It has been proposed that vault may function as a transporter or sequester, but proteins or protein complexes that can be transported or sequestered by this particle have yet to be identified. We have employed the protein-protein interaction approach to screen for signaling molecules potentially modulating the activity of PTEN and found that PTEN associates with both MVP and intact vault particles.

EXPERIMENTAL PROCEDURES

Materials and Cell Lines—Rabbit anti-PTEN polyclonal antibodies were obtained from Upstate Biotechnology Inc. (Lake Placid, NY) and Cell Signaling Technology Inc. (Beverly, MA). Anti-lung resistance-related protein (MVP) monoclonal antibody was obtained from Transduction Laboratories (Lexington, KY). Anti-Myc (clone 9E10) monoclonal antibody and protein A-Sepharose CL-4B were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-hemagglutinin (anti-HA) monoclonal antibody (clone 3F10) used for immunoprecipitation was purchased from Roche Molecular Biochemicals, and anti-HA antibody (clone 12CA5) used for Western blot was prepared from the culture medium of hybridomas (American Type Culture Collection, ATCC). Nitrocellulose membrane Hybond-ECL was from Amersham Biosciences. Anti-mouse IgG-horseradish peroxidase and anti-rabbit IgG-horseradish peroxidase were from Bio-Rad Laboratories (Hercules, CA). Western Lightning chemiluminescence reagent kit was purchased from PerkinElmer Life Sciences. Protease inhibitor mixture tablets were from Roche Diagnostics. HeLa and 293T cells, obtained from ATCC, were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C.

Yeast Two-hybrid Screen—Full-length PTEN was amplified by PCR from a mammary gland cDNA library using *Pfu* DNA polymerase and inserted into pGEM-T vector (Promega Corp., Madison, WI). An aspartic acid 92 to alanine mutant (PTEN-D92A) was generated by PCR-based site-directed mutagenesis. This PTEN-D92A mutant was cloned in-frame downstream of the DNA binding domain of LexA in the pBTM-116-src vector (38) to form the bait construct (LexA-PTEN-D92A). The human lung cDNA library expressed as fusion proteins with the activation domain of GAL4 in the pACT2 vector were obtained from Clontech. The two-hybrid screen was carried out using the Clontech systems according to the manufacturer's recommendations. Briefly, the bait DNA and library DNA were sequentially transformed into the yeast strain L40α. Positive colonies growing on medium lacking leucine, tryptophan, and histidine were further screened for expression of β-galactosidase. The plasmid DNA was recovered from the His+/LacZ+ colonies and identified by DNA sequencing.

Transfection, Immunoprecipitation, and Immunoblot Analysis—To construct a plasmid for the expression of MVP in mammalian cells, a cDNA clone (P-22), isolated in the yeast two-hybrid screen, encoding the amino acid residues Gln^{113} to the end of the C terminus of MVP in pACT2 vector was digested with *Bgl*II, and the DNA fragment was inserted into the pcDNA3 vector (Invitrogen) at the *Bam*HI site. The resulting plasmid, termed pHA-MVP/113–893, expresses an HA-tagged MVP fragment. A C-terminal Myc-tagged PTEN construct (pPTEN-myc) was obtained by inserting the full coding region of PTEN cDNA, amplified by PCR with primers incorporating an *Hin*dIII site, into pcDNA3-myc-His-(-C) vector (Invitrogen) at the same site. The plasmid DNA was transfected into 293T cells using the standard calcium phosphate precipitation method. Forty-eight h after transfection, the cells were washed with cold phosphate-buffered saline once and lysed in Buffer A (50 mM Hepes (pH 7.4), 150 mM sodium chloride, 50 mM KCl, 1% Triton X-100, 5 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, and 1 μg/ml aprotinin). The samples were centrifuged at 14,000 rpm (20,000 $\times g$) for 10 min at 4 °C. An aliquot of this whole cell lysate was removed, and the remaining lysate was subjected to immunoprecipitation as described previously (40). The proteins were resolved on a 7.5% SDS-PAGE gel and transferred to nitrocellulose membranes (Hybond-ECL). The membranes were blocked with 5% milk in Tris-buffered saline (pH 7.6) overnight and then incubated with the first antibodies for 2 h. After washing four times with Tris-buffered saline containing 0.05% Tween-20, the membranes were incubated with the second antibody conjugated to horseradish peroxidase for 1 h and then washed four times with Tris-buffered saline containing 0.05% Tween-20. The blots were developed using the Western Lightning chemiluminescence reagent kit according to the manufacturer's instructions.

