The American Journal of Pathology, Vol. 180, No. 3, March 2012

DOI: 10.1016/j.ajpath.2011.11.021

Growth Factors, Cytokines, and Cell Cycle Molecules

PTEN Regulates PDGF Ligand Switch for β-PDGFR Signaling in Prostate Cancer

M. Katie Conley-LaComb,* Wei Huang,* Shihua Wang,† Dongping Shi,* Young Suk Jung,* Abdo Najy,* Rafael Fridman,* R. Daniel Bonfil,*‡ Michael L. Cher,*‡ Yong Q. Chen,† and Hyeong-Reh Choi Kim*

From the Departments of Pathology and Urology,‡ Barbara Ann Karmanos Cancer Institute, Wayne State University, School of Medicine, Detroit, Michigan; and the Department of Cancer Biology,† Wake Forest University School of Medicine, Winston-Salem, North Carolina*

Platelet-derived growth factor (PDGF) family members are potent growth factors that regulate cell proliferation, migration, and transformation. Clinical studies have shown that both PDGF receptor β (β-PDGFR) and its ligand PDGF D are up-regulated in primary prostate cancers and bone metastases, whereas PDGF B, a classic ligand for β-PDGFR, is not frequently detected in clinical samples. In this study, we examined the role of the tumor suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN) in the regulation of PDGF expression levels using both a prostate-specific, conditional PTEN-knockout mouse model and mouse prostate epithelial cell lines established from these mice. We found an increase in PDGF D and β-PDGFR expression levels in PTEN-null tumor cells, accompanied by a decrease in PDGF B expression. Among Akt isoforms, increased Akt3 expression was most prominent in mouse PTEN-null cells, and phosphatidylinositol 3-kinase/Akt activity was essential for the maintenance of increased PDGF D and β-PDGFR expression. *In vitro* deletion of *PTEN* resulted in a PDGF ligand switch from PDGF B to PDGF D in normal mouse prostate epithelial cells, further demonstrating that PTEN regulates this ligand switch. Similar associations between PTEN status and PDGF isoforms were noted in human prostate cancer cell lines. Taken together, these results suggest a mechanism by which loss of PTEN may promote prostate cancer progression via PDGF D/β-PDGFR signal transduction. *(Am J Pathol 2012, 180:1017–1027; DOI: 10.1016/j.ajpath.2011.11.021)*

Prostate cancer (PCa) is the most diagnosed noncutaneous cancer of men in the United States, and the second leading cause of death, accounting for 10% of cancer-related mortality among men.[1] Studies have suggested a critical role for platelet-derived growth factor (PDGF) signaling during PCa development and progression. The PDGF family consists of four ligands, PDGFs A, B, C, and D, that form homodimers or a heterodimer AB.[2] Tumor-derived PDGFs regulate diverse cellular processes, such as cell proliferation, migration, differentiation, and phenotypic transformation, through activation of their cognate receptors, α and β (α- and β-PDGFR, respectively), involving both autocrine and paracrine signaling mechanisms. Although α-PDGFR can be activated by PDGFs A, B, and C, β-PDGFR is activated by PDGFs B and D. PDGFR signaling may be of particular importance for PCa bone metastasis, because increasing evidence suggests a critical role for PDGF in bone turnover and growth. PDGF regulates commitment of stromal mesenchymal cells to differentiate into osteoprogenitor cells and induces proliferation and migration of osteoblast cells,[3–5] suggesting a role for PDGF in bone formation. PDGF also stimulates bone resorption by increasing the number of osteoclasts and up-regulating matrix-degrading enzyme expression.[6–8] Consistently, our recent study demonstrated that PDGF D/β-PDGFR signaling enhances intraosseous PCa growth and bone reactions in an animal model.

Immunohistochemical (IHC) analysis showed that β-PDGFR is up-regulated in most primary and metastatic PCa cells.[9] More important, expression of β-PDGFR has been identified by microarray analyses as part of a five-

Supported by the Ruth L. Kirschstein National Research Service Award (T32-CA009531 to M.K.C.-L.) and grants from the NIH/National Cancer Institute (R01CA137280 to R.F. and M.C.; R01CA107668 and P01CA106742 to Y.Q.C.; R01CA064139 and R01CA123362 to H.-R.C.K.).

Accepted for publication November 28, 2011.

