PTEN Associates with the Vault Particles in HeLa Cells*

Zhenbao Yu§§, Nasser Fotouhi-Ardakani§§, Liangtang Wu¶, Meryem Maoui‡, Shenglong Wang‡‡, Denis Banville‡‡, and Shi-Hsiang Shen¶¶**

From §Mammalian Cell Genetics, Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada and ¶Department of Medicine, McGill University, Montreal, Quebec H3G 1A4, Canada

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 Ca²⁺-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-P₂ and PtdIns-3,4,5-P₃ (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) (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
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) antibody (clone 12CA5) used for immunoprecipitation was purchased from Roche Molecular Biochemicals, and anti-HA antibody (clone 3F10) used for Western blot was prepared from the culture medium of hybridomas (American Type Culture Collection, ATCC). Nicotrotellum membrane Hybond-K + C+ 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% CO₂ atmosphere at 37 °C.

**Yeast Two-hybrid Screen**—Full-length PTEN was amplified by PCR from a mammary gland cDNA library using Phu DNA polymerase and inserted into pGEM-T vector (Promega Corp., Madison, WI). An asparagine 92 to alanine mutant (PTEN-D92A) was generated by PCR-inserted into pGEM-T vector (Promega Corp., Madison, WI). The resulting plasmid, termed pHA-MVP/113 was constructed by inserting the MVP gene into the pACT2 vector that does not express Src kinase and was confirmed by co-transforming an MVP clone with PTEN into yeast. The interaction of MVP with PTEN was confirmed by co-transforming an MVP clone with PTEN into yeast. The presence of various concentrations of Ca²⁺, 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 Ca²⁺ as that in the binding solution, and the bound proteins were analyzed by SDS-PAGE and Western blot analysis.

**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 MgCl₂, 100 mM KCl, 1 mM dithiothreitol, 1% glycerol, 10% sucrose, 1% Nonidet P-40, and EDTA-free mixture protease inhibitors). The samples were centrifuged at 14,000 rpm (20,000 × 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 Ti65 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 their 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 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 × 10⁶ 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 Glnⁿ to the C terminus of MVP in pACT2 vector was digested with BglII, and the DNA fragment was inserted into the pCDNA3 vector (Invitrogen) at the BamHII site. The resulting plasmid, termed pHA-MVP/113–893, expresses an HA-tagged MVP fragment. A C-terminal Myc-tagged PTEN construct (pPTENmcy) was obtained by inserting the full coding region of PTEN cDNA, amplified by PCR with primers incorporating an HindIII site, into pCDNA3-myc-His-C+) vector (Invitrogen) at the same site. The resulting plasmid 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 × 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% skim 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 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 µl isopropyl-β-D-thiogalactoside at 30 °C for 6 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 × 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 4 h with 1 ml of HeLa cell lysates in the presence of various concentrations of Ca²⁺, 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 Ca²⁺ as that in the binding solution, and the bound proteins were analyzed by 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 their 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 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 × 10⁶ 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 Glnⁿ to the C terminus of MVP in pACT2 vector was digested with BglII, and the DNA fragment was inserted into the pCDNA3 vector (Invitrogen) at the BamHII site. The resulting plasmid, termed pHA-MVP/113–893, expresses an HA-tagged MVP fragment. A C-terminal Myc-tagged PTEN construct (pPTENmcy) was obtained by inserting the full coding region of PTEN cDNA, amplified by PCR with primers incorporating an HindIII site, into pCDNA3-myc-His-C+) vector (Invitrogen) at the same site. 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 Glnⁿ to the end of the C terminus of MVP in pACT2 vector was digested with BglII, and the DNA fragment was inserted into the pCDNA3 vector (Invitrogen) at the BamHII site. The resulting plasmid, termed pHA-MVP/113–893, expresses an HA-tagged MVP fragment. A C-terminal Myc-tagged PTEN construct (pPTENmcy) was obtained by inserting the full coding region of PTEN cDNA, amplified by PCR with primers incorporating an HindIII 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 × 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% skim 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 blots were developed using the Western Lightning chemiluminescence reagent kit according to the manufacturer’s instructions.
Myc antibody or an anti-HA antibody, and the immunoprecipitants were subjected to Western blot analysis. As shown in Fig. 1A, 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. 1B).

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. 3A, 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 Gln113 to the C terminus (Arg893) 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–

---

**Table I**

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

|    | AD-PTEN + Src | BD-PTEN | BD-SHP-1 + Src | BD-SHP-2 + Src |
|----|---------------|---------|---------------|---------------|
| AD-MVP | ++           | ++      | ND            | ND            |
| AD-Gref2 | ND         | ND      | +             | +             |

---

**Figure 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.

**Figure 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.
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. 3B, 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.

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. 4A, 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. 4B). 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
hand, 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 a root mean square deviation of 1.9 A 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.

Acknowledgments—We thank Dr. J. A. Cooper for kindly providing the pBTM-116-src vector, and we thank Jason Boyd and Mark Slater for helpful comments on the manuscript.

