PNUTS Functions as a Proto-Oncogene by Sequestering PTEN

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Abstract

PTEN is a well-defined tumor suppressor gene that antagonizes the PI3K/Akt pathway to regulate a multitude of cellular processes, such as survival, growth, motility, invasiveness, and angiogenesis. While the functions of PTEN have been studied extensively, the regulation of its activity during normal and disease conditions still remains incompletely understood. In this study, we identified the protein phosphatase-1 nuclear targeting subunit PNUTS (PPP1R10) as a PTEN-associated protein. PNUTS directly interacted with the lipid-binding domain (C2 domain) of PTEN and sequestered it in the nucleus. Depletion of PNUTS leads to increased apoptosis and reduced cellular proliferation in a PTEN-dependent manner. PNUTS expression was elevated in certain cancers compared with matched normal tissues. Collectively, our studies reveal PNUTS as a novel PTEN regulator and a likely oncogene. Cancer Res; 73(1); 205–14. ©2012 AACR.

Introduction

PTEN is an important tumor suppressor, which has major roles in cell survival, proliferation, migration, and cell death (1–3). PTEN was initially identified as a gene located in the chromosomal locus 10q23, one of the most frequently mutated or deleted loci in human cancers. Evidence for loss or mutations of the PTEN gene in many diverse tumor types (4) and high susceptibility of PTEN heterozygous mice to a wide range of tumors (5, 6) strongly support the status of PTEN as an important tumor suppressor for many types of cancers. Functionally, PTEN is a dual-specific phosphatase that acts on both lipid and protein substrates (2). The tumor suppressor activity of PTEN is mostly mediated through its lipid substrates (7). One such crucial substrate is phosphatidylinositol-3,4,5-trisphosphate (PIP3), which is converted to phosphatidylinositol-4,5-bisphosphate (PIP2) by PTEN at the cellular membrane (8). PIP3 generated by phosphoinositide 3-kinase (PI3K) is required for the downstream activation of AKT pathway, which further promotes cell growth and survival. Thus, PTEN keeps a check on tumorigenesis by negatively regulating AKT pathway through downregulating the cellular levels of PIP3. PTEN being a very crucial tumor suppressor, it is important to mechanistically understand its regulation during normal and disease conditions. Although PTEN functions were extensively studied, the regulation of PTEN is less understood.

To elucidate potential regulators of PTEN, we recently conducted a tandem affinity purification using PTEN stable cell line and identified several PTEN-associated proteins (9). We repeatedly found PNUTS as one of the potential PTEN-associated proteins. Protein phosphatase-1 nuclear targeting subunit (PNUTS), also called PPP1R10, CAT53, and p99, was originally isolated as a nuclear protein that forms a stable complex with PP1-α and PP1-γ in mammalian cells (10, 11). PNUTS binds to PP1 and potently decreases the catalytic activity of PP1 toward exogenous substrates such as retinoblastoma (Rb) protein in vitro, and reduced expression of PNUTS in mammalian cells affects cell viability (12, 13). However, the exact function of PNUTS in vivo remains to be elucidated.

Materials and Methods

**Plasmids**

Full-length PNUTS and PTEN were cloned into mammalian expressing S-protein/FLAG/SBP (streptavidin-binding protein)—triple-tagged destination vector, and MYC-tagged destination vector using Gateway cloning system (Invitrogen). PNUTS domain deletions and PTEN domain deletions were cloned into S-protein/FLAG/SBP (Streptavidin binding protein)—triple-tagged destination vector. Bacterially expressing glutathione S-transferase (GST)—tagged PTEN, GST-tagged PNUTS, Maltose binding protein (MBP)-tagged PTEN, and MBP-tagged PNUTS vectors were generated by transferring...
their coding sequences into destination vectors by using gateway cloning system.

**Antibodies**

Rabbit anti-PNUTS, anti-Foxo3a, anti-PNUTS [immuno-histochemistry (IHC) specific; Bethyl Laboratories], monoclonal anti-PTEN clone 6H2.1 (Cascade Biosciences), monoclonal anti-MBP (New England Bio Labs), anti-GST, anti-Myc clone 9E10, anti-p53, anti-HDAC2 (all from Santa Cruz Biotechnologies), rabbit anti-pAkt (ser-473), anti-Akt, anti-PTEN, anti-pFoxo3a (ser-253; all from Cell Signalling Technology), anti-HDAC2 (Biomol, used in Fig. 2C), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Imgenex), anti-Rad51 (Bethyl Laboratories), rabbit anti-pAkt (ser-473), anti-Akt, anti-PTEN, anti-MBP (New England Bio Labs), anti-GST, anti-Myc (all from Santa Cruz Biotechnologies), rabbit anti-pAkt (ser-473), anti-Akt, anti-PTEN, anti-pFoxo3a (ser-253; all from Cell Signalling Technology), anti-HDAC2 (Biomol, used in Fig. 2C), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Imgenex), anti-Rad51 (Bethyl Laboratories), anti-Flag, anti-actin (Sigma), antibodies were used in this study.

