Interaction of the Tumor Suppressor PTEN/MMAC with a PDZ Domain of MAGI3, a Novel Membrane-associated Guanylate Kinase*

Yan Wu, Donald Dowbenko, Susan Spencer, Richard Laura, James Lee‡, Qimin Gu‡, and Laurence A. Lasky§

From the Departments of Molecular Oncology and §Molecular Biology, Genentech, Inc., South San Francisco, California 94080

PTEN/MMAC is a phosphatase that is mutated in multiple human tumors. PTEN/MMAC dephosphorylates 3-phosphorylated phosphatidylinositol phosphates that activate AKT/protein kinase B (PKB) kinase activity. AKT/PKB is implicated in the inhibition of apoptosis, and cell lines and tumors with mutated PTEN/MMAC show increased AKT/PKB kinase activity and resistance to apoptosis. PTEN/MMAC contains a PDZ domain-binding site, and we show here that the phosphatase binds to a PDZ domain of membrane-associated guanylate kinase with inverted orientation (MAGI) 3, a novel inverted membrane-associated guanylate kinase that localizes to epithelial cell tight junctions. Importantly, MAGI3 and PTEN/MMAC cooperate to modulate the kinase activity of AKT/PKB. These data suggest that MAGI3 allows for the juxtaposition of PTEN/MMAC to phospholipid signaling pathways involved with cell survival.

Tumor progression is often accompanied by the loss of heterozygosity at a diversity of genetic loci. A notable example of this phenomenon is encountered in advanced gliomas, where homozygous deletions at 10q23 are commonly detected (1). Loss of heterozygosity at an identical chromosomal site is also observed in Cowden’s and Bannayan-Zonana syndromes, two autosomal dominant diseases that result in a predisposition to formation of a variety of malignant tumors (2). Together, these genetic data suggested that a tumor suppressor was likely to be localized at 10q23, and the isolation from this chromosomal region of a gene encoding a phosphatase termed PTEN/MMAC has lent strong support to this idea (3–5). For example, examination of glial, prostate, and endometrial tumors revealed that a high percentage of these cancers contained homozygously mutated PTEN/MMAC loci (6–8). In addition, in vitro analyses of the potential of PTEN/MMAC as a cell growth regulator demonstrated that overexpression of the catalytically active protein in cells that lacked the phosphatase suppressed cell proliferation (9–13). Importantly, recent studies have demonstrated that mice with heterozygous deletions of the PTEN/MMAC locus were extraordinarily resistant to ultraviolet irradiation as well as other inducers of cell death (23). These cell lines also had increased levels of AKT/PKB kinase activity, and both resistance to apoptosis as well as AKT/PKB kinase activity could be controlled by expression of exogenous PTEN/MMAC. In addition, human tumor-derived cell lines missing PTEN/MMAC also showed enhanced AKT/PKB activity and resistance to apoptosis (24–26). It is thus likely that the loss of PTEN/MMAC phosphatase endows tumors with selective growth and survival advantages under stressful conditions such as radiation or chemotherapy.

Although recent crystallographic studies of PTEN/MMAC (27) have suggested a C2 domain-mediated mechanism for phospholipid interaction, an unresolved issue concerning PTEN/MMAC is whether this protein is brought into membranous subcellular sites that are involved with the regulation of cell survival. Here we describe a PDZ domain-mediated association between PTEN/MMAC and MAGI3, a novel protein related to a family of multi-PDZ domain-containing, membrane-associated guanylate kinases (MAGUKs) that are local-

1 The abbreviations used are: PtdIns(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; PRB, protein kinase B; MAGUK, membrane-associated guanylate kinase; GST, glutathione S-transferase; PBS, phosphate-buffered saline; BSA, bovine serum albumin; PCR, polymerase chain reaction; MAGI, membrane-associated guanylate kinase with inverted orientation; HER, human embryonic kidney; MDCK, Madin-Darby canine kidney; AIP, atrophin-interacting protein; NMDA, N-methyl-D-aspartate; PTEN/MMAC, phosphatase with tensin homology mutated in multiple advanced cancers; PDZ, PSD95 DiscsLarge Z01.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) 331900.