Epithelial cell line requests should be addressed to: Yong Q. Chen, Ph.D., Department of Cancer Biology, Wake Forest University Health Sciences, Medical Center Blvd., Winston-Salem, NC 27157. E-mail: yqchen@wfubmc.edu.

Address reprint requests to Hyeong-Reh Choi Kim, Ph.D., Department of Pathology, Wayne State University, 540 E Canfield, Scott Hall, Room 9115, Detroit, MI 48201. E-mail: hrckim@med.wayne.edu.

gene model, along with chromogranin A, *HOXC6*, *IPTR3*, and sialyltransferase-1, that predicts PCa recurrence.[10] Although PDGF B, originally thought to be the sole ligand for β-PDGFR, has not been detected in PCa tissues, our recent IHC study showed that increased PDGF D expression is associated with both higher tumor stage and higher Gleason score,[11] identifying PDGF D as a clinically relevant ligand for β-PDGFR in PCa. PDGF D induces PCa cell motility in an autocrine manner, and PCa-produced PDGF D functions as a chemoattractant for fibroblasts through paracrine signaling.[12] In an animal model, PDGF D expression accelerated early onset of prostate tumor growth and drastically enhanced prostate carcinoma cell invasion and interaction with surrounding stromal cells.[12] Despite increasing evidence for PDGF D in PCa, little is known about the molecular mechanisms by which PDGF D expression is regulated in PCa.

In this study, we identified phosphatase and tensin homologue deleted on chromosome 10 (*PTEN*, also known as *MMAC1/TEP1*) as a key regulator of PDGF D expression in PCa cells. PTEN is a nonredundant, plasma-membrane lipid phosphatase that hydrolyzes the 3-phosphate on phosphatidylinositol 3,4,5-triphosphate and, thereby, negatively regulates phosphatidylinositol 3,4,5-triphosphate–mediated signal transduction pathways, such as the phosphatidylinositol 3-kinase (PI3K)/Akt pathway.[13] By regulating the pathways of the serine/threonine kinase Akt, PTEN regulates many cellular processes, including cell cycle, cell motility/invasion, cell adhesion, protein synthesis, and glucose metabolism. The loss or mutation of the tumor suppressor *PTEN* gene is considered one of the most common genetic abnormalities in PCa.[14] The estimated frequency of monoallelic loss or mutations at the *PTEN* genetic locus is 50% to 80% in primary PCa.[15,16] *PTEN* haploinsufficiency is thought to be an important driving force in the early pathogenesis of many tumors, including PCa. Evidence suggests that complete loss of PTEN function at later stages is associated with more aggressive and metastatic tumors.[14,17] In an animal model, mice with prostate-specific heterozygous PTEN deletion developed mouse prostate intraepithelial neoplasia lesions at 12 to 16 months, with near 100% penetrance. A prostate-specific PTEN homozygous deletion shortened latency, and 100% of the $PTEN^{-/-}$ mice developed mouse prostate intraepithelial neoplasia lesions at the age of 6 weeks. More important, the $PTEN^{-/-}$ mouse prostate intraepithelial neoplasia lesions progressed to invasive adenocarcinomas by the age of 9 weeks,[18] recapitulating the disease progression seen in human PCa, from hyperplasia to prostate intraepithelial neoplasia, then to invasive adenocarcinoma. Herein, we show increased PDGF D expression and β-PDGFR activation in tumor tissues from prostate-specific $PTEN^{-/-}$ mice. By using cell lines established from PTEN knockout mice, we demonstrate that the loss of PTEN results in a ligand switch from PDGF B to PDGF D, resulting in an expression pattern similar to that seen in humans. In addition, we show that PI3K/Akt activities are essential for the maintenance of increased PDGF D expression in both mouse and human prostate epithelial cells in the context of PTEN loss, whereas AMP-activated protein kinase (AMPK) regulates PDGF B expression.

Materials and Methods

Cell Culture

Cell lines were cultured at 37°C in a humidified incubator with 5% CO_2, and all media were supplemented with 2 mmol/L glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin (Life Technologies Inc., Carlsbad, CA). Human prostate carcinoma cell lines PC3 and DU145 were verified by American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum. The mouse cell lines were maintained in advanced Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin.