**Cell lines**

293T, HeLa, K562, MDA-MB231, BPH1, DU145, and PC-3 cells were used in this study. All cell lines were obtained from American Type Culture Collection, which were tested and authenticated by the cell bank using their standard short tandem repeats (STR)–based techniques. Cells were also continuously monitored by microscopy to maintain their original morphology and also tested for mycoplasma contamination by using 4′,6-diamidino-2-phenylindole (DAPI) staining.

**Cell transfections, immunoprecipitation, and immunoblotting**

Cells were transfected with various plasmids using Lipofectamine (Invitrogen) according to the manufacturer’s protocol. For immunoprecipitation assays, cells were lysed with NETN buffer (20 mmol/L Tris–HCl, pH 8.0, 100 mmol/L NaCl, 1 mmol/L EDTA, and 0.5% Nonidet P-40) containing 50 mmol/L β-glycerophosphate, 10 mmol/L NaF, 1 μg/mL of each pepstatin A, and aprotinin on ice for 30 minutes. The whole-cell lysates obtained by centrifugation were incubated with 2 μg of specified antibody bound to either protein A or protein G sepharose beads (Amersham Biosciences) for 1 hour at 4°C. The immunocomplexes were then washed with NETN buffer 4 times and applied to SDS-PAGE. Immunoblotting was conducted following standard protocols.

**Isolation of cytoplasmic and nuclear extracts**

Cells grown in 100-mm dish were resuspended in 400 μL of Buffer A (1 mol/L HEPES pH 7.9, 2 mol/L KCl, 0.5 mol/L EDTA pH 8.0, and 0.1 mol/L EGTA pH 7.0 with protease inhibitors) and incubated in ice for 1 hour. Later 1% NP40 was added and the cytoplasmic extract (supernatant) was collected by centrifugation at 5,000 rpm for 5 minutes. The pellet was resuspended in Buffer B (1 mol/L HEPES pH 7.9, 5 mol/L NaCl, 0.5 mol/L EDTA pH8.0, and 0.1 mol/L EGTA pH 7.0 with protease inhibitors) and the nuclear extract was collected after incubation in ice for 45 minutes followed by centrifugation at 15,000 rpm for 5 minutes.

**GST pull-down and in vitro binding assays**

Bacterially expressed GST-PTEN or control GST bound to Glutathione-Sepharose beads (Amersham) was incubated with 293T cell lysates for 1 hour at 4°C, and the washed complexes were eluted by boiling in SDS sample buffer, separated by SDS-PAGE, and the interactions were analyzed by Western blotting. Similarly, bacterially expressed GST-PNUTS or GST bound to Glutathione-Sepharose beads (Amersham) was incubated with cell lysates of bacteria in which MBP-PTEN is expressed.

**RNA interference**

Control siRNA and the on-target plus individual siRNAs against PNUTS (siRNA#1: ACAAUUGGCGACGUAUUC, siRNA#2: GCAGACCCGUUCACCAGAA) were purchased from Dharmacon Inc. PTEN siRNA was purchased from Qiagen. Retroviral short hairpin RNA (shRNA) set for PNUTS was purchased from Open Biosystems. Transfection was carried out twice 30 hours apart with 20 nmol/L of siRNA using Oligofectamine reagent (Invitrogen) according to the manufacturer’s protocol.

**Retrovirus production and infection**

Virus-containing supernatant was collected 48 and 72 hours after cotransfection of control shRNA or PNUTS shRNA vectors and pcl-ampho helper plasmid into BOSC23 packaging cells, and was used to infect DU-145 or PC-3 cells in the presence of polybrene. Two days later, cells were transferred to puromycin-containing selection medium. The clones stably expressing PNUTS shRNA were screened after 3 weeks and verified by Western blotting using anti-PNUTS antibodies.