Expression, Purification of GST Fusion Proteins, and GST Pull Down—The cDNA fragment encoding amino acid residues 113–474 of MVP was amplified by PCR and inserted into the pGEX-5X1 vector (Amersham Biosciences). The fusion protein was expressed in *Escherichia coli* strain DH10 by induction with 25 μM isopropyl-β-D-thiogalactopyranoside at 25 °C for 16 h and purified as described previously (41). HeLa cells were lysed with Buffer B (50 mM Hepes (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, and EDTA-free mixture protease inhibitors) and centrifuged at 14,000 rpm (20,000 $\times g$) for 10 min at 4 °C. The supernatant was collected as whole cell lysates. For binding assays, glutathione-Sepharose beads with ~1 μg of bound GST or GST fusion protein were incubated at 4 °C for 16 h with 1 ml of HeLa cell lysates in the presence of various concentrations of Ca^{2+}, and as a negative control, with 1 ml of Buffer B, as well. The beads were washed four times with Buffer B containing the same concentration of Ca^{2+} as that in the binding solution, and the bound proteins were analyzed by SDS-PAGE and Western blot.

Sucrose Gradients—HeLa cells were washed once with cold phosphate-buffered saline and lysed with Buffer C (20 mM Hepes (pH 7.9), 2 mM $MgCl_2$, 100 mM KCl, 1 mM dithiothreitol, 15% glycerol, 10% sucrose, 1% Nonidet P-40, and EDTA-free mixture protease inhibitors). The samples were centrifuged at 14,000 rpm (20,000 $\times g$) for 10 min at 4 °C, and 250 μl of whole cell lysates were loaded on the top of 30–60% sucrose gradients (5 ml) prepared in Buffer C and centrifuged for 3 h at 50,000 rpm using a Beckman Vti65 rotor. Twenty fractions (250 μl each) were collected starting from the bottom of the tube to avoid contamination. The fractions were directly used for SDS-PAGE and Western blot analysis.

RESULTS

Identification of MVP as a PTEN-interacting Protein by Yeast Two-hybrid Screen—To identify PTEN-interacting proteins and its potential substrates, we carried out a modified yeast two-hybrid screen with the expression of an exogenous protein-tyrosine kinase (38). The full-length PTEN with a mutation of aspartic acid 92 to alanine (PTEN-D92A), which dramatically reduces the protein-tyrosine phosphatase catalytic activity but retains the binding ability to its substrates (39), was cloned into the plasmid pBTM-116-Src (38). Transformation of the plasmid into yeast results in the expression of the LexA DNA binding domain-PTEN-D92A fusion protein and c-Src kinase. This allows us to identify both tyrosine phosphorylation-dependent and -independent interactions. Starting from 6.9×10^6 primary transformants using a human lung cDNA library, ~60 colonies were positive for both HIS3 and LacZ expression. DNA sequencing revealed that almost half of these positive clones (25 clones) encode the MVP, the major component of vault particles. The shortest MVP clone identified contains an open reading frame encoding amino acid Gln^{113} to the C terminus (Arg^{893}). The interaction specificity was confirmed by co-transforming an MVP clone with PTEN and some unrelated baits such as the SH2 domain-containing protein-tyrosine phosphatases, SHP-1 and SHP-2, respectively, into yeast. The β-galactosidase filter lift assay revealed that MVP specifically interacted with PTEN but not with SHP-1 or SHP-2 though both were well expressed as indicated by their interaction with Grb2 in this assay (Table I). We next determined whether the interaction between PTEN and MVP is tyrosine phosphorylation-dependent. To do so, we cloned PTEN into the pBTM-116 vector that does not express Src kinase and carried out a yeast two-hybrid assay using this construct. As shown in Table I, the presence of Src is not required for the interaction of MVP with PTEN.