Establishment of $PTEN^{+/+}$, $PTEN^{+/-}$, and $PTEN^{-/-}$ Mouse Prostate Epithelial Cell Lines

Prostate-specific deletion of floxed exon 5 of *PTEN* was achieved by Cre recombinase expressed under the control of an androgen-responsive probasin promoter (PB). Mouse genotypes are $PTEN^{loxP/loxP}$*PB-cre4*$^{-/-}$ (wild type), $PTEN^{loxP/+}$*PB-cre4*$^{T/-}$ (heterozygous), and $PTEN^{loxP/loxP}$*PB-cre4*$^{T/-}$ (homozygous) and referred to as $PTEN^{+/+}$, $PTEN^{+/-}$, and $PTEN^{-/-}$, respectively, as described.[19] The $PTEN^{+/+}$, $PTEN^{+/-}$, and $PTEN^{-/-}$ mouse prostate epithelial cells were isolated from prostates of corresponding mice at the age of 8 weeks using the previously described method.[20] Cell lines were established by a serial dilution method and subsequent clonal selection,[21] and the PTEN status in these cell lines was confirmed by genotyping and immunoblot analyses.

In Vitro *Deletion of PTEN in Mouse Prostate Epithelial Cell Line*

$PTEN^{loxP/loxP}$ cells were isolated from the prostates of 8-week-old mice. *In vitro* deletion of PTEN was achieved by expression of Cre recombinase by a self-deleting lentiviral vector (PTEN viral knockout, or PTEN-vKO). PTEN-knockout clones (PTEN-vKO-#2 and PTEN-vKO-#3) were then selected from the pooled population (PTEN-vKO-pp). A pooled population of wild-type PTEN control cells was also established by infection with a control lentivirus ($PTEN^{L/L}$).

Reagents

The generation of antibody that recognizes the growth domain of PDGF D was previously described.[22] Antibodies against PTEN, phosphorylated β-PDGFR (Y751), phosphorylated Akt (pAkt; T308 and S473), phosphorylated S6 kinase (S6K) (T389), total S6K, phosphorylated c-Jun kinase (JNK; T183/Y185), total JNK, phosphorylated extracellular signal–regulated kinase (T202/Y204),

total extracellular signal–regulated kinase, phosphorylated AMPKα (T172), and total AMPKα were obtained from Cell Signaling Technology (Boston, MA). The Akt1, Akt2, and Akt3 antibodies were obtained from Upstate (Billerica, MA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The AMPK activator, 5-aminoimidazole-4-carboxyamide ribonucleoside (AICAR), was obtained from Cell Signaling Technology; and the AMPK inhibitor, Compound C, was obtained from Calbiochem (Gibbstown, NJ).

Inhibition of the PTEN Downstream Signaling Pathways

Cells were cultured with complete growth media and treated with indicated concentrations of inhibitors or vehicle control. Cells were treated with 1 or 25 μmol/L JNK Inhibitor II (Fisher Scientific, Waltham, MA), 1 or 25 μmol/L PD98059 (Sigma-Aldrich, St Louis, MO), 1 to 50 μmol/L LY294002 (Sigma-Aldrich), 10 to 50 nmol/L wortmannin (Calbiochem), or 1 to 15 μmol/L Akt Inhibitor IV (Fisher Scientific) for 18 hours. Cells were treated with 10 nmol/L rapamycin (Sigma-Aldrich) for 48 hours. mRNA was collected from cells at the designated time point and subjected to RT-PCR and real-time RT-PCR analyses.