**PTEN-lipid binding assay**

Phosphatidylserine (PS) or phosphatidylethanolamine (PE) or phosphatidylcholine (PC) or PS:PE:PC mix (1:1:1) dissolved in 2:1:0.8 ratio of methanol/chloroform/water were spotted onto nitrocellulose membranes and dried at room temperature for 1 hour. After blocking (50 mmol/L Tris–HCl pH 7.5, 150 mmol/L NaCl, 0.1% Tween, and 2 mg/mL bovine serum albumin) the membranes for 1 hour, they were incubated with GST-PTEN either in the presence or absence of PNUTS overnight at 4°C. The membranes were then washed 10 times for 5 minutes each with 1 × TBS-T and incubated with horse-radish peroxidase (HRP)–conjugated anti-GST antibody. The binding of PTEN to the lipid was detected by enhanced chemiluminescence.

**Apoptosis assays**

Cells were transfected with control, PNUTS, or PTEN and PNUTS siRNAs. After 72 hours, the cells were washed with PBS and stained with fluorescein isothiocyanate (FITC)-Annexin V and propidium iodide (PI) according to the manufacturer’s protocol (BD Biosciences Annexin V Kit). Apoptotic cells (Annexin V–positive, PI-negative) were then determined by flow cytometry.

**Soft agar colony assays**

Cells were resuspended in RPMI containing 10% FBS along with 0.5% low-melting agarose and seeded on a plate coated with 1% agarose in RPMI and 10% FBS. Viable colonies were scored after 3 weeks of incubation and the quantified data were presented from 3 independent experiments.
Quantitative RT-PCR

Tumor samples were collected following patient informed consent and approval from the ethics committee of each collaborating hospital. Total RNA was isolated using Trizol reagent (Invitrogen) as per manufacturer’s instructions. One microgram of total RNA was reverse transcribed in the presence of anchored oligo dT (CyScribe Post Labeling Kit; Ambion) using Superscript-II (Invitrogen) as per manufacturer’s protocol. Quantitative PCR (qPCR) was then initiated using the SYBR Pre mix Ex Taq (perfect real time) kit (Takara Biochemistry) as per manufacturer’s protocol with 40 cycles of 95°C/30 seconds and 60°C/1 minute. The threshold cycle (Ct) values for PTEN and PNUTS were normalized to GAPDH for each sample and the ΔCt values thus obtained were plotted. Pearson correlation (r) between the genes was calculated using Microsoft Excel. Following Primers are used for quantitative real-time PCR (qRT-PCR). PNUTS_F: CTGCGAGCTAAAGGTAAGATA, PNUTS_R: AAGTGGACATTTTGTCTTTTTT, PTEN_F: AAAACAGGCAGAAACCA, PTEN_R: GGCTCCAAAGGCGCCTGTAT, GAPDH_F: CAATGACCCCTTCATTGACC, GAPDH_R: GATCTCGTCTCTGTGAAGATG.

Immunofluorescence staining

Cells grown on coverslips were fixed with 3% paraformaldehyde solution in PBS containing 50 mmol/L sucrose at room temperature for 15 minutes. After permeabilization with 0.5% Triton X-100 buffer containing 20 mmol/L HEPES pH7.4, 50 mmol/L NaCl, 3 mmol/L MgCl2 and 300 mmol/L sucrose at room temperature for 5 minutes, cells were incubated with a primary antibody at 37°C for 20 minutes. After washing with PBS, cells were incubated with rhodamine-conjugated secondary antibody at 37°C for 20 minutes. Nuclei were counterstained with DAPI. After a final wash with PBS, coverslips were mounted with glycerin containing paraphenylenediamine.

Immunohistochemistry

Sections of matched tumor and normal tissues were placed on a single frosted coated slide (Fisher). Antigen retrieval was conducted using the pressure cooker method in citrate buffer (pH 6.0) and endogenous peroxidase was blocked using 2% H2O2. The slides were incubated with PNUTS antibody (Bethyl; 1:250 dilution) for 1 hour followed by incubation with HRP-conjugated secondary antibody (DAKO). Visualization was conducted using Envision Plus HRP kit using 3',3'-diaminobenzidine (DAB) substrate (DAKO) as per manufacturer’s instructions. The slides were counterstained with hematoxyline and mounted. The IHC results were evaluated for both nuclear stain intensity [on a scale of 1 (negative stain) to 5 (very strong)] and for percentage-positive epithelial cells. Overall staining intensity (OSI) was calculated as the product of intensity score and percentage of positive cells scored. The tumor/normal OSI-fold change was converted to log2 scale and plotted.