MVP Associates with PTEN in 293T Cells—To determine whether MVP interacts with PTEN in mammalian cells, we co-transfected PTEN-myc (C-terminal Myc-tagged PTEN) with HA-MVP/113–893 (N-terminal HA-tagged MVP fragment containing the amino acid residues Gln^{113} to the C terminus) into 293T cells. Immunoprecipitations were performed with an anti-

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TABLE I
MVP interacts with PTEN but not with SHP-1 in yeast two-hybrid assay

++, yeast colonies turned blue after 30 min of incubation. +, yeast colonies turned blue after 2 h of incubation. −, yeast colonies did not turn blue after 6 h of incubation. ND, not determined.

	BD-PTEN + Src	BD-PTEN	BD-SHP-1 + Src	BD-SHP-2 + Src
AD-MVP	++	++	−	−
AD-Grb2	ND	ND	+	+

Myc antibody or an anti-HA antibody, and the immunoprecipitants were subjected to Western blot analysis. As shown in Fig. 1*A*, HA-MVP/113–893 was detected in the anti-myc immunoprecipitant, indicating that HA-MVP/113–893 was co-precipitated with PTEN-myc. As a negative control, mouse IgG did not precipitate HA-MVP/113–893. The reciprocal experiment further confirmed the association of these two proteins in 293T cells, as PTEN-myc was co-immunoprecipitated with HA-MVP/113–893 by the anti-HA antibody (Fig. 1*B*).

Endogenous PTEN Associates with the Vault Particles in HeLa Cells—MVP is the major component of the vault particle, which is comprised of three proteins and at least one small untranslated RNA molecule. To investigate whether PTEN interacts with the assembled vault complex, we isolated vault particles from HeLa cells by centrifugation on a 30–60% sucrose gradient. Western blot analysis of the gradient fractions with anti-MVP antibody showed that the vault particles were present in fraction 11 to fraction 17 with a peak concentration in fraction 13. Very little MVP was detected in the top gradient fractions containing the original whole cell lysates, indicating that most MVP molecules are assembled into the vault particles in HeLa cells. Western blot analysis of the same fractions with anti-PTEN antibody indicated that PTEN was distributed in the top gradient fractions of the original whole cell lysates, as well as in the fractions 12–15 where the vault particles were located. PTEN was not detected in fractions 8–11, indicating that the PTEN co-localized with the vault fractions was not contamination derived from the top fractions. To further exclude the possibility of contamination of PTEN in the vault fractions 12–15 from the whole cell lysates, we analyzed two unrelated proteins Dok1 and Erk1 in the same fractions. As shown in Fig. 2, *C* and *D*, neither Dok1 nor Erk1 was detected in the vault fractions. These results strongly suggest that endogenous PTEN associates with intact vault particles in HeLa cells.

The C2 Domain of PTEN Interacts with the EF Hand Pair of MVP—The association of endogenous PTEN with vault particles prompted us to define the mechanism of the association between PTEN and MVP. We first mapped the MVP binding region of PTEN by deletion analysis in the yeast two-hybrid system. PTEN is composed of an N-terminal catalytic domain, followed by a C2 domain, two PEST segments, and a PDZ domain binding motif. We made mutations with sequential deletion of each C-terminal functional region. As shown in Fig. 3*A*, the deletion of the PDZ domain binding motif (the last three amino acid residues) and the PEST segments did not affect the interaction. However, the deletion of the C2 domain completely abolished the interaction. This result suggests that the C2 domain is necessary for the interaction of PTEN with MVP.

