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Vinculin Controls PTEN Protein Level by Maintaining the Interaction of the Adherens Junction Protein β-Catenin with the Scaffolding Protein MAGI-2*

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M. Cecilia Subauste‡§, Perihan Nalbant‡, Eileen D. Adamson¶, and Klaus M. Hahn‖

From the ‡Department of Cell Biology, The Scripps Research Institute, La Jolla, California 92037, ¶The Burnham Institute, La Jolla Cancer Research Center, La Jolla, California 92037, and ‖Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina 27599-7365

PTEN is a frequently mutated tumor suppressor in malignancies. Interestingly, some malignancies exhibit undetectable PTEN protein without mutations or loss of PTEN mRNA. The cause(s) for this reduction in PTEN is unknown. Cancer cells frequently exhibit loss of cadherin, β-catenin, α-catenin and/or vinculin, key elements of adherens junctions. Here we show that F9 vinculin-null (vin$^{-/-}$) cells lack PTEN protein despite normal PTEN mRNA levels. Their PTEN protein expression was restored by transfection with vinculin or by inhibition of PTEN degradation. F9 vin$^{-/-}$ cells express PTEN protein upon transfection with a vinculin fragment (amino acids 243–1066) that is capable of interacting with α-catenin but unable to target into focal adhesions. On the other hand, disruption of adherens junctions with an E-cadherin blocking antibody reduced PTEN protein to undetectable levels in wild-type F9 cells. PTEN protein levels were restored in F9 vin$^{-/-}$ cells upon transfection with an E-cadherin-α-catenin fusion protein, which targets into adherens junctions and interacts with β-catenin in F9 vin$^{-/-}$ cells. β-Catenin is known to interact with MAGI-2. MAGI-2 interaction with PTEN in the cell membrane is known to prevent PTEN protein degradation. Thus, MAGI-2 overexpression in F9 vin$^{-/-}$ cells restored PTEN protein levels. Moreover, expression of vinculin mutants that reinstated the disrupted interactions of β-catenin with MAGI-2 in F9 vin$^{-/-}$ cells also restored PTEN protein levels. These studies indicate that PTEN protein levels are dependent on the maintenance of β-catenin-MAGI-2 interaction, in which vinculin plays a critical role.

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN)[1] is a lipid phosphatase that acts as a tumor suppressor by negatively controlling the phosphoinositide 3-kinase/Akt signaling pathway (1). PTEN belongs to a class of "gatekeeper" tumor suppressors together with p53, retinoblastoma, and adenomatous polyposis coli (1). These proteins are involved in the regulation of normal cell growth, and their mutations contribute directly to carcinogenesis (1). PTEN is one of the most frequently mutated proteins in cancer, but reduction in PTEN protein levels cannot be attributed only to observed mutations (2, 3). In cases of prostatic carcinoma, leukemia, and lymphoma, PTEN protein levels are severely reduced without detectable PTEN mutations or altered PTEN mRNA expression (2, 3). The cause of the reduced PTEN protein expression in these malignancies remains largely unknown.

A prevalent feature in various malignancies is decreased expression of adherens junctions proteins, including E-cadherin, α-catenin, β-catenin, and/or vinculin (4–6). Adherens junctions are multiprotein complexes with one of the highest cellular concentrations of signaling proteins (7). Among these signaling proteins are members of the membrane-associated guanylate kinase family (8, 9). These scaffold proteins play a critical role in signal transduction by optimizing the functional activity and stability of their ligands (10, 11). PTEN interaction with members of the membrane-associated guanylate kinase family, such as membrane-associated guanylate-kinase inverted 2 (MAGI-2), prevents PTEN degradation (12, 13). MAGI-2, also known as synaptic scaffolding molecule (14), has been shown to interact with β-catenin (15), a key element of the adherens junction (16, 17). β-Catenin is involved in the adherens junction assembly through its interaction with α-catenin and with cadherin. α-Catenin directly links the Cadherin-β-catenin complex to the cytoskeleton. The interactions of α-catenin with various cytoskeletal proteins are indispensable for adherens junction assembly and function (16–18). Among these cytoskeletal proteins is vinculin (16–18). In this study, we found that cells devoid of vinculin have severely reduced PTEN levels despite having normal levels of PTEN mRNA. This was due to a disruption in the interaction of β-catenin with MAGI-2. This study reveals a novel mechanism of PTEN regulation whereby its protein level is controlled through the maintenance of β-catenin-MAGI-2 interaction, with the ubiquitous cytoskeletal protein vinculin playing a central role.

EXPERIMENTAL PROCEDURES

Cell Culture—The following cell lines were used: wild-type (WT) F9 cells; F9 vin$^{-/-}$ cells; and F9 vin$^{-/-}$ cells stably transfected with plasmid vectors expressing full-length vinculin (F9 vin$^{-/-}$ rescue), a fragment containing the sequences of the head portion of vinculin (F9 vin$^{-/-}$ head; amino acids 1–821); and cells expressing the vinculin fragment 243–1066 in which the talin binding sites were deleted (F9 vin$^{-/-}$ Δ talin BS) (19). Cells were cultured as reported previously (19).