RT-PCR

mRNA was purified from cells using the RNeasy kit (Qiagen, Valencia, CA). cDNA synthesis was performed with a Superscript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA), followed by PCR using GoTaq Flexi DNA Polymerase (Promega, Madison, WI). Forward and reverse murine-specific primers used are as follows: PDGF B, 5′-GCCTGTGACTAGAAGTCCTG-3′ (forward) and 5′-GTCATGGGTGTGCTTAAACT-3′ (reverse); PDGF D, 5′-CAGGGAAGACAGTGAAGAAG-3′ (forward) and 5′-GAGCTGCAGATACAGTCACA-3′ (reverse); β-PDGFR, 5′-CATCATGAGGGACTCAAACT-3′ (forward) and 5′-GATGGCATTGTAGAACTGGT-3′ (reverse); PTEN, 5′-ACACCGCCAAATTTAACTGC-3′ (forward) and 5′-TGAGGTTTCCTCTGGTCCTG-3′ (reverse); Akt1, 5′-GACCCACGACCGCCTCTG-3′ (forward) and 5′-GACACAATCTCCGCACCATAGAAG-3′ (reverse); Akt2, 5′-GAGGACGCCATGGATTACAAG-3′ (forward) and 5′-GACAGCTACCTCCATCATCTCAGA-3′ (reverse); and Akt3, 5′-GAGTACCTGGCACCAGAGGT-3′ (forward) and 5′-AGAAAGGCAACCTTCCACAC-3′ (reverse). Forward and reverse human-specific primers used are as follows: PDGF B, 5′-CATTCCCGAGGAGCTTTATG-3′ (forward) and 5′-CTCAGCAATGGTCAGGGAAC-3′ (reverse); PDGF D, 5′-GAACAGCTACCCCAGGAACC-3′ (forward) and 5′-CTTGTGTCCACACCATCGTC-3′ (reverse); PTEN, 5′-GGACGAACTGGTGTAATGATATG-3′ (forward) and 5′-TCTACTGTTTTTGTGAAGTACAGC-3′ (reverse); Akt1, 5′-ATGAGCGACGTGGCTATTGTGAAG-3′ (forward) and 5′-GAGGCCGTCAGCCACAGTCTGGATG-3′ (reverse); Akt2, 5′-ATGAATGAGGTGTCTGTCATCAAAGAAGGC-3′ (forward) and 5′-TGCTTGAGGCTGTTGGCGACC-3′ (reverse); and Akt3, 5′-ATGAGCGATGTTACCATTGT-3′ (forward) and 5′-CAGTCTGTCTGCTACAGCCTGGATA-3′ (reverse). Forward and reverse primers recognizing both murine and human GAPDH were 5′-ATCACCATCTTCCAGGAGCGA-3′ and 5′-GCCAGTGAGCTTCCCGTTCA-3′, respectively.

Real-Time RT-PCR

mRNA was purified from cells using the RNeasy kit (Qiagen). cDNA synthesis was performed with a Superscript III First-Strand Synthesis System (Invitrogen). Real-time RT-PCR was performed using SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA) and the Stratagene MX4000 qPCR System, according to the manufacturer's protocol. Relative values of gene expression were normalized to GAPDH and calculated using the $2^{-\Delta\Delta C_T}$ method, where $\Delta\Delta C_T = (\Delta C_{T\text{target gene}}\text{-}\Delta C_{TGAPDH})_{sample}\text{-}(\Delta C_{T\text{target gene}}\text{-}\Delta C_{TGAPDH})_{control}$. The fold change in relative expression was then determined by calculating $2^{-\Delta\Delta C_T}$.

IHC Analysis of Murine Prostate Tissues

Slides of formalin-fixed, paraffin-embedded prostate sections from $PTEN^{+/+}$ and $PTEN^{-/-}$ mice at the age of 8 weeks were deparaffinized with xylene, then rehydrated sequentially with decreasing concentrations of EtOH from 100% to 70%, followed by water. Endogenous peroxidase activity was blocked with 3% H_2O_2, and antigen retrieval was performed by steaming for 20 minutes in Antigen Retrieval Citra Plus Solution (BioGenex, Freemont, CA). Slides were then washed twice with PBS and blocked with Cas-Block solution (Invitrogen). Slides were incubated overnight at 4°C in a humidified chamber with either anti-PDGF D polyclonal antibody (Ab; 8D2, 1:500 dilution) or anti-phosphorylated-β-PDGFR polyclonal Ab (1:100 dilution). Sections were then washed twice with PBS and incubated with ABC Vectastain Kit (Vector Labs, Burlingame, CA), according to manufacturer's protocol, followed by incubation with 3,3′-diaminobenzidine tetrahydrochloride (Vector Labs). Mayer's hematoxylin (Sigma-Aldrich) was used to counterstain the nuclei. Sections were then dehydrated with increasing concentrations of EtOH, washed with xylene twice, and mounted with Permount (Sigma-Aldrich).

IHC Analysis of Human Prostate Carcinoma

Serial sections of formalin-fixed, paraffin-embedded prostate sections from human PCa were stained for PDGF D and PTEN, as previously described.[23] Slides were incubated overnight at 4°C with anti-PDGF D polyclonal Ab (8D2, 1:50 dilution) or anti-PTEN monoclonal Ab (1:250 dilution, clone 6H2.1; Cascade Bioscience, Winchester, MA).