Results

PNUTS Is a Negative Regulator of PTEN

We have shown the interaction of endogenous PTEN with PNUTS by immunoprecipitating HEK293T cell lysates using a PNUTS polyclonal antibody or a PTEN monoclonal antibody (Fig. 1A). By transiently expressing exogenous Flag-PTEN and Myc-PNUTS, we further confirmed their interaction in HEK293T cells (Supplementary Fig. S1A). In addition, an in vitro GST pull-down assay with a bacterially expressed PTEN clearly shows the association of PNUTS with PTEN (Supplementary Fig. S1B). Importantly, bacterially purified recombinant GST-PNUTS and MBP-PTEN proteins interacted with each other suggesting a direct interaction between PTEN and PNUTS (Fig. 1B). To map the binding region of PTEN, we generated expression constructs for SFB-tagged PTEN and a series of domain deletion mutants (Fig. 1C). Our immunoprecipitation results indicated that PNUTS interacts with the C2-domain of PTEN (Fig. 1D and Supplementary Fig. S1C). On the other hand, to map the PTEN-binding region in PNUTS, we coexpressed SFB-tagged PNUTS constructs (Fig. 1E) along with Myc-tagged PTEN. Deletion of the N-terminal TF2S domain of PNUTS (∆TF2S PNUTS) disrupted its binding to PTEN (Fig. 1F). Collectively, these interaction data suggest that PTEN C2 domain and PNUTS TF2S domain are both necessary and sufficient for the interaction between PTEN and PNUTS.

PNUTS sequesters PTEN in the nucleus

Previous studies have shown that PTEN is localized in cytoplasm and nucleus (14), whereas PNUTS is exclusively a nuclear protein (11). We further tested whether PTEN and PNUTS colocalize in the cell. PTEN is mainly localized in cytoplasm with sparse nuclear localization. Interestingly, expression of full-length PNUTS, but not ∆TF2S PNUTS leads to drastic relocalization of PTEN to the nucleus, in which it colocalizes with PNUTS (Fig. 2A and Supplementary Fig. S1D). On the other hand, PTEN mutant defective of PNUTS binding (PTEN D1) is exclusively localized in cytosol and overexpression of PNUTS has no effect on its localization. Furthermore, expression of PNUTS mutant (W401A) that is defective in PP1 binding also relocalized PTEN to the nucleus indicating PP1 independent role of PNUTS in PTEN localization. Overexpression of PNUTS has no effect on PHHPP, another phosphatase in Akt pathway, indicating the specificity of PNUTS–PTEN interaction. We further showed the positive role of PNUTS in recolocalization of PTEN into the nucleus by extracting the cytoplasmic and nuclear fractions. Overexpression of PNUTS enhanced nuclear pool of PTEN (Fig. 2B), whereas siRNA-mediated knockdown of PNUTS reduced nuclear PTEN levels (Fig. 2C) with no significant changes in the half-life of PTEN (Supplementary Fig. S1E). Previous studies have shown that ubiquitination and deubiquitination of PTEN plays a critical role in nuclear-cytoplasmic shuttling of PTEN (14, 15). We used PTEN K289E mutant that is defective in ubiquitination to further study the role of PNUTS in PTEN localization. PTEN K289E mutant is predominantly cytoplasmic but coexpression of PNUTS lead to its nuclear recolocalization similar to wild-type PTEN (Fig. 2D and Supplementary Fig. S1F), thus suggesting the ubiquitin-independent PTEN nuclear localization by PNUTS. Collectively these data suggest the PNUTS sequesters PTEN in the nucleus by directly interacting with PTEN.
PNUTS is upregulated in human cancers and positively correlates with PTEN expression