Reverse Transcription-PCR—Total RNA was isolated from both WT F9 and F9 vin$^{-/-}$ cells (3×10^6 cells) using RNeasy Mini Kit from Qiagen according to the manufacturer's instructions. First-strand

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§ To whom correspondence should be addressed: Dept. of Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: 858-784-7108; Fax: 858-784-7333; E-mail: mcsub@scripps.edu.

[1] The abbreviations used are: PTEN, phosphatase and tensin homologue deleted on chromosome 10; WT, wild-type; Ab, antibody; ERK, extracellular signal-regulated kinase; Δ talin bs, deleted talin binding sites; E-Cad-α-cat, E-cadherin-α-catenin; MAGI, membrane-associated guanylate-kinase inverted.

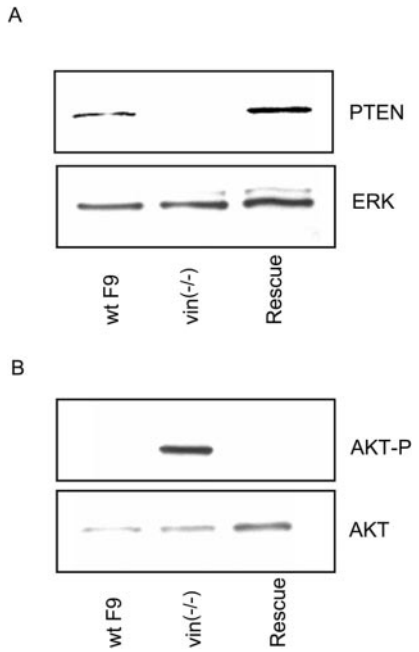

FIG. 1. **PTEN is restored to WT level and activity in F9 vin$^{-/-}$ cells transfected with vinculin.** *A*, detergent lysates of WT F9 cells, F9 vin$^{-/-}$, and F9 vin$^{-/-}$ rescue cells were analyzed by immunoblotting using anti-PTEN and anti-ERK1/2 Ab. The result shown is a representative example from 10 experiments. *B*, cell lysates of WT F9, F9 vin$^{-/-}$, and F9 vin$^{-/-}$ rescue cells were analyzed by immunoblotting using anti-Akt and anti-phospho-Akt Ab. Results of one representative experiment of five are shown.

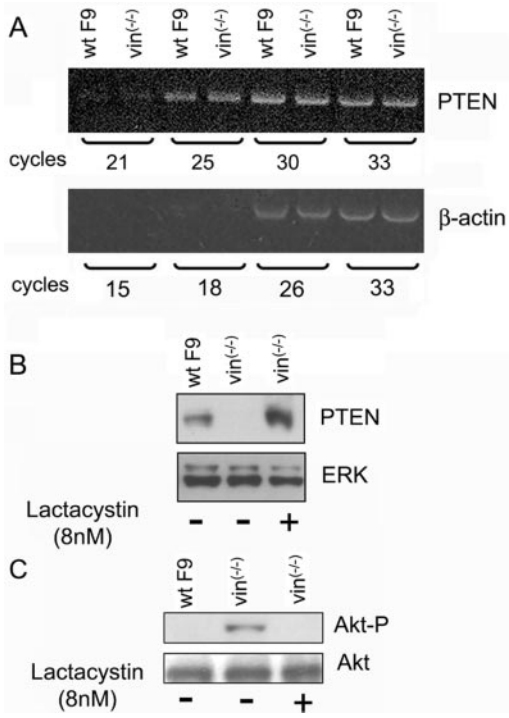

FIG. 2. **PTEN mRNA levels are the same in WT F9 cells and F9 vin$^{-/-}$ cells, and PTEN levels are restored in F9 vin$^{-/-}$ cells upon proteasome inhibition.** *A*, levels of PTEN and β-actin mRNA in WT F9 and F9 vin$^{-/-}$ cells were assessed by reverse transcription-PCR. Aliquots drawn at the indicated cycles were visualized on an agarose gel. WT F9 cells, *lanes 1*, *3*, *5*, and *7*. F9 vin $^{-/-}$ cells, *lanes 2*, *4*, *6*, and *8*. Representative results from one of two experiments are shown. *B*, detergent lysates from WT F9, F9 vin$^{-/-}$, and F9 vin$^{-/-}$ cells treated for 36 h with lactacystin (8 nM) were analyzed by immunoblotting using anti-PTEN and anti-ERK Ab. The result shown is a representative example from three experiments. *C*, cell lysates from WT F9, F9 vin$^{-/-}$, and F9 vin$^{-/-}$ cells treated for 36 h with lactacystin (8 nM) were analyzed by immunoblotting using anti-Akt and anti-phospho-Akt Ab. Results of one representative experiment of three are shown.

