TSC2 Deficiency Increases PTEN via HIF1α

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Lenin Mahimainathan†, Nandini Ghosh-Choudhury*†, Balachandar Venkatesan‡, Falguni Das†, Chandi C. Mandal†, Nirmalya Dey†, Samy L. Habib§∥, Balakuntalam S. Kasinath†‡∥, Hanna E. Abboud†‡∥, and Goutam Ghosh Choudhury*†

From †Veterans Affairs Research and §Geriatric Research, Education, and Clinical Center, South Texas Veterans Health Care System, San Antonio, Texas 78229 and the Departments of †Medicine and ‡Pathology, University of Texas Health Science Center, San Antonio, Texas 78229-3900

Substantial evidence suggests roles of TSC2 and PTEN in the development of cancer predisposition syndromes. Loss of TSC2 results in benign tumors, neurological disorders, and angiomylipomas. We found that PTEN mRNA and protein levels are elevated in Tsc2−/− mouse embryo fibroblasts with concomitant reduction in Akt phosphorylation. Reconstitution of TSC2 in Tsc2−/− mouse embryo fibroblasts decreases PTEN levels. Interestingly, increased HIF1α activity present in Tsc2 null cells is required for PTEN transcription and protein expression. We identified a canonical hypoxia-responsive element in the PTEN promoter, which regulates the transcription of this tumor suppressor protein in a TSC2-dependent manner. Finally, we demonstrate a positive correlation between expression of HIF1α and PTEN in renal angiomylipomas from TSC patients. Our results reveal a unique function of HIF1α in up-regulation of PTEN and provide a new mechanism of reduced Akt phosphorylation in Tsc2 null cells. These data suggest that PTEN may safeguard against developing malignant tumors in patients with TSC deficiency.

Mutations in the tuberous sclerosis tumor suppressor genes (TSC1 and TSC2) result in autosomal dominant diseases characterized by benign tumors (hamartomas) in the kidney, heart, brain, and other organs (1). Approximately 1 in 6000 live births is linked to a germ line-inactivating mutation of either of the two genes, TSC1 or TSC2. Thus, each mutated gene accounts for ~50% of the cases (2). TSC2 associates with TSC1 to form the active signaling complex (3). The C terminus of TSC2 contains a GTPase-activating protein domain, which blocks mTOR activity by increasing hydrolysis of GTP associated with the small G-protein Rheb (Ras homolog enriched in the brain) (4, 5). Growth factor-stimulated Akt and other kinases phosphorylate TSC2 at specific sites, resulting in its dissociation from the TSC1/2 complex (6–9).

The lipid phosphatase activity of the tumor suppressor PTEN dephosphorylates the second messenger phosphatidylinositol (PI)3,4,5-trisphosphate, thus negatively regulating the PI 3-kinase signaling pathway (10). Germ line mutations in PTEN cause cancer predisposition syndromes, such as Cowden disease (11–13). Also, loss of PTEN is common in many tumors, including sporadic glioblastoma, endometrial carcinoma, melanoma, meningioma, and renal, breast, prostate, and small cell lung cancer (11–13). Whereas PTEN deficiency predisposes to malignancy, it is rare in TSC patients (11, 13, 14). In PTEN-deficient cancer cells, even in the absence of growth factors, Akt is constitutively active, which results in phosphorylation and inactivation of TSC2 and activation of mTOR (8, 10, 15). Similarly, the disruption of TSC2 results in significantly increased mTOR activity (16–18). In the latter case, the mTOR activation leads to inactivation of Akt through a negative feedback loop involving IRS (insulin receptor substrate) proteins (17, 19–21). However, additional signaling pathway(s) probably contribute to reduced Akt activation in TSC2 deficiency.

In this report, we demonstrate that TSC2 deficiency results in increased expression of PTEN. As a mechanism, we show that HIF1α positively regulates the transcription of PTEN, using a canonical HIF-responsive element. Furthermore, we demonstrate that renal angiomylipomas in TSC patients express elevated levels of HIF1α and PTEN protein. Thus, increased levels of PTEN in renal angiomylipomas of patients with TSC may mute the malignant potential of these tumors by decreasing Akt activation.