PNUTS is localized on chromosome 6p21.3, a region with frequent aberrations such as chromosome amplifications and increase in gene copy number reported in various human cancers (16, 17). In addition, we have shown that PNUTS readily interacts with PTEN, a well-known tumor suppressor, thus we speculated that PNUTS may play a critical role in
To test the role of PNUTS in tumorigenesis, we have analyzed the expression of PNUTS in tissues derived from various types of cancers. Our IHC results clearly showed that PNUTS expression is significantly upregulated in tumors derived from squamous cell carcinomas and adenocarcinomas of esophagus compared with their matched normal tissues (Fig. 3A and B). In addition, gene expression datasets from publicly available cancer database (Oncomine)
show increased expression of PNUTS in various tumors, including prostate, skin, renal, and brain compared with that in normal tissues (Supplementary Fig. S2A). Interestingly, we observed a positive correlation between the expression of PTEN and PNUTS at the transcript level in esophageal, colon, and pancreatic tumors along with normal tissues (Fig. 3C). In addition, PNUTS expression was significantly higher in triple-positive breast cancer (with high PTEN levels; ref. 18, 19) compared with the triple-negative breast cancers (low PTEN; Fig. 3D), which again support a strong association of PNUTS and PTEN. Together these data suggest that PNUTS is concurrently expressed along with PTEN to regulate the survival and growth signaling in cells.

**PNUTS control Akt activation and apoptosis in a PTEN dependent manner**

Because PNUTS binds the lipid-binding domain (C2 domain) of PTEN, we investigated whether PNUTS interaction with PTEN blocks the association between PTEN and lipids. We conducted an *in vitro* PTEN–lipid binding assay to test if PNUTS interferes with PTEN binding to lipids. As expected, GST-PTEN but not GST alone, strongly associated with the lipids. Interestingly, addition of full-length PNUTS dramatically reduced the association of PTEN with the lipids (Fig. 4A). But, addition of ΔTF2S PNUTS showed modest effects on PTEN binding to lipids. To further explore the effect of PNUTS on the PTEN access to lipids at the membrane, we tested whether Akt signaling downstream of PTEN is affected when PNUTS levels were modulated in cells. Indeed, knockdown of PNUTS by specific siRNAs in HeLa cells resulted in decreased Akt phosphorylation (Fig. 4B) with no significant effect on PTEN and total Akt levels (Fig. 4B). PNUTS regulates Akt activation in a PTEN-dependent manner, because a simultaneous depletion of PNUTS along with PTEN by siRNA reversed PNUTS effect on Akt phosphorylation (Fig. 4C). As PNUTS relocalized PTEN to the nucleus, we further analyzed the effect of PNUTS on the

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**Figure 3.** PNUTS is differentially expressed in normal and tumor tissues. A, sections of matched tumor and normal tissues were stained with PNUTS antibody and the representative IHC images were shown. B, tissue samples obtained from esophageal squamous cell carcinoma and adenocarcinoma were subjected to IHC with specific PNUTS antibody. Data represent the fold change in the overall staining density in tumor sample compared with matched normals. C, a qRT-PCR analysis was done after extracting RNA from tissues derived from esophageal, pancreatic and colon cancers (n = 52), and normal tissues (n = 32). Data represent the relative transcript abundance of PNUTS and PTEN normalized against GAPDH. The Pearson correlation coefficient is also shown. The trend line depicts the distribution of the data points with respect to perfect correlation (r = 1). D, tissue samples obtained from triple-positive (Er+, PR+, Her2/neu+) and triple-negative (Er−/C0, PR−/C0, Her2/neu−) breast cancers (n = 20 samples each) were subjected to IHC with specific PNUTS antibody. Fisher exact test (2-tailed) P value is also shown.
nuclear functions of PTEN. Expression of PTEN in PTEN null PC-3 prostate cancer cells enhanced rad51 and p53 protein levels, but surprisingly coexpression of PNUTS along with PTEN reduced rad51 and p53 to normal levels (Fig. 4D). These results suggest that PNUTS sequesters PTEN in an inactive state in the nucleus. It is well known that PTEN promotes apoptosis (20), and as PNUTS acts as a negative regulator of PTEN function, we hypothesized that depletion of PNUTS might induce apoptosis. To test this hypothesis, we depleted PNUTS by siRNA in HeLa cells and checked for cell death. Knockdown of PNUTS by siRNA readily induced cellular apoptosis (Fig. 4E), but a simultaneous depletion of PNUTS and PTEN rescued cells from apoptosis when compared with PNUTS depletion alone (Fig. 4F). Similar results were observed in K562 myeloid leukemic cells (Supplementary Fig. S3A–S3C), MDA-MB231 breast cancer cells (Supplementary Fig. S4A–S4C) and BPH1 prostate epithelial cells (Supplementary Fig. S4D–S4F) depleted with PNUTS and PTEN. In addition to reduced Akt phosphorylation, depletion of PNUTS also affects Foxo3A phosphorylation, a downstream substrate of Akt (Supplementary Fig. S4A and S4D). Taken together, these results indicate that PNUTS negatively regulates apoptosis in a PTEN-dependent manner.