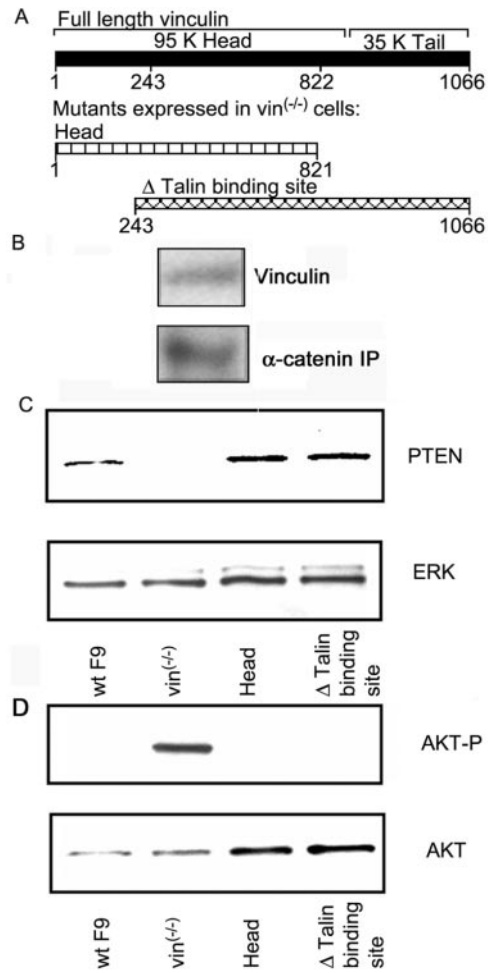

FIG. 3. **PTEN expression and activity in F9 vin$^{-/-}$ cells transfected with various vinculin constructs.** *A*, vinculin mutants. *Top line*, full-length vinculin. *Second line*, the head fragment (amino acids 1–821). *Third line*, the vinculin fragment in which talin binding sites were deleted (amino acids 243–1066). *B*, cell lysates from F9 vin$^{-/-}$ Δ talin bs cells were immunoprecipitated with an anti-α-catenin antibody, followed by immunoblotting with an antibody against vinculin. Representative results from one of two experiments are shown. *C*, detergent lysates of these cell lines were analyzed by immunoblotting using anti-PTEN and anti-ERK1/2 Ab. Results from one representative experiment of three are shown. *D*, cell lysates of WT F9, F9 vin$^{-/-}$, F9 vin$^{-/-}$Δ talin bs, and F9 vin$^{-/-}$ head cells were analyzed by immunoblotting using anti-Akt and anti-phospho-Akt Ab. Representative results from one of three independent experiments are shown.

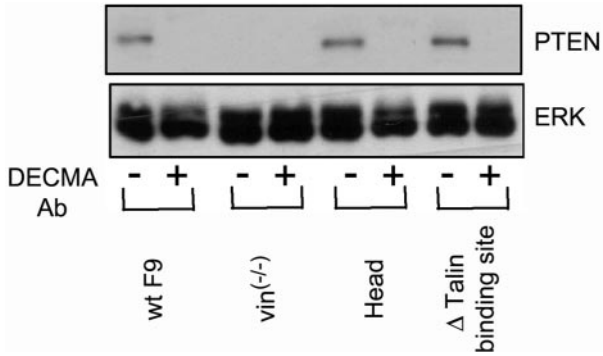

FIG. 4. **Disruption of adherens junction with a function-perturbing anti-E-cadherin antibody, DECMA-1, reduces PTEN protein expression in WT F9, F9 vin$^{-/-}$ Δ talin bs, and F9 vin$^{-/-}$ head cells.** Detergent lysates of WT F9, F9 vin$^{-/-}$, F9 vin$^{-/-}$ Δ talin bs, and F9 vin$^{-/-}$ head cells treated with or without the neutralizing antibody against E-cadherin (DECMA-1) were analyzed by immunoblotting using anti-PTEN and anti-ERK1/2 Ab. Results from one representative experiment of three are shown.