Results

Up-Regulation of PDGF D and β-PDGFR in Prostate Tumors from PTEN-Null Mice

We recently demonstrated PDGF D expression in human tissues and a positive association with grade and stage.[11] We also demonstrated that PDGF D drives PCa aggressiveness in animal models.[12] Increased PDGF D expression and its potential oncogenic activity in PCa progression raised a question as to how PDGF D expression is regulated in prostate tumor cells. Given the well-known functions of PTEN in the regulation of growth factor signaling[24,25] and its frequent loss in PCa, we hypothesized that the PDGF D/β-PDGFR pathway is driven by PTEN deficiency. To address this hypothesis, we first examined PDGF D expression in normal prostate tissues from control $PTEN^{+/+}$ mice and prostate adenocarcinomas from $PTEN^{-/-}$ mice. As shown in Figure 1A, IHC analysis showed higher expression levels of PDGF D in the prostate tumor tissues with prostate-specific PTEN deletion ($PTEN^{-/-}$) compared with the prostate of $PTEN^{+/+}$ mice. Although PDGF D was detected mostly in the ventral prostate, increased PDGF D expression was detected in all prostate lobes, including the dorsolateral prostate and anterior prostate. Increased PDGF D expression was accompanied by higher levels of phosphorylated forms of β-PDGFR in prostate carcinomas in $PTEN^{-/-}$ mice, indicating active β-PDGFR signaling (Figure 1B). In contrast, β-PDGFR activation was barely detected in normal prostate tissues. Unlike PDGF B, a classic ligand for β-PDGFR, PDGF D is secreted as a latent homodimer, and serine protease–mediated proteolytic removal of the N-terminal domain is required for the growth factor domain dimer to induce β-PDGFR dimerization and phosphorylation.[26,27] To determine whether the active form of PDGF D dimer is present in these prostate tumor tissues, immunoblot analysis was performed. As expected from activated β-PDGFR, the growth factor domain dimer of PDGF D was readily detected in tumor tissues isolated from the prostates of $PTEN^{-/-}$ mice (Figure 1C), indicating activation of PDGF D/β-PDGFR signaling in these tumor cells.