Similarly, we determined which region of MVP was responsible for the interaction. For this, we first searched any putative structural or functional domains in MVP. As reported by van Zon *et al.* (42), the MVP molecule contains three putative EF hand repeats in the N-terminal half and a coiled-coil conformation in the C-terminal half. EF hand is a helix-loop-helix motif. In general, six amino acid residues in position 1, 3, 5, 7, 9, and 12 of the loop structure are involved in calcium binding. These amino acid residues are conserved in the first two EF hand motifs, but not all are conserved in the third one though the overall homology between the second and the third EF hands (52% identity) is higher than that between the first and the second ones (42% identity). The shortest MVP clone (P-22) we identified in the yeast two-hybrid screen encodes the peptide sequence from Gln^{113} to the C terminus (Arg^{893}) and contains both the EF hand repeats and the coiled-coil sequence. Based on this clone, we made constructs that consist of the N-terminal part of P-22 (MVP113–620) including the EF hands and its C-terminal part (MVP621–893) including the coiled-coil domain, respectively. The β-galactosidase assay experiments showed that PTEN interacted with the N-terminal fragment (MVP113–620) but not with the C-terminal part (MVP621–

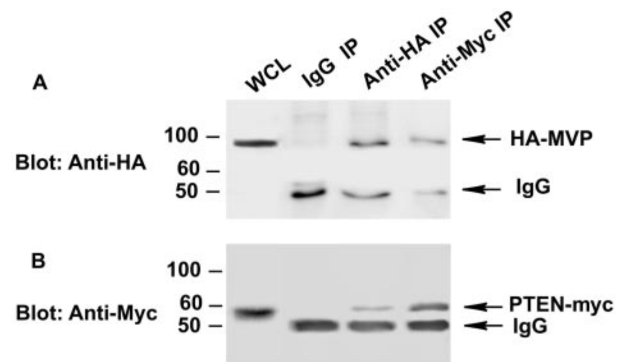

FIG. 1. **PTEN associates with MVP when co-expressed in 293T cells**. 293T cells were transfected with Myc-tagged PTEN (*PTEN-myc*) and HA-tagged C-terminal region of MVP (*HA-MVP*). Forty-eight h post-transfection, the cells were lysed, and whole cell lysates were subjected to immunoprecipitation and Western blot analysis with anti-HA (*A*) or anti-Myc (*B*) antibodies as described under "Experimental Procedures." Molecular mass (kDa) is indicated to the *left* of the gels. *WCL*, whole cell lysate; *IP*, immunoprecipitation.

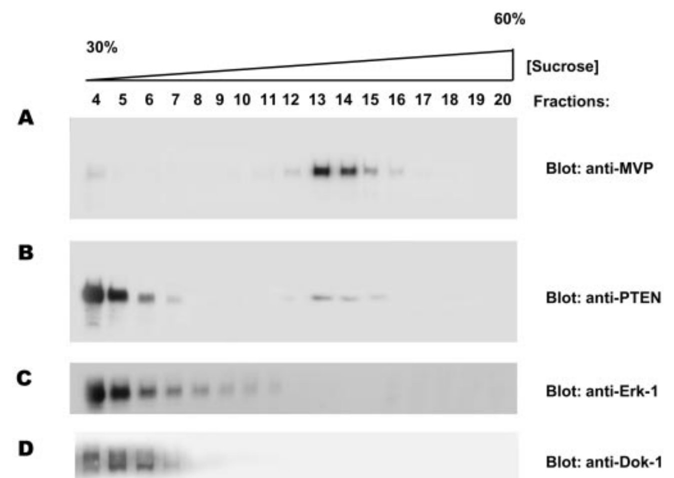

FIG. 2. **Endogenous PTEN associates with the vault particles in HeLa cells.** HeLa cells were lysed with Buffer C, and 250 μl of whole cell lysates were used for sucrose gradients as described under "Experimental Procedures." The fractions collected were subjected to Western blot analysis with anti-MVP (*A*), anti-PTEN (*B*), anti-Erk1 (*C*), and anti-Dok1 (*D*) antibodies.