MATERIALS AND METHODS

Cell Culture and Adenovirus Infection—Tsc2+/+ and Tsc2−/− MEFs, generously provided by Dr. D. J. Kwiatkowski (Harvard University), were grown in DMEM with low glucose with 10% fetal bovine serum (22, 23). 293 cells were grown in DMEM with high glucose-containing 10% serum. All cell stocks were maintained in the presence of plasmocin and primocin. Tsc2−/− MEFs were infected with adenovirus vector expressing TSC2. This viral vector also expressed green fluorescence protein to detect efficient infection. As a control, an adenovirus vector expressing β-galactosidase was used.

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* Recipient of a Research Career Scientist Award from the Department of Veterans Affairs. To whom correspondence should be addressed: Dept. of Medicine, Mail Code 7882, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900. E-mail: choudhuryg@uthscsa.edu.

The abbreviations used are: PI, phosphatidylinositol; RT, reverse transcription; siRNA, small interfering RNA; MEF, mouse embryo fibroblasts; HRE, HIF1α-responsive element; RLA, relative luciferase activity.
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were immunoblotted with the indicated antibodies. Along with hydrogenase mRNAs, as indicated. Green fluorescent protein-TSC2 (HIF1α/H11032)

**FIGURE 1. Increased Pten expression in TSC2+/− MEFS.** A, lysates of Tsc2+/+ and Tsc2−/− MEFS were immunoblotted with the indicated antibodies. B, Tsc2−/− MEFS were transfected with Pten siRNA or scrambled RNA (scr RNA) from two different sources (left and right), as described under “Materials and Methods.” Cell lysates were immunoblotted with the indicated antibodies. C, Tsc2−/− MEFS were infected with Ad green fluorescent protein-tuberin and Adβ-galactosidase adenovirus vectors. Lysates of infected cells were immunoblotted with indicated antibodies. D and E, RNAs isolated from Tsc2−/− and Tsc2+/− (D) and Tsc2−/− MEFS infected with Ad green fluorescent protein-TSC2 (E) were used in RT-PCR to detect Pten and glyceraldehyde-3-phosphate dehydrogenase mRNAs, as indicated. F, Pten-Luc reporter plasmid was transfected into Tsc2+/− and Tsc2−/− MEFS along with Renilla plasmid. Luciferase activity was determined as described under “Materials and Methods.” Relative luciferase activity (RLA) of mean ± S.E. of triplicate measurements is shown. *, p < 0.05 versus Tsc2+/− MEFS. G, Tsc2−/− MEFS were transfected with Pten-Luc plus increasing doses of Tsc2 expression vector. Luciferase activity is presented as described in F, *, p < 0.001 versus vector-transfected.

**Tissue Samples**—Kidney angiomyolipoma tissues from TSC patients with angiomyolipoma and normal kidney tissues were obtained from the Brain and Tissue Bank for Developmental Disorders (University of Maryland). This study has been approved by the Institutional Review Board of the University of Texas Southwestern Medical Health Science Center at San Antonio.

**Antibodies and Reagents**—Antibodies against PTEN and TSC2 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phospho-Akt (Ser-473) antibody was from Cell Signaling. HIF1α antibodies recognizing mouse and human proteins, respectively, were obtained from Novus Biologicals and BD Bioscience. Akt antibody was obtained from Upstate. Green fluorescent protein and actin antibodies were purchased from Sigma. Anti-hemagglutinin epitope-specific antibody was obtained from Covance. HIF1α inhibitor tetrahydroisouquinoline alkaloid emetin (6′,7′,10,11-tetramethoxy-emetan, 2HCl) was purchased from Sigma (24). Another HIF1α inhibitor, YC-1 (3-(5′-hydroxymethyl-2′furyl)-1-benzylindazole), was obtained from Calbiochem (25, 26). Plasmocin and primocin were purchased from Invivogen. Nuclear extract isolation and luciferase assay kits were purchased from Pierce and Promega, respectively. The QuickChange II site-directed mutagenesis kit was obtained from Stratagene. RNAzol and OneStep RT-PCR kits were purchased from Invitrogen. Primers to detect PTEN mRNA were obtained from SuperArray.