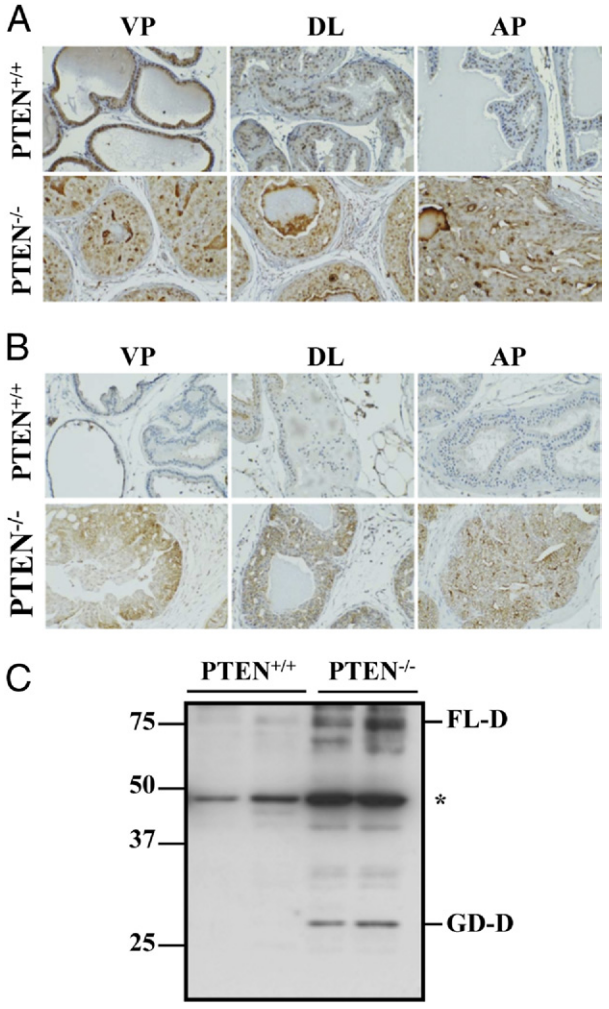

Figure 1. Activation of PDGF D/β-PDGFR in prostate tumors in $PTEN^{-/-}$ mice. **A** and **B:** Prostate tissue sections from $PTEN^{+/+}$ and $PTEN^{-/-}$ mice were immunostained with antibodies against PDGF D (**A**) or phosphorylated β-PDGFR (**B**). Original magnification, ×20. AP, anterior prostate; DL, dorsolateral prostate; VP, ventral prostate. **C:**Prostate tissue lysate from $PTEN^{+/+}$ and $PTEN^{-/-}$ mice was resolved on nonreducing SDS-PAGE, followed by immunoblot analysis with anti-PDGF D antibody. The **asterisk** indicates nonspecific. FL-D, full-length dimer; GFD-D, growth factor domain dimer.

PTEN Loss-Mediated PDGF Ligand Switch and a Critical Role for PI3K/Akt in PDGF D Up-Regulation

To further investigate the role of PTEN in the regulation of PDGF expression, we used mouse prostate epithelial cell lines derived from $PTEN^{+/+}$, $PTEN^{+/-}$, and $PTEN^{-/-}$ mice harboring prostate-specific deletion of *PTEN* exon 5. The exon 5 encodes the phosphatase domain; thus, deletion of this exon results in loss of PTEN phosphatase activity. As shown in Figure 2A, RT-PCR analysis using a forward primer in exon 4 and a reverse primer in exon 8 confirmed wild-type and exon 5–deleted PTEN mRNA expression in $PTEN^{+/+}$ and $PTEN^{-/-}$ cells, respectively. As expected, $PTEN^{+/-}$ cells expressed both wild-type and exon 5–deleted PTEN mRNAs. Exon 5–deleted PTEN protein was undetectable, suggesting loss of PTEN function in $PTEN^{-/-}$ cells. When we examined the mRNA levels of β-PDGFR and its ligands, PDGF B and PDGF D, PDGF B expression was markedly down-regulated in $PTEN^{-/-}$ cells compared with $PTEN^{+/+}$ and $PTEN^{+/-}$ cells. Conversely, PDGF D and β-PDGFR levels were elevated in $PTEN^{-/-}$, but not $PTEN^{+/+}$ or $PTEN^{+/-}$, cells (Figure 2A). Immunoblot analysis also showed increased PDGF D and decreased PDGF B expression in $PTEN^{-/-}$ cells compared with $PTEN^{+/+}$ or $PTEN^{+/-}$ cells (Figure 2B). These results showed that homozygous deletion of PTEN is associated with a β-PDGFR ligand switch from PDGF B to PDGF D, closely recapitulating expression patterns of PDGF ligands in human prostate tumor tissues.

To identify the PTEN pathways responsible for the regulation of PDGF gene expression, $PTEN^{-/-}$ cells