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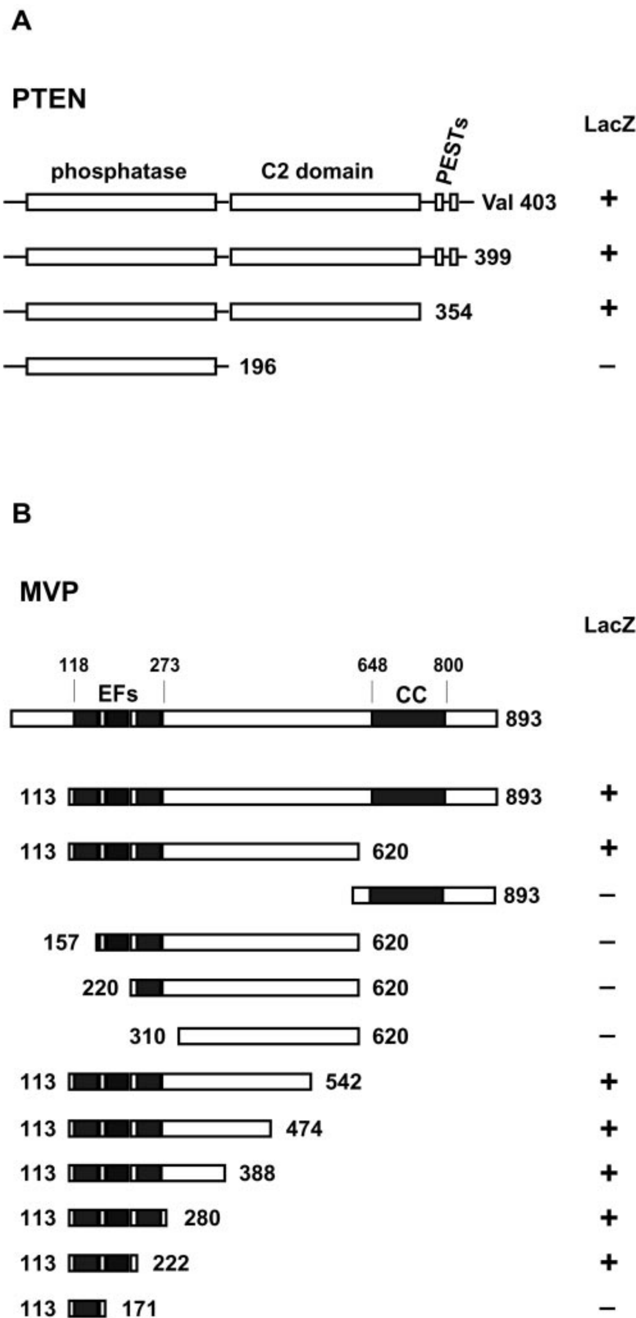

FIG. 3. **The C2 domain of PTEN interacts with the EF hands of MVP in the yeast two-hybrid assay.** The yeast strain L40α was co-transformed with pACT2-MVP and a series of pBTM-116-PTEN constructs (*A*) or with pBTM-116-PTEN and a series of pACT2-MVP constructs (*B*). The transformed yeast colonies were restreaked on a master plate and subjected to a standard colony-lift filter assay as described in the Clontech protocols. +, yeast colony turned blue in less than 30 min of incubation; −, yeast colony did not show any visible blue color during the experimental process (more than 6 h). *PESTs*, proline-, glutamic acid-, serine-, and threonine-rich regions; *CC*, coiled coil.

893) (Fig. 3*B*). Based on these results, we further made sequential deletions of the N-terminal fragment starting from both the N terminus and the C terminus. As shown in Fig. 3*B*, deletion of a short N-terminal region (from amino acid 113 to amino acid 156) containing part of the first EF hand sequence resulted in complete disruption of the interaction between MVP and PTEN, suggesting that the first EF hand is essential for the interaction. With the C-terminal deletion analysis, the interaction region for MVP was finally narrowed down to a fragment containing residues 113–222 that is composed of the first two EF hand repeats. Taken together, PTEN interacts with MVP through the C2 domain of PTEN and the first two EF hand domains of MVP.