**siRNAs and Plasmids**—Scrambled control and siRNA recognizing human TSC2 were obtained from Dharmacon, as smart pools of four siRNAs. Another set of human TSC2 siRNAs, a pool of three, was purchased from Santa Cruz Biotechnology. Smart pool PTEN siRNAs (four siRNAs) and another pool of three siRNAs were purchased from Dharmacon and Santa Cruz Biotechnology, respectively. Mouse HIF1α siRNA and scrambled siRNA plasmids were kind gifts from Dr. R. R. Ratan (Winifred Masterson Burke Medical Research Institute). A second set of siRNAs as a pool of three siRNAs against Hif1α was obtained from Santa Cruz. pCMV-TSC2 was kindly provided by Dr. I. de Belle (Burnham Institute). Reporter plasmid containing the PTEN promoter-driven firefly luciferase gene was a kind gift from Dr. I. de Belle (Burnham Institute). Adenovirus vector expressing TSC2 and green fluorescent protein was a kind gift from Dr. G. N. Finlay (New England Medical Center, Boston, MA).

**Cell Lysis and Immunoblotting**—MEFS and 293 cells were washed with PBS and harvested in radioimmune precipitation buffer (27–31). Whole cell lysates prepared by centrifugation at 12,000 × g at 4 °C for 30 min were resolved by SDS gel electrophoresis, transferred to polyvinylidene difluoride membrane, and immunoblotted with the indicated antibodies, as described (27, 29, 31).

**RNA Extraction and Reverse Transcription (RT)-PCR**—Total RNAs were isolated using the RNAzol kit according to the vendor’s protocol. RT-PCR was performed using the OneStep RT-PCR kit according to the manufacturer’s instructions.

**Electrophoretic Mobility Shift Assay**—Nuclear extracts were prepared using the kit according to the vendor’s protocol. The HRE-3 element from the PTEN promoter was made by annealing the oligonucleotide 5′-GAG CAG CGT GGT CA-3′ with its
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FIGURE 2. TSC2 regulates PTEN expression in 293 cells. A, lysates of scrambled or TSC2 siRNA-transfected 293 cells were immunoblotted with TSC2 and actin antibodies to detect the level of TSC2 down-regulation. B, lysates of TSC2 siRNA-transfected 293 cells were immunoblotted with the indicated antibodies. Two independent sources of siRNA pools were used (left and right). C, lysates of two independent (left and right) PTEN-targeted siRNA-transfected 293 cells were immunoblotted with the indicated antibodies. D and E, 293 cells were transfected with PTEN-Luc and scrambled or TSC2 siRNAs from two independent sources (D, left and right) or PTEN-Luc plus pCMV-TSC2 expression vector (E), as indicated. RLA (mean ± S.E. of triplicate measurements) is shown. *p < 0.05 versus scrambled in D; *p < 0.001 versus vector in E.

complementary strand. The probe was labeled with [γ-32P]ATP using T4 polynucleotide kinase. The electrophoretic mobility shift assay was performed using 10 μg of nuclear extracts, as described (27, 29, 31). To determine the specificity of interaction, the nuclear extracts were incubated with cold double-stranded oligonucleotide prior to incubation with the radiolabeled probe. For antibody reaction, the nuclear extracts were incubated with Hif1α antibody, followed by incubation with the labeled probe, as described (28). The DNA protein complex was resolved by 5% polyacrylamide gel electrophoresis.

Site-directed Mutagenesis—The HRE-3 sequence was mutated using the QuikChange II site-directed mutagenesis kit, as described (32). The mutation was verified by sequencing the DNA. The mutated bases in the HRE-3 core sequence are shown in Fig. 5C.

Transient Transfection and Luciferase Activity—The cells were transfected with the reporter plasmid, along with the indicated vector and the indicated expression vectors or siRNAs. Luciferase activity was determined in the cell lysate using a luciferase assay kit according to the manufacturer’s protocol (27, 29–31, 33).

Statistics—Statistical significance of the data was calculated using analysis of variance followed by Student-Newman-Keuls analysis, as described previously (27, 29, 30). Significance was considered at a p value of <0.05.