‡ To whom correspondence should be addressed: Dept. of Molecular Oncology, Genentech, Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080. Tel.: 650-225-1123; Fax: 650-225-6127; E-mail: lal@gene.com.

Received for publication, December 6, 1999, and in revised form, February 4, 2000
Published, JBC Papers in Press, March 23, 2000, DOI 10.1074/jbc.M909741199
PTEN/MMAC Interacts with MAGI3

FIG. 1. Sequence and expression of MAGI3. A, shown is the amino acid sequence of the novel PTEN/MMAC interacting protein (MAGI3) isolated from a human fetal brain cDNA library as compared with MAGI1 (originally MAGI (29) and MAGI2 (originally AIP/S-SCAM (32, 33))). The various domains (PDZ, guanylate kinase (GUK), and WW) are illustrated with bold over lines. Note the high degree of conservation of the functional domains, including the PTEN/MMAC-binding PDZ domain 2, as compared with the interdomain spacer regions. B, the tissue distribution of MAGI3 was examined using the Multiple Tissue cDNA Panel (CLONTECH, Inc.). DNA primers specific for MAGI3 and the housekeeping transcript glucose-3-phosphate dehydrogenase (G3PDH) were utilized. Polymerase chain reaction was performed using 35 cycles.

MATERIALS AND METHODS

Yeast Two-hybrid Mapping—In order to identify proteins that interact with PTEN/MMAC, a catalytically inactive form of the phosphatase was fused after the DNA-binding domain of lexA. A human fetal brain cDNA library was screened using CLONTECH's MATCHMAKER lexA two-hybrid system, and positive clones were isolated and sequenced. Full-length MAGI3 was isolated by screening a fetal brain cDNA library with a radioactively labeled probe derived from the yeast two-hybrid positives. In order to map the specific PDZ domain of MAGI3 that interacts with PTEN/MMAC, the individual PDZ domains were fused after the activation domain and tested in the two-hybrid system. Carboxyl-terminal regions of BAI-1 and the NMDA 2B receptor were cloned by polymerase chain reaction (PCR) and inserted after the activation domain. Mutants lacking the carboxyl-terminal PDZ-binding sites were produced by PCR.

In Vitro Binding Assays—In vitro analysis of the MAGI3 and PTEN protein interaction was performed using the TnT rabbit reticulocyte transcription/translation system (Promega) and affinity-purified GST fusion proteins (Amersham Pharmacia Biotech). One microgram of plasmid was translated in the reticulocyte system in a total volume of 0.025 ml. Samples were diluted into 0.5 ml of 50 mM HEPES, pH 7.2, 1% Triton X-100, 10% glycerol, 100 mM NaCl, and complete protease inhibitors (Roche Molecular Biochemicals). GST fusion proteins were then added and incubated for 60 min at 4 °C with shaking. The GSH-
FIG. 1—continued
In addition, the initial two-hybrid-positive region obtained with PTEN/MMAC as bait is also illustrated (MAGI3 including the six PDZ domains, the one WW domain, and the guanylate kinase (GUK) domain. PDZ domains are numbered as previously described previously (51), and the immunoprecipitated PTEN/MMAC was detected using an affinity purified polyclonal anti-PTEN/MMAC antibody directed against a bacterially expressed GST fusion protein.