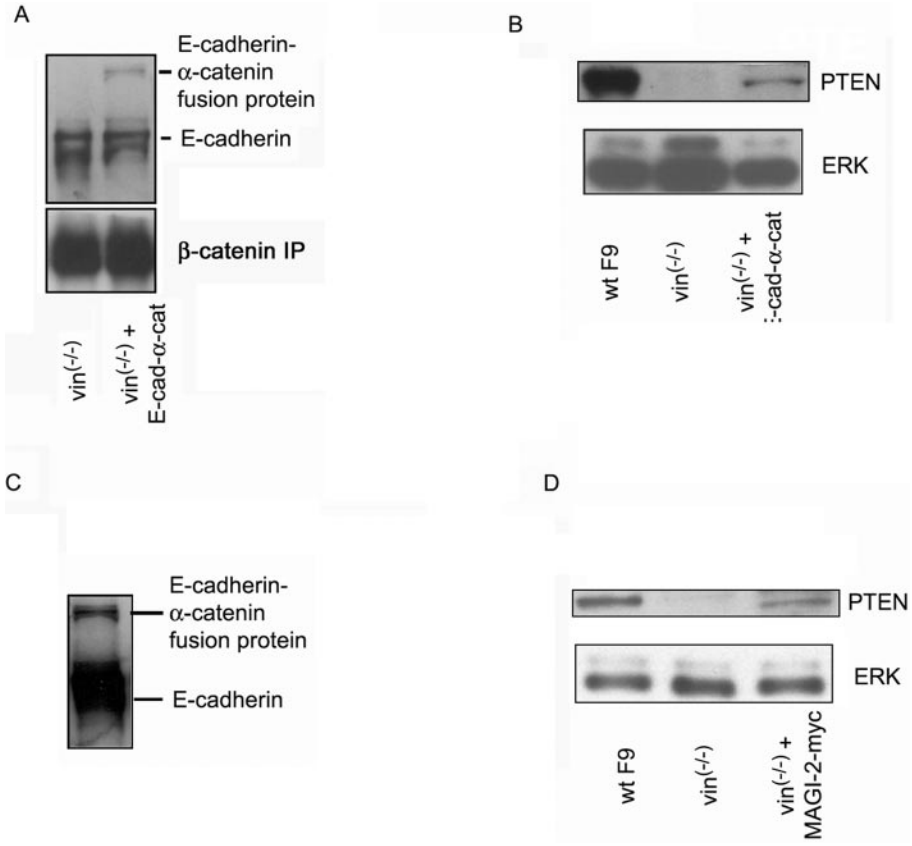

FIG. 5. **Transfection of E-cadherin-α-catenin fusion protein or MAGI-2 leads to PTEN expression in F9 vin$^{-/-}$ cells.** *A*, cell lysates from F9 vin$^{-/-}$ cells transfected with an E-cadherin-α-catenin (*E-cad-α-cat*) fusion protein were immunoprecipitated with an anti-β-catenin antibody, followed by immunoblotting with an antibody against E-cadherin. Representative results from one of two experiments are shown. Cell lysates from F9 vin$^{-/-}$ cells transfected with E-cadherin-α-catenin protein were analyzed by immunoblotting using anti-PTEN and anti-ERK1/2 Ab (*B*) and anti-E-cadherin Ab (*C*). The results shown in *B* and *C* are representative examples from three independent experiments. *D*, detergent lysates of F9 vin$^{-/-}$ cells transfected with MAGI-2-myc-tagged protein were analyzed by immunoblotting using anti-PTEN and anti-ERK1/2 Ab. Results from one of two experiments are shown.

cDNA was reverse-transcribed from 1 μg of total RNA using avian myeloblastosis virus reverse transcriptase (Promega) with oligo(dT)$_{15}$ as primer. The reaction volume was 20 μl, of which 5 μl were used to amplify a fragment of the β-actin gene for semiquantitative analysis of the amount of total RNA added to the PCRs. Amplification of the 285-bp PTEN fragment was performed with 10 μl of the reverse transcription reaction. Oligonucleotide primers for the PCR amplification were based on the published PTEN sequence (GenBank™ accession no. U93051) and β-actin sequence (GenBank™ accession no. X00351), respectively. The primer sequences are as follows: PTENf, GATTTCTATGGGGAAGTAAGGA; PTENrev, GTAACGGCTGAGGGAACTC; actinf, GATATCGCCGCGCTCGTCGTCGAC; and actinrev, CAGGAAGGAAGGCTGGAAGAGTGC. PCRs were performed in a 50-μl volume containing 10 mM Tris (pH 8.4), 50 mM KCl, 1 mM $MgCl_2$, oligonucleotide primers (0.1 μM of each), 0.2 mM dATP, 0.2 mM dTTP, 0.2 mM dGTP, 0.2 mM dCTP, and 2.5 units of *Taq* DNA polymerase. PCR cycles were as follows: 94 °C for 2 min; followed by 30–36 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; and a final cycle of 72 °C for 5 min. Samples (6 μl) were drawn from the PCR at the indicated cycles to be visualized on an agarose gel.

Preparation of Protein Extracts, Immunoblotting, and Immunoprecipitation—For immunoblotting analyses, cells were lysed in a buffer containing 20 mM Tris-HCl (pH 8), 100 mM NaCl, 10% glycerol, 1% Triton X-100, 50 mM NaF, 1 mM sodium vanadate, 1 mM benzamidine, 5 μg ml^{-1} leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The cell lysates were clarified at 10,000 × *g* for 10 min at 4 °C. Samples were separated on SDS-polyacrylamide gels (Bio-Rad). Proteins were transferred to Immobilon-P transfer membranes (Millipore Corp.) blocked and probed with the following antibodies (Abs): rabbit anti-ERK1/2 Ab, rabbit anti-Akt Ab, rabbit anti-phospho-Akt Ab, and rabbit anti-PTEN Ab (all from Cell Signaling Technology); rabbit anti-α-catenin Ab (Chemicon Corp.); mouse monoclonal anti-E-cadherin Ab and mouse monoclonal anti- β-catenin Ab (both from BD Biosciences); rabbit anti-MAGI-2 (Santa Cruz Biotechnology). Bound antibody was detected by incubation with secondary antibodies conjugated with horseradish peroxidase (rabbit and mouse horseradish peroxidase-derived Ab were from Cell Signaling, and rabbit horseradish peroxidase-derived Ab was from Santa Cruz Biotechnology) and visualized by enhanced chemiluminescence (Pierce). Equal loading in each lane was verified by assessing ERK1/2 concentration.