RESULTS

TSC2 Inhibits Expression of PTEN—Previous studies have established that a feedback mechanism in cells lacking TSC2 inhibits PI 3-kinase activity, which results in decreased activation of Akt (20, 21, 34). The level of PI 3-kinase product PIP3 is regulated by PTEN. Inactivating mutation or deficiency of PTEN leads to Akt activation and tumorigenesis (12). Therefore, we investigated a potential role for PTEN in regulating Akt phosphorylation in murine embryonic fibroblasts (MEFs) lacking Tsc2. Immunoblot analysis showed significantly increased abundance of Pten in Tsc2−/− MEFs compared with wild type cells (Fig. 1A). Increased Pten resulted in reduced phosphorylation of Akt (Fig. 1A). Transfection with Pten siRNA, however, showed an increase in Akt phosphorylation (Fig. 1B, left). Transfection of a pool of three siRNAs from a different source targeting Pten produced the same results (Fig. 1B, right). To demonstrate that the increased expression of Pten was a consequence of the lack of the Tsc2 gene, we reconstituted Tsc2−/− MEFs with human TSC2 using an adenovirus vector. Expression of TSC2 significantly reduced the level of Pten protein in these cells with a concomitant increase in phosphorylation of Akt (Fig. 1C). Expression of PTEN has been shown to be regulated by transcriptional mechanisms (35, 36). RT-PCR analysis showed increased expression of Pten mRNA in Tsc2−/− MEFs relative to the levels expressed in wild type MEFs (Fig. 1D). Furthermore, reconstitution of TSC2 in Tsc2−/− MEFs lowered the Pten mRNA level (Fig. 1E). To evaluate whether TSC2 regulates PTEN transcription, we used a reporter plasmid in which the luciferase gene is driven by the PTEN promoter (PTEN-Luc) (36). Transient transfection assays with this reporter plasmid showed significantly elevated transcription of PTEN in Tsc2−/− MEFs as compared with that in wild type cells (Fig. 1F). Cotransfection of TSC2 with PTEN-
Luc into Tsc2−/− MEFs inhibited transcription of the PTEN reporter in a concentration-dependent manner (Fig. 1G).

The above results from Tsc2−/− MEFs indicate that Tsc2 inhibits expression of Pten. To examine whether the increase in the PTEN expression is a direct effect of TSC2 mutation or a result of other mutations present in the Tsc2−/− MEFs, we disrupted TSC2 signaling in human embryonic kidney epithelial 293 cells. Transfection of TSC2 siRNA significantly lowered TSC2 protein expression (Fig. 2A). Compared with scrambled siRNA-treated cells, PTEN expression was increased following knockdown of TSC2 expression, which resulted in reduced Akt phosphorylation (Fig. 2B, left). Transfection of a different set of siRNAs targeting TSC2 showed similar results (Fig. 2B, right). Down-regulation of PTEN in 293 cells using two different siRNAs increased Akt phosphorylation (Fig. 2C, left and right). Down-regulation of TSC2 with two independent sets of siRNAs also significantly increased transcription of PTEN, as judged by reporter transfection assays using PTEN-Luc (Fig. 2D). These results provide evidence that TSC2 negatively regulates expression of the PTEN tumor suppressor gene.