**Immunofluorescence Analyses—**MDCK cells were plated on glass chamber slides and then transfected with LipofectAMINE Plus (Life Technologies, Inc.). Immunofluorescence was performed 24 h post-transfection. Cells were fixed for 20 min in 4% paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100 in 200 mM sucrose for 10 min. Cells were blocked with 10% fetal bovine serum in PBS and incubated with the appropriate fluorescein isothiocyanate or Cy3-conjugated secondary antibody for 30 min. Polyclonal antibodies from guinea pig were generated against GST fused to amino acids 244–295 of MAGI1. The specificity of the antisera was determined by immunoblot analysis of HEK 293 cell extracts from cells that overexpressed V5-tagged MAGI1, MAGI2, or MAGI3. The resulting immunoreactivity was specific and recognized MAGI1 but not MAGI2 or MAGI3. The MAGI1 antibodies were affinity purified prior to use in immunocytochemistry. Antibody reactive against GST was first depleted from the antisera using GST-Sepharose (Pierce). MAGI1-specific antibody was then isolated from the depleted antisera by affinity chromatography using a GST fusion protein containing MAGI1 amino acids 244–295 immobilized on CNBr-Sepharose 4B. The commercial antibodies against ZO-1 (rabbit) and E-cadherin (rat) are from Zymed Laboratories Inc. and Sigma, respectively. Secondary antibodies for immunofluorescence are from Jackson ImmunoResearch Laboratories. CACO-2 cells were grown on 0.4-μm collagen-coated polycarbonate filters until polarized as determined by resistance measurements. Cells were then fixed in cold methanol for 25 min, permeabilized for 10 min with 0.25% Triton X-100 in PBS, and blocked for 1 h with 10% horse serum in PBS. Cells were then double labeled with anti-MAGI1 (5 μg/ml) and anti-ZO-1 (2.5 μg/ml) diluted in 2% BSA, 0.02% azide, washed with 2% BSA in PBS, incubated with the appropriate secondary antibodies for 30 min, and washed again. The filters were then cut out with a razor and mounted on slides with 20 μl of Vectashield (Vector Laboratories) in preparation for analysis by immunofluorescence microscopy.

**Analysis of AKT/PKB Activity—**Epitope-tagged AKT/PKB and different combinations of PTEN and MAGI3 constructs were transfected into human 293 cells. After 36 h, the cells were lysed using 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.1% Nonidet P-40, 10% glycerol. Proteins were co-precipitated as described previously (51), and the immunoprecipitated PTEN/MMAC was detected using an affinity purified polyclonal anti-PTEN/MMAC antibody directed against a bacterially expressed GST fusion protein.

**RESULTS AND DISCUSSION**

**Isolation of a PTEN/MMAC-interacting MAGUK**—In order to determine the potential mechanism by which PTEN/MMAC is brought into close proximity to subcellular sites involved with survival, a yeast two-hybrid screen was performed using a human fetal brain cDNA library. This resulted in the isolation of a partial protein with significant homology to multi-PDZ domain containing proteins in the membrane-associated gua-
nylate kinase (MAGUK) family that includes the neuronal PSD 95 protein, the Discs Large tumor suppressor protein, and the tight junction protein ZO-1 (28–30). The highest degree of homology was to the central PDZ domains of MAGI (membrane-associated guanylate kinase with inverted orientation) (31) and the atrophin-interacting protein (AIP) (32) or S-SCAM (33), two recently described members of the MAGUK family. The two-hybrid fragment encoding the novel PTEN/MMAC-binding protein was used to isolate cDNAs containing the full-length sequence that is shown in Fig. 1A. The novel PTEN/MMAC-binding protein contained a domain structure similar to that observed for MAGI and AIP/S-SCAM, with 6 PDZ domains (numbered according to Ref. 33), a guanylate kinase domain, and a WW domain. Overall, the homology of the structural domains shared between these three proteins is relatively high, whereas the interdomain spacer regions showed lower sequence conservation, and two-hybrid analysis has revealed that the homologous PDZ domains in MAGI1 and AIP/S-SCAM also bind to PTEN in a carboxyl-terminal-dependent manner (data not shown).2 Because both AIP/S-SCAM and the novel PTEN/MMAC-interacting protein bear significant resemblance to MAGI, we propose to rename these proteins MAGI2, MAGI3, and MAGI1, respectively. Northern analysis of MAGI1 and MAGI2 expression revealed that MAGI1 is widely expressed (31), whereas MAGI2 is transcribed predominantly in the brain (32, 33). In order to determine the tissue distribution of MAGI3, a sensitive PCR analysis was performed. Fig. 1B reveals that MAGI3 is widely expressed in various fetal and adult tissues, including a variety of tumors, with only adult skeletal muscle, leukocytes, and spleen and fetal liver and spleen showing low or undetectable transcript levels. Finally, the gene encoding MAGI3 was mapped to chromosomal region 1p21 using a radiation hybrid panel (data not shown).