Immunoprecipitation studies were carried out according to a method reported previously (17), with some minor modifications, using the mouse anti-β-catenin Ab, rabbit anti-α-catenin Ab, and rabbit anti-MAGI-2 Ab mentioned in the immunoblotting studies, as well as a mouse anti-PTEN Ab (Santa Cruz Biotechnology) and a mouse anti-paxillin Ab (BD Biosciences) at a concentration of 1:300.

Plasmids and Transfection—E-cadherin-α-catenin construct (18) was kindly provided by Dr. Akira Nagafuchi, and pcDNA3 MAGI-2-myc-tagged protein (11) was a gift from Dr. Charles Sawyers. Transient transfections were done using Lipofectamine and Plus Reagent (Invitrogen) following the manufacturer's instructions. In brief, F9 cell lines were transfected with 8 μl of Lipofectamine, 16 μl of Plus Reagent, and 7 μg of the plasmids. Cells were used 48 h after transfection. The protein expression of MAGI-2-myc-tagged protein and E-cadherin-α-catenin (E-cad-α-cat) was confirmed by immunoblotting with a mouse anti-myc Ab (Sigma) and a mouse anti-E-cadherin Ab (BD Biosciences), respectively.

Cadherin Blocking Experiments—Neutralizing antibody against E-cadherin (DECMA-1 Ab; Accurate Chemical & Scientific Corp.) was mixed at a concentration of 30 μg ml^{-1} with confluent cells in α-minimum Eagle's medium containing 10% fetal bovine serum (Invitrogen). After 18 h, cells were lysed, and PTEN protein levels were assessed by immunoblotting.

RESULTS

PTEN Protein Expression Is Undetectable in F9 vin$^{-/-}$ Cells and Is Restored upon Proteasome Inhibition—Mouse F9 vin$^{-/-}$ embryonal carcinoma cells had undetectable PTEN protein levels (Fig. 1*A*). F9 vin$^{-/-}$ cells re-expressing vinculin to WT levels (F9 vin$^{-/-}$ rescue cells) (19, 20) exhibit a restoration of PTEN expression to the levels seen in WT F9 cells. The restoration of PTEN activity in F9 vin$^{-/-}$ rescue cells was reflected in reduction of the increased Akt phosphorylation seen in F9 vin$^{-/-}$ cells (Fig. 1*B*). Thus, vinculin is involved in the regulation of PTEN protein expression.

PTEN protein levels can be controlled by different mechanisms (3). In this regard, PTEN protein levels can be regulated at the transcriptional level by transcription factors such as p53 or Egr-1 (21, 22). As seen in Fig. 2*A*, PTEN mRNA

levels were similar in F9 vin$^{-/-}$ cells and WT F9 cells, so decreased PTEN in F9 vin$^{-/-}$ cells could not be explained by loss of mRNA expression. PTEN protein levels can also be regulated by a proteasome-mediated proteolytic degradation (13, 23). As seen in Fig. 2*B*, treatment with lactacystin (Sigma), a potent proteasome inhibitor, led to restoration of PTEN expression in F9 vin$^{-/-}$ cells. Lactacystin treatment also led to a reduction of the increased Akt phosphorylation seen in F9 vin$^{-/-}$ cells (Fig. 2*C*). These data indicated that vinculin regulates PTEN protein stability by affecting its proteasome-mediated proteolytic degradation.

PTEN Levels Are Restored in F9 vin$^{-/-}$ Cells Transfected with Vinculin Constructs That Interact with α-Catenin, Whereas Disruption of Adherens Junctions Reduces PTEN Expression in Wild-type Cells—Vinculin is a major cytoskeletal protein present in focal adhesions and adherens junctions (24). In order to address how vinculin controls PTEN protein expression, F9 vin$^{-/-}$ cells were stably transfected with a vinculin head fragment (F9 vin$^{-/-}$ head cells) or a vinculin fragment with the talin binding sites deleted (F9 vin$^{-/-}$ Δ talin bs cells) (19) (Fig. 3*A*), and the PTEN protein levels in these cells were compared with the levels seen in WT F9 cells and F9 vin$^{-/-}$ cells. Vinculin head domain is known to target into focal adhesions as well as adherens junctions (17). The vinculin Δ talin bs fragment cannot target into focal adhesions (25) but can still interact with α-catenin, a protein present only in the adherens junctions (Fig. 3*B*). As shown in Fig. 3, *C* and *D*, PTEN protein level and activity were restored to wild-type levels in F9 vin$^{-/-}$ cells expressing the vinculin head as well as Δ talin bs fragments. On the other hand, incubation with DECMA-1 Ab, an antibody known to block E-cadherin-mediated adherens junction formation (26), led to a loss of PTEN protein expression in WT F9 cells as well as in F9 vin$^{-/-}$ head and F9 vin$^{-/-}$ Δ talin bs cells (Fig. 4). These data indicated that regulation of PTEN protein level by vinculin is at the adherens junction.