**PNUTS is required for tumorigenicity of cells**

To further test whether endogenous PNUTS influences cellular survival and growth and to establish its role as a proto-oncogene, we stably depleted PNUTS with shRNA in a PTEN-positive prostate cancer cell line (DU145) and a PTEN-negative prostate cancer cell line (PC3). DU-145 PNUTS shRNA expressing cells showed decreased phosphorylation of Akt and Foxo3a, increased p27 levels, (Supplementary Fig. S5A and S5B)
and reduced cell proliferation (Fig. 5A) when compared with control shRNA expressing cells. In contrast, levels of phosphorylated Akt (pAkt) and extent of cell proliferation were not significantly changed in PC-3 PNUTS shRNA expressing cells compared with control shRNA (Fig. 5B and Supplementary Fig. S5C and S5D). In addition, we analyzed the cell-transforming ability of PNUTS by carrying out soft agar colony formation assays. Depletion of PNUTS significantly reduced the transforming capability of DU-145 cancer cells when compared with control shRNA (Fig. 5C), whereas the transforming capability of PC-3 cancer cells was unaffected with depletion of PNUTS (Fig. 5D). Conversely, stable expression of full-length PNUTS but not ΔTF2S PNUTS in normal BPH1 prostate epithelial cells (Supplementary Fig. S5E) enhanced the proliferation (Fig. 5E) and the transforming ability of cells (Fig. 5F). Interestingly, the expression of PNUTS W401A mutant that is defective in

![Graphs and images](image.png)
binding to PP1 also shows increased proliferation and transformation although less compared with full-length PNUTS suggesting that PNUTS mediates its cellular functions mainly through PTEN inhibition and to some extent via PP1 inhibition. Collectively, these results indicate that PNUTS acts as a potential oncogene by sequestering PTEN.

Discussion

Because PTEN is a very critical tumor suppressor and trivial changes in the PTEN levels make it susceptibility for malignancy, it is essential to define the regulatory mechanisms that control PTEN functions. Several studies have indicated that PTEN is regulated by multiple mechanisms either at the transcriptional or posttranslational level. PTEN expression is regulated by transcription factors such as p53, PPAR-γ, and egr-1 at transcription level (21–23), whereas PTEN ubiquitination and phosphorylation regulate its protein levels and activity at the posttranslational level (9, 24). In this study, we show that PTEN activity can be regulated through a direct protein–protein interaction.

PNUTS tightly associates with PTEN and block the lipid access at the membrane, as PNUTS via its N-terminal TF2S domain binds directly to the lipid-binding C2 domain of PTEN. In fact, we have shown that expression of PNUTS sequesters PTEN in the nucleus as compared with the nonexpressing cells, in which PTEN is mainly cytoplasmic and membrane associated. Thus, PNUTS might affect the PI3K metabolism at the membrane by nuclear sequestration of PTEN. In addition, our data also suggested that PNUTS negatively regulates nuclear PTEN-induced expression of rad51 and p53. These results suggest that PNUTS sequesters PTEN in an inactive state in the nucleus.

Our studies also suggested that PNUTS might be acting as a potential oncogene by positively regulating the Akt pathway, promoting cell growth and proliferation, and suppressing cellular apoptotic potential in a PTEN-dependent manner. Importantly, we have shown that PNUTS expression is significantly upregulated in tumors derived from squamous cell carcinomas and adenocarcinomas of esophagus compared with their matched normal tissues. Furthermore, we observed a positive correlation between the expression of PTEN and PNUTS at the transcript level both in tumors (esophageal, colon, and pancreas) and normal tissues, thus suggesting a tight association of PNUTS with PTEN in regulating its function. PTEN is known to be downregulated by different mechanisms, such as PTEN deletions and mutations in tumors in which PTEN in inactivated by mutations, deletions, etc. Earlier studies have shown that depletion of PNUTS increased apoptosis in PT1- and Rb-dependent manner (12). Our results suggest that it would be possible that PNUTS may exert its cellular functions via acting through multiple pathways. In addition to its role in human cancers, PI3K–Akt–PTEN axis has multitudes of functions in regulating various pathologic conditions, such as diabetes, neurologic disorders, aging, and heart diseases (25). Hence, the identification of PNUTS as a critical regulator of PTEN through our studies has provided an additional line of potential therapeutic intervention for human diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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