Characterization of the PTEN/MMAC-MAGI3 Interaction—Type 1 PDZ domains are short, compact structures that interact predominantly with the minimal carboxyl-terminal sequence (S/T)XV (34–36). Although this short consensus sequence appears in a large number of proteins, it is likely that other more amino-terminal residues are also involved in the specificity of PDZ domain-binding (34–36). Examination of the PTEN/MMAC sequence revealed that the carboxyl terminus of the protein encoded the sequence TKV, consistent with the involvement of this region in binding to one or more PDZ domains of MAGI3. As shown in Fig. 2, the original two-hybrid clone encompassed PDZ domains 2 and 3 and a portion of domain 4. Yeast two-hybrid mapping studies were thus performed to examine the nature of the PDZ domain that binds to PTEN/MMAC as well as to examine the requirement for the carboxyl-terminal TKV sequence for this interaction. As Fig. 2

![Fig. 3](image-url)

**In vitro and transfected cell interactions between PTEN/MMAC and MAGI3.** A, either 1 or 10 μg of GST alone or GST fusion proteins containing either PDZ domains 2, 3, and part of 4 (GST MAGI3 PDZ 2–4) or PDZ domain 2 alone (GST MAGI3 PDZ 2) were incubated with *in vitro* translated PTEN containing a hemagglutinin (HA) epitope tag at its amino terminus (*PTEN W.T.*) or the same protein with a deletion of the carboxyl-terminal 3 residues (*PTEN-TKV*) as described previously (47). The lysates were pelleted using glutathione beads and analyzed by SDS-gel electrophoresis and autoradiography. Antibody against the hemagglutinin epitope was used in immunoprecipitations as a control for total PTEN/MMAC protein levels. B, increasing amounts of a 20-residue peptide derived from the carboxyl terminus of PTEN/MMAC (+TKV peptide, DSDPENPFDEQDHTQITKV) or a peptide missing the carboxyl-terminal 3 residues (~TKV peptide, DTDSDPENPFDEQHTQI) were added to a GST precipitation reaction containing a GST fusion of PDZ domain 2 and 3 of MAGI3 and *in vitro* translated, hemagglutinin-tagged PTEN/MMAC. C, human embryonic kidney (293) cells were transfected with various combinations of wild type (*HA-PTEN W.T.*) or a carboxyl-terminal deletion mutant (*HA-PTEN-TKV*) of PTEN/MMAC and a FLAG epitope-tagged version of MAGI3 as shown at the top of the figure. Cell lysates were immunoprecipitated (IP) and blotted as shown using previously described techniques (51). Note that PTEN/MMAC can be co-precipitated with FLAG MAGI3, and the carboxyl-terminal mutant of PTEN/MMAC cannot, demonstrating that the co-precipitation is due to a specific interaction between the PTEN/MMAC carboxyl terminus and a MAGI3 PDZ domain. *Mab,* monoclonal antibody.

---

2 C. Sawyers, personal communication.
illustrates, only PDZ domain 2 interacted specifically with PTEN/MMAC. This figure also shows that, as predicted from a variety of structure-function studies, deletion of the carboxyl-terminal TKV sequence of PTEN/MMAC resulted in a diminished interaction between this protein and the third PDZ domain of MAGI3 in yeast. In order to examine the specificity of PDZ domain binding, other proteins were examined for their ability to interact with the MAGI3 PDZ motifs. Previous data suggested that the carboxyl terminus of a seven-transmembrane, G protein-coupled receptor termed BA-I interacted with the fourth PDZ domain of MAGI3 (37). Because this MAGI2 PDZ domain shows a high degree of sequence conservation with the fourth PDZ domain of MAGI3, we tested for binding of the carboxyl terminus of BA-I with the various PDZ domains from MAGI3. Fig. 2 shows that the carboxyl terminus of BA-I interacts with the fourth PDZ domain of MAGI3, consistent with results found for MAGI2. Examination of the carboxyl terminus of BA-I reveals the PDZ-binding site consensus sequence TEV, and the interaction between PDZ domain 4 and the carboxyl terminus of BA-I is decreased when these three residues are deleted (Fig. 2). Finally, the fifth PDZ domain of MAGI3 interacts with the carboxyl terminus of the NMDA receptor subunit 2B (Fig. 2), consistent with previous results found for the interaction of this cell surface receptor with the fifth PDZ domain of MAGI2 (33). As with PTEN/MMAC and BA-I, the carboxyl terminus of the NMDA receptor 2B encodes a PDZ-binding site consensus sequence, SDV. These data demonstrate that MAGI3 binds to several proteins, including intracellular and cell surface molecules, and these interactions are mediated by sequence-specific associations between multiple PDZ domains and carboxyl-terminal regions.