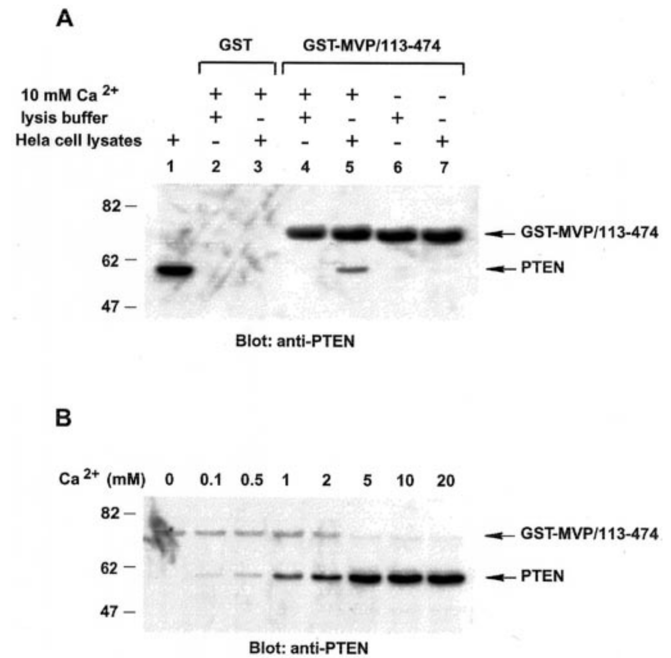

FIG. 4. **PTEN interacts with MVP *in vitro* in a Ca^{2+}-dependent manner.** GST or GST-MVP/113–474 was incubated with HeLa cell lysates or lysis buffer at 4 °C for 16 h in the presence of Ca^{2+} or in its absence as indicated. The beads were washed four times, and the bound proteins were subjected to Western blot analysis as described under "Experimental Procedures." Molecular mass (kDa) is indicated to the *left* of the gels.

The Interaction of MVP with PTEN Requires Ca^{2+}—Because EF hands are Ca^{2+} binding motifs, and the binding of Ca^{2+} induces the conformation change of EF hands, we were interested in testing whether the interaction of MVP with PTEN requires Ca^{2+}. We carried out GST pull down experiments using a GST fusion protein containing amino acid residues 113–474 of MVP (GST-MVP/113–474). As shown in Fig. 4*A*, PTEN was pulled down by GST-MVP/113–474 from HeLa cell lysates in the presence of Ca^{2+} (*lane 5*) but not in its absence (*lane 7*). In the same conditions, PTEN was not precipitated by GST alone (*lane 3*). As a negative control, we also incubated GST fusion proteins with lysis buffer in the pull down experiment to exclude the unspecific binding of anti-PTEN antibody to the GST fusion proteins or the co-purified bacteria proteins in the Western blot analysis. The band with a molecular mass of ~50 kDa recognized by anti-PTEN antibody in the Western blot analysis was not detected in the control samples (pull down of lysis buffer) (*lanes 2*, *4*, and *6*). Further experiments revealed that 5 mM Ca^{2+} is required for maximum binding (Fig. 4*B*). These results suggest that PTEN interacts with MVP in a Ca^{2+}-dependent manner.