Transfection of E-Cadherin-α-Catenin Fusion Protein or MAGI-2 Restores PTEN Levels in F9 vin$^{-/-}$ Cells—It has been demonstrated previously that adherens junction organization is impaired in F9 vin$^{-/-}$ cells (16). Vinculin plays an important role in adherens junction organization and function through its interaction with the cytoskeleton (18). Therefore, PTEN protein levels were examined in F9 vin$^{-/-}$ cells transfected with an E-cadherin-α-catenin fusion protein because this protein produces cadherin-based cell adhesion activity even in the absence of vinculin (18). This fusion protein includes the complete α-catenin protein; therefore, the β-catenin binding site is preserved (27). In fact, we found that this fusion protein interacts with β-catenin in F9 vin$^{-/-}$ cells (Fig. 5*A*). As shown in Fig. 5*B*, expression of the E-cadherin-α-catenin fusion protein restored PTEN levels in F9 vin$^{-/-}$ cells. Restoration was not complete, likely because the fusion protein was poorly expressed compared with native E-cadherin (Fig. 5*C*).

The activity and stability of PTEN are dependent on its interaction with the PSD-95/Discs-large/ZO-1 homology domains of proteins belonging to the membrane-associated guanylate kinase family, such as MAGI-2 or -3 (11, 13, 28). These scaffold proteins are known to localize into adherens junction and interact with β-catenin (8, 15). Overexpression of MAGI-2 led to restoration of PTEN protein expression in F9 vin$^{-/-}$ cells (Fig. 5*D*).

Expression of Vinculin Mutants That Restored PTEN Protein Levels Also Restored the Disrupted Interactions of β-Catenin with MAGI-2 in F9 vin$^{-/-}$ Cells—Thus far, we have found that the control of PTEN protein expression by vinculin is at the level of the adherens junction (Figs. 3, 4, and 5*B*). Furthermore, PTEN protein stability is restored upon overexpression of