REFERENCES

1. Li, J., Yen, C., Liaw, D., Podeszpanina, K., Bose, S., Wang, S., Li, P., Miliarexis, C., Rodgers, L., McCombie, R., Bigner, S. H., Giovannella, B. C., Ittmann, M., Tycko, B., Hulisboho, H., Wigler, M. H., and Parsons, R. (1997) Science 275, 1943–1947
2. Steck, P. A., Parisoule, M. A., Jasser, S. A., Yung, W. K., Lin, H., Ligon, A. H., Langford, L. A., Baumgard, M. L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D. H., and Tavtigian, S. V. (1997) Nat. Genet. 15, 356–362
3. Li, D., and Sun, H. (1997) Cancer Res. 57, 2124–2129
4. Marsh, D. J., Dahia, P. L., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R. J., and Eng, C. (1997) Nat. Genet. 16, 333–349
5. Liaw, D., Marsh, D. J., Li, J., Dahia, P. L., Wang, S.-I., Zheng, Z., Bose, S., Call, K. M., Tsou, H. C., Peacock, M., Eng, C., and Parsons, R. (1997) Nat. Genet. 16, 64–67
6. Lynch, E. D., Osterrner, E. A., Lee, M. K., Arena, J. F., Ji, H., Dann, J., Swaminathan, K., Suchard, D., MacLeod, P. M., Ruland, C., Beitsch, P., Finlay, D. T., Haimstad, K., Lubs, H., Malert, P., and King, M. C. (1997) Am. J. Hum. Genet. 61, 1254–1260
7. Nemen, M. R., van Staveren, C. G., Peters, E. A. J., Ben Hassel, M., Gorlin, R. J., Hammad, L., Lindseth, C. F., Fryns, J. P., Sjödin, R. H., Woods, D. G., Mariman, E. C. M., Padberg, G. W., and Kremer, H. (1997) Hum. Mol. Genet. 6, 1383–1387
8. Di Cristofano, A., Pesce, B., Carden-Cordos, C., and Pandolfi, P. P. (1998) Nat. Genet. 19, 348–355
9. Stambolic, V., Suzuki, A., de la Pompa, J. L., Brothers, G. M., Mirtos, C., Sassaki, T., Ruland, J., Penninger, J. M., Siderovski, D. P., and Mak, T. W. (1998) Cell 95, 29–39
10. Podeszpanina, K., Ellenhoff, L. N., Nemes, A., Gu, J., Tamura, M., Yamada, K. M., Carden-Cordos, C., Catorretti, G., Fisher, P. E., and Parsons, R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1563–1568
11. Myers, M. P., Stolarow, J. P., Eng, C., Li, J., Wang, S. I., Wigler, M. H., Parsons, R., and Tonks, N. K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9052–9057
12. Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R., and Yamada, K. (1998) Science 280, 1614–1617
13. Machama, T., and Dixon, J. E. (1998) J. Biol. Chem. 273, 13375–13378
14. Machama, T., Taylor, G. S., and Dixon, J. E. (2001) Arterioles: Res. Biochem. 20, 477–729
15. Simpson, L., and Parsons, R. (2001) Exp. Cell Res. 264, 29–41
16. Wu, X., Hepner, K., Castelino-Prabhu, S., Do, D., Kaye, M. B., Yuan, X. J., Mills, G. B., Lu, Y., Fang, X., Wang, H., Eder, A., Mao, M., Swaby, R., Cheng, K. W., Stokoe, D., Siminovitch, K., Jaffe, R., and Gray, J. (2001) J. Biol. Chem. 276, 844–851
17. Yamada, K. M., and Araki, M. (2001) Biochem. Soc. Trans. 29, 2147–2150
18. Wu, X., Hepner, K., Castelino-Prabhu, S., Do, D., Kaye, M. B., Yuan, X. J., Mills, G. B., Lu, Y., Fang, X., Wang, H., Eder, A., Mao, M., Swaby, R., Cheng, K. W., Stokoe, D., Siminovitch, K., Jaffe, R., and Gray, J. (2001) Semin. Oncol. 28, 125–141
19. Georgescu, M. M., Kirsch, K. H., Akagi, T., Shishido, T., and Hanauska, H. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 10182–10187
20. Lee, J. O., Yang, H., Georgescu, M. M., Di Cristofano, A., Machama, T., Shi, Y., Dixon, J. E., Pandolfi, P., and Pavletich, N. P. (1999) Cell 99, 323–334
21. Wu, X., Hepner, K., Castelino-Prabhu, S., Do, D., Kaye, M. B., Yuan, X. J., Wu, J., Ross, C., Sawaya, C. I., and Wang, Y. (2001) Proc. Natl. Acad. Sci. U. S. A. 97, 4233–4238
22. Wu, X., Dowdenko, D., Spencer, S., Laura, R., Lee, J., Gu, Q., and Lasyk, L. A. (2000) J. Biol. Chem. 275, 21477–21485
23. Wu, X., Huang, L., Ormonde, P. A., Baumgard, M. L., Pero, R., Byreddy, D. V., Tavtigian, S. V., and Bartel, P. L. (2000) Cancer Res. 60, 35–37
24. Lee, N. R., Gray, A., Pass, I., Orchiston, E. A., and Downes, C. P. (2000) Biochem. J. 346, 827–833
25. Kedersha, N. L., and Rome, L. H. (1986) J. Cell Biol. 103, 699–709
26. Kedersha, N. L., Heuser, J. E., Chuang, D. C., and Rome, L. H. (1991) J. Cell Biol. 113, 225–235
27. Kickhoefer, V. A., Siva, A. C., Kedersha, N. L., Inman, E. M., Ruland, C., Streuli, M., and Rome, L. H. (1999) J. Cell Biol. 146, 917–928
28. Kickhoefer, V. A., Stephen, A. G., Harrington, L., Robinson, M. O., and Rome, L. H. (1999) J. Biol. Chem. 274, 32715–32717
29. Kickhoefer, V. A., Searles, R. P., Kedersha, N. L., Garber, M. E., Johnson, D. L., and Rome, L. H. (1999) J. Biol. Chem. 274, 7868–7872
30. Chiang, D. C., Rome, L. H., and Kedersha, N. L. (1993) J. Cell Sci. 106, 23–29
31. Kong, L. B., Siva, A. C., Rome, L. H., and Stewart, P. L. (1999) Structure 7, 371–379
Interaction of PTEN with Vaults