In vitro interaction experiments were next performed to confirm the yeast two-hybrid studies. Fig. 3A illustrates that GST fusion proteins containing the original two-hybrid-derived MAGI3 fragment (encoding PDZ domains 2, 3, and part of 4) as well as a GST fusion protein containing only PDZ domain 2 were both effective at interacting with PTEN/MMAC in vitro. As predicted from the yeast two-hybrid analysis, deletion of the carboxyl-terminal three amino acids (PTEN-TKV) resulted in a significant decrease in the interactions between the phosphatase and the PDZ domain-containing fusion proteins. In order to determine if the carboxyl terminus was sufficient for binding, peptide inhibition studies were performed (Fig. 3B). This analysis demonstrated that a 20-residue peptide derived from the carboxyl terminus of PTEN was capable of inhibiting the in vitro interaction between the third PDZ domain of MAGI3 and the phosphatase, whereas a peptide lacking the carboxyl-terminal 3 residues did not inhibit the interaction. The levels of peptide required to inhibit completely the in vitro interaction were relatively high (μM) suggesting that sequences upstream of the carboxyl-terminal 20 residues of PTEN/MMAC may also be involved with PDZ domain binding.

Binding studies using transfected cells were next performed to examine the interaction between PTEN/MMAC and MAGI3 under more physiological conditions. HEK 293 epithelial cells were transfected with an epitope-tagged form of MAGI3 together with either wild type PTEN/MMAC or PTEN/MMAC lacking the carboxyl-terminal three residues (PTEN-TKV). Fig. 3C shows that transfection of wild type PTEN/MMAC together with MAGI3 resulted in a complex that could be co-precipitated. As was found in the yeast two-hybrid and in vitro interaction studies, deletion of the carboxyl-terminal three residues resulted in a significant decrease in the in vivo interaction between these two proteins. These binding data support the conclusion that PTEN/MMAC and MAGI3 form a physiologically significant complex in transfected cells. Whereas insufficient levels of endogenous MAGI3 and PTEN are found in cells or tissues for co-precipitation studies, Sawyers and colleagues have demonstrated co-precipitation of PTEN with endogenous MAGI2 in brain extracts.
Subcellular Localization of MAGI3—Thus far, all MAGUKs have been found to be associated with the cell surface. This includes, for example, the localization of PSD 95, as well as other post-synaptic density MAGUKs, with the tight junctional complexes found at epithelial cell borders (30–38). The subcellular localization of MAGI3 was thus investigated by expressing a green fluorescent protein-tagged form of the protein in MDCK epithelial cells, since these cells form a single cell layer-thick epithelial sheet with distinctive tight junction. Fig. 4A illustrates that, as expected, the endogenous ZO-1 MAGUK is predominantly localized to these tight junctional sites (38). This figure also shows that MAGI3 is found to be predominantly localized at the tight junctions and appears to partially co-localize with ZO-1 at these sites. These studies demonstrate that MAGI3, like other MAGUKs, localizes to a specific cell surface domain of epithelial cells. Although the examination of the subcellular localization of endogenous MAGI3 is important, we have been unable to find cell lines or tissues where this protein is expressed at detectable levels. However, screening of various cell lines for expression of MAGI1, a MAGUK that is closely related to MAGI3 and which binds to PTEN/MMAC in an identical manner (data not shown), revealed high levels of expression in a CACO-2 colon carcinoma cell line. Importantly, Fig. 4B illustrates that endogenous MAGI1 co-localizes with endogenous ZO-1 to the apical tight junctions of CACO-2 colon carcinoma epithelial cells. Together, these data demonstrate that MAGI-type MAGUKs are cell surface localized proteins that are targeted to epithelial cell tight junctions.