HIF1α Regulates PTEN Expression—The results described above demonstrate transcriptional regulation of PTEN in a TSC2-sensitive manner. A recent report demonstrated increased expression of Hif1α transcription factor in Tsc2−/−/− MEFs (37). We also confirmed the elevated levels of Hif1α in these cells (supplemental Fig. S1). Incubation of Tsc2−/−/− MEFs with two independent inhibitors of Hif1α (24–26), emetine and YC-1, significantly reduced the expression of Pten protein and resulted in increased Akt phosphorylation (Fig. 3, A–D). To confirm these results genetically, we used plasmid-derived Hif1α siRNA expression targeting Hif1α. Transfection of Hif1α siRNA plasmid in Tsc2−/−/− MEFs inhibited Hif1α protein expression, resulting in significantly reduced expression of Pten protein (Fig. 3E). Using a second set of siRNAs containing a pool of three siRNAs also showed the same results (Fig. 3E, right). Down-regulation of HIF1α with plasmid-derived or oligonucleotide pool siRNAs increased phosphorylation of Akt (Fig. 3F, left). After transfection of siRNAs from two different sources to knock down HIF1α, RT-PCR analysis showed reduced Pten mRNA expression in Tsc2−/−/− MEFs (Fig. 3G). Since VHL tumor suppressor
protein induces degradation of Hif1α through the proteasomal pathway, we used a plasmid expressing FLAG-tagged VHL. Expression of VHL in Tsc2−/− MEFs inhibited Pten protein expression (supplemental Fig. S2). Furthermore, in 293 cells, ectopic expression of HIF1α significantly increased the expression of PTEN mRNA and protein (Fig. 3, H and I). Collectively, these results demonstrate that up-regulated Hif1α in Tsc2-deficient cells positively regulates PTEN expression.

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**HIF1α Regulates PTEN Transcription**—We next examined the mechanism by which HIF1α regulates PTEN. Analysis of the 5′-flanking sequence of the PTEN gene upstream of the start codon revealed the presence of three putative HIF1α-responsive elements (HREs), which share homology with VEGF, endothelin-1, and Nur-77 HREs (Fig. 4A) (38–42). Transfection of Tsc2−/− MEFs with Hif1α siRNA plasmid or oligonucleotide pool recognizing Hif1α reduced transcription of PTEN in PTEN-Luc reporter assays (Fig. 4B, left and right). In contrast, expression of HIF1α increased transcription of PTEN in 293 cells (Fig. 4C). In addition, VHL, which induces degradation of HIF1α, significantly inhibited both basal and HIF1α-induced PTEN transcription (Fig. 4D). Since HRE-1 and HRE-2 are present in the untranslated region of the PTEN mRNA (35), we used 3′ deletion of the PTEN promoter (from −2 to −778), which retains the transcription start site at −1031 bp and HRE-3 (Fig. 4F). The effect of HIF1α on transcription of this reporter was tested in 293 cells. As expected, HIF1α increased the transcription of PTEN from PTEN-Luc containing all three HREs (Fig. 4F). But no significant difference in response to HIF1α was found in transcription of PTEN using HRE-3-Luc reporter plasmid (Fig. 4F). These data suggest that HRE-3 at −1391 bp (Fig. 4A) from the start codon regulates the transcription of PTEN.

In order to test the involvement of HRE-3 specifically, we employed electrophoretic mobility shift assays using nuclear extracts from Tsc2+/+ and Tsc2−/− MEFs. Double-stranded oligonucleotide representing the HRE-3 from the PTEN promoter was used as a probe. Formation of protein-DNA complexes was detected with nuclear extract isolated from Tsc2−/− MEFs as compared with those from Tsc2+/+ cells (Fig. 5A, compare lane 3 with lane 2). Incubation of nuclear extracts with
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Expression of PTEN and Hif1α in Human Renal Angiomyolipomas—The results described above demonstrate a correlation between PTEN expression and TSC2 deficiency. TSC patients commonly develop renal angiomyolipomas, relatively benign tumors of the kidney, which consist of adipose tissue, muscle cells, and blood vessels (43, 44). We examined expression of PTEN in renal angiomyolipoma samples from six TSC patients. RT-PCR analysis showed significantly increased expression of PTEN mRNA in the angiomyolipoma samples relative to the healthy kidney tissues (Fig. 6, A and B). Since our data showed that Hif1α in Tsc2−/− MEFs up-regulates PTEN, we determined the expression of these two proteins in the angiomyolipoma samples. Expression of PTEN was markedly elevated in the renal angiomyolipomas (Figs. 6, C (top) and D). These increased PTEN levels were directly correlated with increased HIF1α expression (Figs. 6, C (middle) and E). Co-elevation of HIF1α and PTEN in angiomyolipomas and in Tsc2−/− MEFs suggests an antagonistic relationship between TSC2 and PTEN, which may contribute to the relatively benign nature of angiomyolipomas in patients with TSC.