40252

36. Scheffer, G. L., Wijngaard, P. L. J., Flens, M. J., Izquierdo, M. A., Slovak, M. L., Pinedo, H. M., Meijer, C. J. L. M., Clevers, H. C., and Scheper, R. J. (1995) *Nat. Med.* 1, 578–582

37. Scheffer, G. L., Schroeijers, A. B., Izquierdo, M. A., Wiemer, E. A., and Scheper, R. J. (2000) *Curr. Opin. Oncol.* 12, 550–556

38. Keegan, K., and Cooper, J. A. (1996) *Oncogene* 12, 1537–1544

39. Flint, A. J., Tiganis, T., Barford, D., and Tonks, N. K. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1680–1685

40. Wu, L., Fu, J., and Shen, H.-S. (2002) *Mol. Cell. Biol.* 22, 2673–2686

41. Yu, Z., Su, L., Hoglinger, O., Jaramillo, M. L., Banville, D., and Shen, S.-H. (1998) *J. Biol. Chem.* 273, 3687–3694

42. van Zon, A., Morsink, M. H., Schoester, M., Scheffer, G. L., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2002) *Biochem. Biophys. Res. Commun.* 291, 535–541

43. Abbondanza, C., Rossi, V., Ruscigno, A., Gallo, L., Belsito, A., Piluso, G., Medici, N., Nigrin, V., Molinari, A. M., Moncharmont, B., and Puca, G. A. (1998) *J. Cell Biol.* 141, 1301–1310

44. Gimn, O., Perren, A., Weng, L. P., Marsh, D. J., Yeh, J. J., Ziebold, U., Gil, R., Hinze, R., Delbridge, L., Lees, J. A., Robinson, B. G., Komminoth, P., Dralle, H., and Eng, C. (2000) *Am. J. Pathol.* 156, 1693–1700

45. Lachyanvark, M. B., Sultana, N., Schonherr, C. M., Mitra, P., Poluha, W., Lambert, S., Quesenberry, P. J., Litofsky, N. S., Recht, L. D., Nabi, R., Miller, S. J., Ohta, S., Neel, B. G., and Ross, A. H. (2000) *J. Neurosci.* 20, 1404–1413

46. Perren, A., Komminoth, P., Saremaslani, P., Matter, C., Feurer, S., Lees, J. A., Heitz, P. U., and Eng, C. (2000) *Am. J. Pathol.* 157, 1097–1103

47. Whiteman, D. C., Zhou, X. P., Cummings, M. C., Pavey, S., Hayward, N. K., and Eng, C. (2002) *Int. J. Cancer* 99, 63–67

48. Kitazono, M., Sumizawa, T., Takehayaishi, Y., Chen, Z. S., Furukawa, T., Nagayama, S., Tani, A., Takao, S., Aikou, T., and Akiyama, S. (1999) *J. Natl. Cancer Inst.* 91, 1647–1653

49. Cheng, S. H., Lam, W., Lee, A. S., Fung, K. P., Wu, R. S., and Fong, W. F. (2000) *Toxicol. Appl. Pharmacol.* 164, 134–142

50. Hu, Y., Stephen, A. G., Cao, J., Tanzer, L. R., Slapak, C. A., Harrison, S. D., Devanarayan, V., Dantzig, A. H., Starling, J. J., Rome, L. H., and Moore, R. E. (2002) *Int. J. Cancer* 97, 149–156

51. Williams, R. L. (1999) *Biochim. Biophys. Acta* 1441, 255–267