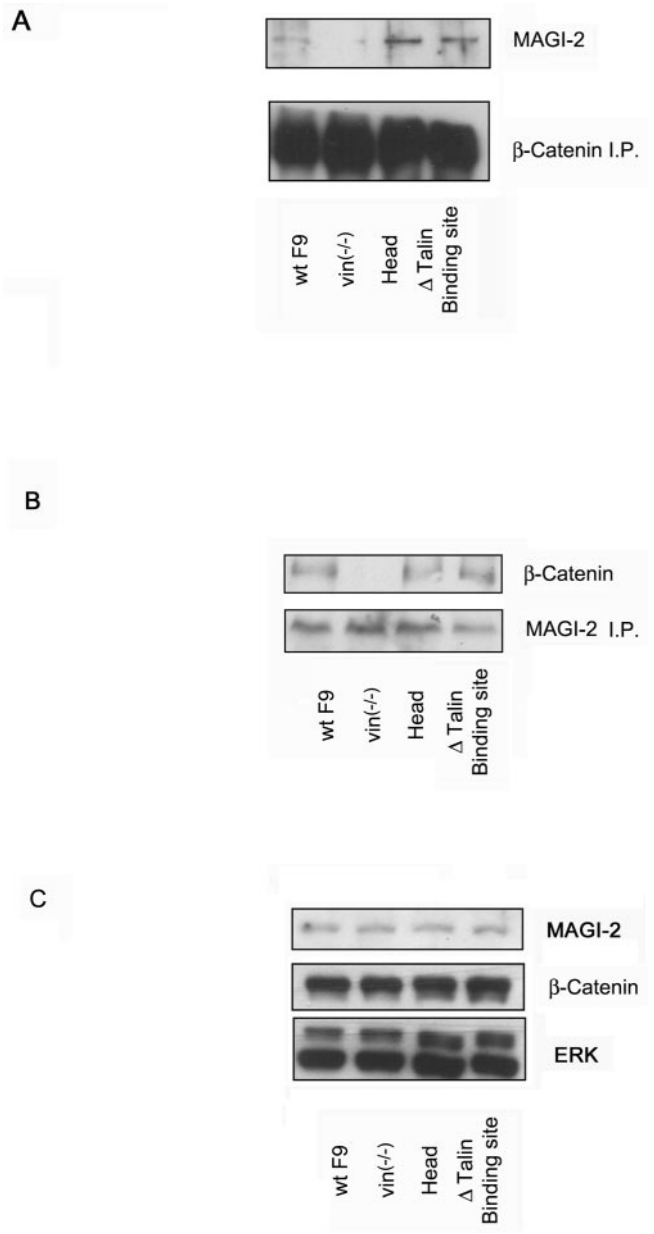

FIG. 6. **Decreased binding of β-catenin MAGI-2 in F9 vin$^{-/-}$ cells is restored to WT levels in F9 vin$^{-/-}$Δ talin bs and F9 vin$^{-/-}$ head cells.** *A*, cell lysates from WT F9, F9 vin$^{-/-}$, F9 vin$^{-/-}$Δ talin bs, and F9 vin$^{-/-}$ head cells were immunoprecipitated with an anti-β-catenin antibody, followed by immunoblotting with an antibody against MAGI-2. Representative results from one of two independent experiments are shown. *B*, cell lysates from WT F9, F9 vin$^{-/-}$, F9 vin$^{-/-}$Δ talin bs, and F9 vin$^{-/-}$ head cells were immunoprecipitated with an anti-MAGI-2 antibody, followed by immunoblotting with an antibody against β-catenin. Representative results from one of two independent experiments are shown. *C*, detergent cell lysates from WT F9, F9 vin$^{-/-}$, F9 vin$^{-/-}$Δ talin bs, and F9 vin$^{-/-}$ head cells were analyzed by immunoblotting using anti-β-catenin, anti-MAGI-2, and anti-ERK Ab. Representative results from one of three independent experiments are shown.

MAGI-2 (Fig. 5*D*). This scaffold protein is known to bind to the adherens junction protein β-catenin. Because F9 vin$^{-/-}$ cells exhibit a disruption of adherens junction organization (16), their loss of PTEN protein expression could be associated with an impaired β-catenin-MAGI-2 interaction. To gauge the level of MAGI-2-β-catenin interaction in different cell lysates, either MAGI-2 or β-catenin was immunoprecipitated, and immunoblotting was used to probe for the presence of the binding partner (Fig. 6, *A* and *B*). As shown in Fig. 6, *A* and *B*, inter-

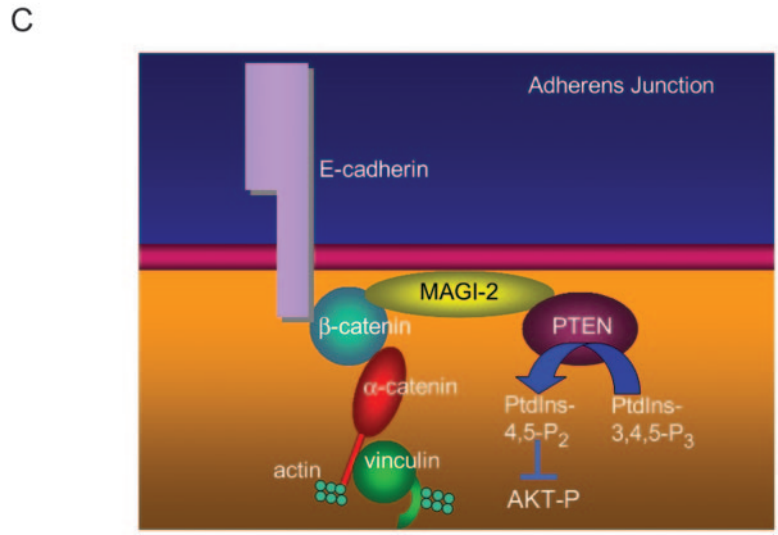

FIG. 7. **Vinculin regulates PTEN protein level by maintaining the interaction of β-catenin with MAGI-2.** *A*, cell lysates from WT F9 cells were immunoprecipitated with an anti-PTEN antibody, followed by immunoblotting with an antibody against MAGI-2 as well as β-catenin. Representative results from one of two independent experiments are shown. *B*, cell lysates from WT F9 cells were immunoprecipitated with an anti-paxillin antibody, followed by immunoblotting with an antibody against MAGI-2 as well as β-catenin. Representative results from one of two independent experiments are shown. *C*, model for the role of vinculin in controlling PTEN protein expression and function by maintaining the interaction of β-catenin with MAGI-2.

action of β-catenin with MAGI-2 was decreased in F9 vin$^{-/-}$ cells. MAGI-2-β-catenin interaction was restored to WT levels in the PTEN-expressing F9 vin$^{-/-}$ Δ talin bs and F9 vin$^{-/-}$ head cells (Fig. 6, *A* and *B*). There was no difference in β-catenin and MAGI-2 protein levels among WT F9, vin$^{-/-}$, vin$^{-/-}$ head, and vin$^{-/-}$ Δ talin bs cells (Fig. 6*C*). Therefore, vinculin mutants that restored PTEN protein levels also restored the disrupted MAGI-2-β-catenin interaction in F9 vin$^{-/-}$ cells.

DISCUSSION

PTEN is a tumor suppressor involved in the control of fundamental biological processes including the cell cycle, apoptosis, and motility (29–33). Loss of PTEN is commonly found in highly aggressive tumors (3). Multiple mechanisms leading to altered PTEN protein levels, besides PTEN mutations, have been entertained (3). In this regard, our results demonstrate that tumor cells devoid of vinculin have undetectable PTEN protein levels due to a disrupted interaction between β-catenin and MAGI-2.