DISCUSSION

In this study we found that endogenous PTEN associates with the vault particle, the largest intracellular ribonucleoprotein particle described to date. The precise cellular function(s) of the vault complex are not yet completely revealed. However, its unique structure and subcellular localization indicate that vault may be involved in the transport/sequestration of cellular molecules. Although the majority of vaults are present in the cytoplasm, a subset of vaults localizes to the nuclear membrane at or near the nuclear pore complexes (34). The structure of vault shows a hollow interior that is big enough to enclose a

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complex as large as an intact ribosome (35). Identification of vault cargos would verify its role in molecular transportation. It has been reported recently that the vaults associate with the estrogen receptor in the nucleus of MCF7 cells, and this association increases in response to estrogen stimulation (43). At present, the physiological function of the association between PTEN and vault is not clear. It is known that the substrate (PtdIns-3,4,5-P_3) of PTEN is localized to the membrane, whereas PTEN is predominantly distributed in the cytoplasm. However, in some cell lines, PTEN is also localized in the nucleus (12, 44–47). PTEN does not contain a nuclear localization signal, and the mechanism by which PTEN is localized to the nucleus is unknown. Because the endogenous PTEN interacts with vault that can be localized in the nuclear membrane, it is possible that vault mediate the cellular localization of PTEN.

Evidence has been emerging recently that both MVP and the vault particle are frequently up-regulated in multidrug-resistant cancer cells (reviewed in Ref. 37). Although it has not been firmly proven that the drug-resistance phenotype is directly caused by the overexpression of vaults, some studies have demonstrated that reduction of MVP expression reversed the drug-resistance phenotype, and overexpression of MVP could induce drug resistance (48–50). Proteins responsible for drug resistance usually belong to the transmembrane transporters, such as P-glycoprotein (P-gp), MDR protein 1 (MRP1), and breast cancer-resistance protein (BCRP), which act by decreasing intracellular drug concentrations (reviewed in Ref. 37). How vaults mediate drug resistance in cancer cells is unclear. It may act by binding drugs or drug-binding proteins and sequestering them or transporting them to a cellular location where the drug targets are not located. Alternatively, it may act by regulating the function of other proteins indirectly related to drug resistance. PTEN, as an enzyme that dephosphorylates PtdIns-3,4,5-P_3, negatively regulates the phosphoinositide 3-kinase pathway and cell growth. Down-regulation of PTEN activity and/or its transient translocalization to membrane will induce uncontrolled cell growth that could result in resistance to drug treatment. Hence, vaults may inhibit PTEN function through their interaction to promote drug resistance in cancer cells.

The sequential deletion experiments suggest that the interaction between these two proteins is through the C2 domain of PTEN and the two EF hands of MVP. The pair of EF hands is both necessary and sufficient for the interaction. As described above, vault consists of multiple copies of three proteins, and MVP is the major vault component. Each vault particle contains ~eight VPARP, two telomerase-associated protein 1, and 96 MVP molecules. Recently, van Zon *et al.* (42) characterized the function of each structural domain of these three proteins for the assembly of the vault particles. It was found that MVP self-associates through its C-terminal coiled-coil sequence and interacts with VPARP through its N-terminal region. MVP does not directly interact with telomerase-associated protein 1. Because the ratio of VPARP to MVP molecules in the vault particle is 8/96, the N terminus of MVP will not be completely occupied by VPARP. As a result, some of the EF hand motifs may be exposed, making it possible for vault to associate with other proteins. Both the C2 domains and the EF hands are characterized as Ca^{2+} binding domains although the C2 domain of PTEN seems not to bind Ca^{2+}. We found that the *in vitro* interaction between PTEN and MVP requires Ca^{2+}, implying that Ca^{2+} may regulate the association of PTEN with the vault particles *in vivo*. Protein-protein interaction through C2 domain and EF hands is not a general phenomenon. However, some proteins do contain both the C2 domains and the EF hands, such as the phospholipase C (PLC) family proteins (51). The crystal structure revealed that the second lobe of the EF hands of PLC-δ1 makes an interaction with its C2 domain (51). The C2 domain of PTEN has structural similarity to that of PLC-δ1 with an root mean square deviation of 1.9 Å for 75 Cα atoms (21). It would be interesting to find out whether the EF hands of MVP interact with the C2 domain of PTEN with a mechanism similar to that of the intramolecular interaction of the EF hands and the C2 domain of PLC-δ1.

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