Regulation of AKT/PKB Kinase Activity by PTEN/MMAC and MAGI3—Examination of tumor-derived mutations of the PTEN/MMAC locus has demonstrated a number of changes that affect the carboxyl terminus. Notably, several glioblasto-
mas and endometrial tumors contain mutations in exons 8 and 9 that result in truncated proteins that lack between 19 and ~60 carboxyl-terminal residues as well as an extended protein due to mutation of the stop codon (41, 42). As demonstrated here, these deletions would result in a loss of the PDZ domain-binding site, and these tumor-derived PTEN/MMAC mutants would thus not efficiently associate with MAGI3. In addition, some of these mutations result in changes in PTEN/MMAC protein stability as well (43), although removal of the carboxyl-terminal 3 residue PDZ-binding site clearly does not affect protein expression levels (Fig. 3C). An accumulating body of evidence suggests that a major role for PTEN/MMAC is the control of plasma membrane-associated PtdIns(3,4,5)P3 levels and downstream AKT/PKB kinase activity (12, 19, 20, 23, 24, 26). If association between PTEN/MMAC and MAGI3 is involved with the control of AKT/PKB, then the efficiency of kinase regulation by the phosphatase should be enhanced by co-expression of the PDZ domain-containing protein with the phosphatase. In order to test this hypothesis, very low levels of PTEN/MMAC were expressed either with or without MAGI3, and the effects on AKT/PKB kinase activity were examined. Fig. 5A illustrates that the regulation of AKT/PKB kinase by PTEN/MMAC is significantly enhanced in the presence of MAGI3. Thus, the ability of the AKT/PKB kinase to phosphorylate histone H2B is decreased when both PTEN/MMAC and MAGI3 are co-transfected together, suggesting that PTEN/MMAC more efficiently dephosphorylates PtdIns(3,4,5)P3 when it associates with the membrane-associated protein MAGI3. Importantly, this figure also illustrates that this enhancement requires a PDZ domain-mediated interaction, since the mutant form of PTEN/MMAC missing the carboxyl-terminal 3-amino acid PDZ-binding site is no longer able to regulate AKT/PKB activity in the presence of MAGI3. It should be noted that the enhancement effect of MAGI3 on the ability of PTEN/MMAC to regulate AKT/PKB kinase activity is less important when higher levels of PTEN/MMAC are expressed in transfected cells, suggesting that elevated levels of enzyme expression obviate the requirement for this type of membrane localization mechanism, at least in heterologous systems (data not shown).

The simplest interpretation of these results is that membrane-localized MAGI3 brings the phosphatase in close proximity to the cell surface to allow for efficient phospholipid dephosphorylation. In order to test this hypothesis, PTEN/MMAC was artificially targeted to the plasma membrane by the incorporation of an amino-terminal myristoylation site derived from p60src. Fig. 5B illustrates that localization of PTEN/MMAC to the plasma membrane by amino-terminal myristoylation results in an enhanced ability to down-regulate AKT/PKB kinase activity, consistent with the suggestion that plasma membrane localization is critical for efficient PTEN/MMAC PtdIns(3,4,5)P3 phosphatase activity. Together, these data are consistent with the hypothesis that MAGI3 serves to bring PTEN/MMAC into close proximity to membrane phospholipid substrates in order to regulate downstream AKT/PKB kinase activity.