DISCUSSION

The current study demonstrates a previously unrecognized role of TSC2 deficiency for attenuation of Akt phosphorylation by increased expression of PTEN expression. Moreover, we have delineated a molecular pathway by which the increased level of HIF1α in TSC2 deficiency regulates PTEN expression (Fig. 7). We identified a HIF1α-responsive element in the PTEN promoter, which regulates PTEN transcription. Finally, using renal angiomyolipoma tumor samples from TSC patients, we provide the first evidence for the presence of a positive correlation between HIF1α and PTEN expression.

Inactivation of TSC2 results in inhibition of insulin/IGF1-stimulated Akt phosphorylation (34). This deficiency in Akt phosphorylation involves a negative feedback regulation of IRS-1/IRS-2, resulting in attenuation of PI 3-kinase activity and Akt phosphorylation.

cold HRE-3 oligonucleotide inhibited the DNA-protein complex formation, demonstrating the specificity of the interaction (Fig. 5A, lanes 4 and 5). To identify Hif1α in this protein-DNA complex, we performed an electrophoretic mobility shift assay in the presence of a Hif1α-specific antibody. Incubation of nuclear extracts from Tsc2−/− MEFs with Hif1α antibody completely blocked DNA binding relative to that in the presence or absence of control IgG (Fig. 5B, lane 4 versus lanes 3 and 5). Next, we examined the requirement of HRE-3 for the transcription of PTEN. We mutated the HRE-3 in the HRE-3-Luc reporter plasmid containing the 3′ deletion (Fig. 5C). Transient transfection assays using this plasmid in Tsc2−/− MEFs showed significantly low reporter activity, compared with the wild type HRE-3-containing promoter (Fig. 5D). These results suggest that increased Hif1α present in Tsc2−/− MEFs is not sufficient to induce transcription of PTEN if HRE-3 is mutated. We also examined the activity of this mutant HRE-3 reporter in 293 cells in response to HIF1α, which mimics the increased levels of HIF1α protein in Tsc2−/− MEFs. The mutant HRE-3-containing reporter was significantly less responsive to HIF1α relative to the wild type HRE-3 reporter (Fig. 5E). To further confirm the role of HRE-3, we mutated this site in the context of the full-length promoter (PTEN-Luc-HRE-3 Mut) (supplemental Fig. S3A) Transfection of this reporter plasmid into 293 cells showed significantly reduced response to Hif1α as compared with PTEN-Luc reporter (supplemental Fig. S3B). Thus, HRE-3 is necessary and sufficient for regulation of PTEN transcription by HIF1α.

FIGURE 5. HRE-3 regulates HIF1α responsiveness of PTEN promoter. A and B, nuclear extracts from Tsc2−/− or Tsc2−/− MEFs were used in the electrophoretic mobility shift assay using the HRE-3 from the PTEN promoter as a probe, as described under “Materials and Methods.” Nuclear extracts in lanes 4 and 5 in A were incubated with cold HRE-3 oligonucleotide, as indicated. Nuclear extracts in B were incubated with Hif1α antibody or IgG, as indicated. The arrow indicates the protein-DNA complex. C, schematic diagram shows the reporter constructs with wild type and mutated HRE-3 sequence in the HRE-3-Luc and HRE-3-Luc-Mut reporter plasmids. The mutated bases in HRE-3-Luc and HRE-3-Luc-Mut are underlined. D, Tsc2−/− MEFs were transfected with HRE-3-Luc or HRE-3-Luc-Mut reporter plasmids. E, 293 cells were transfected with HIF1α and HRE-3-Luc or Hif1α plus HRE-3-Luc-Mut plasmids. RLA (mean ± S.E. of triplicate measurements) is shown. *p < 0.001 versus HRE-3-Luc.
phosphorylation (20, 21, 45). Furthermore, the reduced expression of platelet-derived growth factor receptors in Tsc2/H11002/H11002 MEFs also contributes to negative regulation of PI 3-kinase-mediated Akt phosphorylation (23, 46).