Vinculin-null cells exhibit undetectable PTEN protein concentration despite their normal mRNA levels. Thus, regulation of PTEN protein by vinculin is not at the transcriptional level. In fact, our findings reveal that vinculin regulates PTEN protein levels by affecting ubiquitin-mediated proteolytic degradation of PTEN. Interestingly, inactivation of PTEN through protein degradation is reminiscent of other tumor suppressors such as p53 and p27Kip1, which have reduced stability in some cancer cells (34, 35).

Our results demonstrate that the effect of vinculin on PTEN protein expression was at the level of the adherens junction. This was demonstrated through transfection of vinculin-null cells with a vinculin mutant that could not localize into focal adhesions (25) but could interact with α-catenin. This mutant was capable of restoring PTEN protein to wild-type levels. Furthermore, expression of an E-cadherin-α-catenin fusion protein restored PTEN protein expression. This fusion protein is known to localize in adherens junctions and restore cadherin-based cell adhesion activity (18). Moreover, our studies demonstrate that it can interact with β-catenin in F9 vin$^{-/-}$ cells. These findings are apparently in contradiction with previous studies, in which no interaction of this fusion protein with β-catenin was found. These differences could be due to the fact that we used epithelial cells, whereas the previous study used fibroblasts (18). It is known that the adherens junction organization is different in these two cell types (18). β-Catenin has been shown to interact with MAGI-2, besides being involved in adherens junction assembly (15). In this regard, we found that MAGI-2 interaction with β-catenin was disrupted in vinculin-null cells. Interestingly, a previous study has found that the localization of zonnula occludens-1, another member of the membrane-associated guanylate kinase family, in the apical junctional complex was disrupted in these cells and that its targeting into these complexes was restored upon vinculin re-expression (16). In our studies, the disrupted MAGI-2-β-catenin interaction was restored in vinculin-null cells upon expression of vinculin mutants capable of interacting with α-catenin. MAGI-2 interaction with PTEN is known to prevent PTEN degradation (12, 13). In our studies, overexpression of MAGI-2 in vinculin-null cells led to restoration of their PTEN protein

levels. Moreover, restoration of the disrupted MAGI-2-β-catenin interaction in these cells was associated with re-expression of PTEN protein.

Based on previous studies and our results, we propose a model (Fig. 7*C*) in which β-catenin present in the E-cadherin-β-catenin-α-catenin complex provides a link for MAGI-2 in the adherens junction. PTEN will then be recruited through its direct interaction with MAGI- 2 (11). Thus, a PTEN-MAGI-2-β-catenin heterotrimeric complex will be formed as reflected by the specific capacity of anti-PTEN Ab (Fig. 7*A*), but not a nonspecific (anti-paxillin) Ab (Fig. 7*B*), to co-immunoprecipitate PTEN, MAGI-2, and β-catenin (Fig. 7*A*). In E-cadherin complexes, β-catenin interacts directly with p85, the regulatory subunit of phosphoinositide 3-kinase (36). Thus, in the proposed complex, PTEN will inhibit the phosphoinositide 3-kinase/Akt pathway by efficiently dephosphorylating phosphatidylinositol 3,4,5-triphosphate (1). The interaction of MAGI-2 with PTEN will also stabilize PTEN (11). Certain PTEN mutants incapable of binding to MAGI-2 have reduced protein stability (12), which is restored by adding back the minimal PSD-95/Discs-large/ZO-1 homology-binding motif of PTEN (12). The unstable PTEN protein is degraded through a ubiquitin-associated proteolytic process (13, 23). By maintaining MAGI-2-β-catenin interaction, vinculin could restrict ubiquitin-mediated degradation of PTEN.

This study also suggests a mechanistic explanation for the loss of PTEN in multiple cases of malignancies that do not exhibit mutations or loss of PTEN mRNA expression (2, 3). A common feature of these tumors is decreased expression of E-cadherin, α-catenin, or β-catenin, key elements in adherens junctions (5, 6). In this regard, disruption of adherens junction formation with an E-cadherin blocking antibody led to loss of PTEN protein expression in WT F9 cells. Future work could address the prevalence of this mechanism as a cause for loss of PTEN expression in malignancies. These findings could also be relevant in the understanding of physiological processes because adherens junction organization is a highly dynamic process. In this regard, adherens junction assembly has been implicated in developmental morphogenesis (37). Interestingly, very recent studies have also shown a role for PTEN in neuronal development (38, 39). A minimal decrease in PTEN protein levels in neuronal progenitors significantly enhances the activity of the Akt/phosphoinositide 3-kinase kinase pathway (40). Thus, future work could be directed to addressing whether the dynamic changes of adherens junction assembly play an important role in neurite development by directly regulating PTEN protein levels. In summary, this work has uncovered an important mechanism of PTEN regulation, in which PTEN protein levels and activity are controlled through the maintenance of β-catenin-MAGI-2 interaction.

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