The catalytic activity of PTEN/MMAC appears to be a critical component of tumor suppression, and the control of PtdIns(3,4,5)P3 levels by this enzyme is likely to have important effects on the survival and proliferation of tumors (12, 19, 20, 23, 24, 26). It is therefore of significant interest that the interaction of this enzyme with a specific PDZ domain of a membrane-associated MAGUK is involved with subcellular localization of the phosphatase and the regulation of the AKT/PKB kinase. MAGUKs and other multi-PDZ domain-containing proteins have been previously implicated in a diversity of cell signaling pathways. For example, the Drosophila Discs Large MAGUK is located at epithelial cell tight junctions, and mutations in either the guanylate kinase or SH3 domains were found to mislocalize this protein and result in lethal embryonic tumors with disrupted adherens junctions (40, 44). In addition, a complex of PDZ domain-containing proteins, including one MAGUK, is involved with the appropriate apical localization of the LET 23 tyrosine kinase receptor that induces Caenorhabditis elegans vulval development (45). Finally, the multi-PDZ domain-containing protein INAD assembles the various components of the Drosophila visual system in close proximity to allow for increased efficiency of signal transduction (46). The results reported here suggest that MAGI3 performs a similar critical role by bringing PTEN/MMAC in close proximity to cell surface phospholipid signaling pathways at subcellular sites such as the tight junctions. The present results, as well as other studies, also suggest that MAGIs assemble a diversity of PDZ domain interacting proteins into a cell surface complex. For MAGIEL and 3, this complex includes membrane associating factors such as GKAP/SAPAP SAPAP (47) (data not shown), G-coupled receptors such as BAI-1 (37), and ion channels such as the NMDA receptor (33). The inclusion of PTEN/MMAC into such a multi-component “signalosome” complex suggests that this phosphatase may be implicated in a diversity of pathways including those involved with angiogenesis (37) and excitatory neuron function (48). It is likely that this complex will become more intricate as the binding partners for the other domains of MAGI3 are identified. Interestingly, the interaction between PTEN/MMAC and MAGI3 might provide for a mechanism to regulate the ability of the phosphatase to modulate AKT/PKB kinase activity. For example, the interaction between PTEN/MMAC and MAGI3 could be modulated by phosphorylation of the threonine residue contained within the carboxyl-terminal PDZ recognition site. Importantly, a recent report has demonstrated that the AKT/PKB survival kinase is found at the identical tight junctional location in epithelial cells as the MAGI3-PTEN/MMAC complex, consistent with the hypothesis that this signaling complex is located at a specific subcellular site involved with the regulation of cell survival (49). In addition, another recent study has clearly demonstrated the importance of E-cadherin, another cell junction protein, in the regulation of the activity of the AKT/PKB survival kinase (50).

In conclusion, the results reported here describe a novel mechanism for the localization of a critical intracellular tumor suppressor to a multicomponent signaling complex at epithelial cell-cell junctions.

Acknowledgments—We thank Dr. Wenlu Li for help with confocal and fluorescence microscopy, Dr. Ying Li for suggesting the myristoylation experiment, Dr. Charles Sawyer for communication of preliminary data, Dr. Vishva Dixit for careful reading of the manuscript, and David Wood for help with figures.

REFERENCES
1. Bigner, S., Mark, J., Burger, P., Mahaley, M., Bullard, D., Mulhbiaer, L., and Bigner, D., (1998) Cancer Res. 48, 405–411
2. Eng, C., (1998) Int. J. Oncol. 12, 701–710
3. Li, J., Yen, C., Liaw, D., Podsypanina, K., Wang, S. I., Puc, J., Miliaresis, C., Tycko, B., Hibshoosh, H., Wigler, M. H., and Parsons R. (1997) Science 275, 1945–1947
4. Li, D. M., and Sun, H. (1997) Cancer Res. 57, 2124–2129
5. Steck, P., Pershouse, M. A., Jassar, S. A., Yung, W. K. A., Lin, H., Ligon, A. H., Langford, I. A., Baumbard, M. L., Hattier, T., Davis, T., Frye, C., Hu, R., Sweldun, B., Teng, D. H., and Tavtigian, S. V. (1997) Nat. Genet. 15, 356–362
6. Cairns, P., Okami, K., Halachmi, S., Halachmi, N., Esteller, M., Herman, J. G., Kout, D., Bova, G. S., Bignell, G. R., and Parsons R. (1999) Cancer Res. 59, 4997–5000
7. Rasheed, B. K. A., Stenzel, T. T., McEwen, R. E., Parsons, R., Friedman, A. H., Friedman, H. S., Bigner, D. D., and Bigner, S. H. (1997) Cancer Res. 57, 4187–4190
8. Tashiro, H., Blazer, M. S., Wu, R., Cho, K. R., Bose, S., Wang, S. I., Li, J., Parsons, R., and Ellenson, L. H. (1997) Cancer Res. 57, 3855–3848
Interaction of the Tumor Suppressor PTEN/MMAC with a PDZ Domain of MAGI3, a Novel Membrane-associated Guanylate Kinase

Yan Wu, Donald Dowbenko, Susan Spencer, Richard Laura, James Lee, Qimin Gu and Laurence A. Lasky

J. Biol. Chem. 2000, 275:21477-21485.
doi: 10.1074/jbc.M909741199 originally published online March 23, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M909741199

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 51 references, 26 of which can be accessed free at http://www.jbc.org/content/275/28/21477.full.html#ref-list-1