The requirement of increased Akt phosphorylation due to inactivating mutations of PTEN or constitutive activation of growth factor receptors and PI 3-kinase for cell growth in different cancer and cancer predisposition syndromes is well established (11–13). In support of this, Akt1 has been shown to be necessary for the development of tumors in Pten/H11001/H11002 mice (47). The observation that Akt phosphorylation is dramatically reduced in Tsc2/H11002/H11002 cells may represent the major mechanism explaining why Tsc2/H11002/H11002 MEFs do not lead to malignancy. Although TSC2 represents a tumor suppressor gene, its loss is not seen in highly proliferative and invasive cancers. In this study, we show that TSC2 deficiency increases expression of PTEN, thus contributing to the inhibition of phosphorylation of Akt (Figs. 1 and 7). Our results provide a further mechanism explaining why Tsc2/H11002/H11002 MEFs are more sensitive to apoptosis, since PTEN induces apoptosis in many cells (12, 19, 21, 48, 49).

Recently, Manning and co-workers (50) described a novel positive regulation of TORC2-mediated Akt Ser-473 phosphorylation by TSC2. Direct input from PI 3-kinase may not be necessary for TORC2-mediated Ser-473 phosphorylation of Akt. Specific inhibition of TORC1, which regulates the negative feedback loop integrating IRS-1 into the lipid kinase, has been shown to partially increase Akt Ser-473 phosphorylation in Tsc2/H11002/H11002 MEFs in response to insulin (50). Furthermore, direct inhibition of PI 3-kinase by wortmannin partially blocks TORC2 kinase activity, suggesting a role for PI 3-kinase in TORC2-mediated phosphorylation of Akt (50). Our results also support this notion. Increased PTEN expression would inhibit PI 3-kinase signaling, leading to attenuation of Akt phosphorylation in TSC2 deficiency.

Pten haploinsufficiency is required for increased Akt activation and virulent tumorigenesis in Tsc2/H11001/H11002 mice (51, 52). This demonstration indicates that PTEN may contribute to regulation of Akt phosphorylation in the event of TSC2 loss. Our results in TSC2-deficient cells support this notion that PTEN contributes to reduced Akt phosphorylation. Furthermore, our results provide a positive correlation between elevated PTEN levels and the benign nature and relative limited proliferative capacity of the TSC tumors.

Elevated expression of angiogenic factors has been detected in tumors from TSC patients (53). In humans, loss of VHL tumor suppressor is associated with highly vascular renal cell carcinomas and hemangiomas similar to those found in animal models of TSC (54, 55). Increased angiogenic activity found in the TSC tumors is mediated by VEGF (53, 56). Recently, it was shown that increased levels of HIF1α present in Tsc2/H11002/H11002 MEFs are necessary for the production of VEGF in these cells as well as in the TSC mouse model (37, 56). Similarly, we show a requirement of Hif1α in inducing the expression of Pten in Tsc2/H11002/H11002 MEFs (Fig. 3). These results indicate that HIF1α may produce opposing activities by inducing angiogenic/mitogenic phosphorylation (20, 21, 45). Furthermore, the reduced expression of platelet-derived growth factor receptors in Tsc2/H11002/H11002 MEFs also contributes to negative regulation of PI 3-kinase-mediated Akt phosphorylation (23, 46).
Angiomyolipomas represent benign tumors with loss of heterozygosity of one or both TSC genes (43, 59, 60). Loss of TSC2 is also found in sporadic angiomyolipomas (59, 60). More than half of the TSC patients develop angiomyolipomas of the kidney, which contribute to morbidity secondary to destruction of renal parenchyma and hemorrhage. Although patients lacking TSC1 develop angiomyolipomas, TSC2 deficiency-associated pathology is more severe than TSC1 cases (2). In this study, we demonstrate increased HIF1α protein levels, which correlate with increased PTEN mRNA and protein expression in renal angiomyolipomas (Fig. 6). Our findings in TSC2-deficient cells that HIF1α transcriptionally regulates expression of PTEN (Figs. 4 and 5) are also consistent with our data in tissue samples from patients with renal angiomyolipomas. Since PTEN inhibits PI3-kinase signal transduction, leading to reduced Akt activation (Fig. 7), our results demonstrating increased PTEN expression in TSC2 deficiency may represent a mechanism for the presence of less malignant tumors in TSC patients.

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