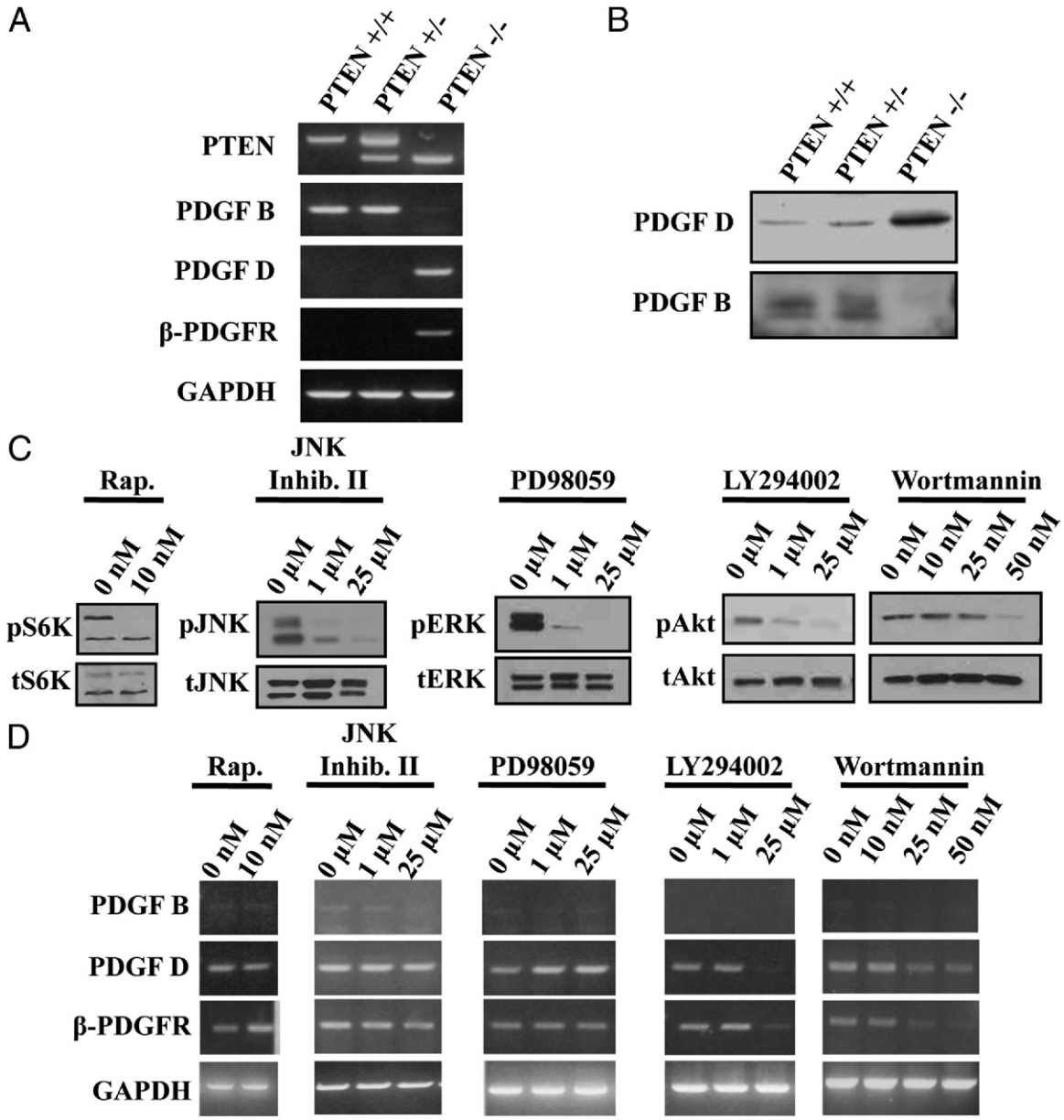

Figure 2. Loss of PTEN modulates expression of PDGFs/PDGFR through the PI3K pathway. **A:** mRNA expression levels of PTEN, PDGF B, PDGF D, and β-PDGFR in $PTEN^{+/+}$, $PTEN^{+/-}$, and $PTEN^{-/-}$ cells were analyzed by RT-PCR. **B:** Conditioned medium was collected from serum-starved $PTEN^{+/+}$, $PTEN^{+/-}$, and $PTEN^{-/-}$ cells; resolved on reducing SDS-PAGE; and immunoblotted with anti-PDGF D or anti-PDGF B antibodies. **C** and **D:** $PTEN^{-/-}$ cells were treated for 2 days with the mTOR inhibitor rapamycin (Rap.) or overnight with increasing concentrations of JNK inhibitor (Inhib.) II, the MAPK inhibitor PD98059, the PI3K inhibitor LY294002, or wortmannin. In **C**, cell lysates were collected and resolved on reducing SDS-PAGE and immunoblotted with antibodies directed against phosphorylated and total S6K (pS6K and tS6K, respectively; T389), JNK (pJNK and tJNK, respectively; T183/Y185), extracellular signal–regulated kinase (pERK and tERK, respectively; T202/Y204), and Akt (pAkt and tAkt, respectively; S473). In **D**, mRNA expression levels of PDGF B, PDGF D, and β-PDGFR were analyzed by RT-PCR.

were treated with established pharmacological inhibitors of PTEN downstream signaling molecules and examined for their effects on PDGF gene regulation. Although inhibitors of mammalian target of rapamycin (mTOR), JNK, and MAPK/Erk kinase (MEK) kinase had little effect on PDGF regulation, inhibition of PI3K with either LY294002 or wortmannin resulted in decreased expression of both PDGF D and β-PDGFR (Figure 2, C and D). These results suggest that the PI3K pathway, activated on PTEN loss, is critical for up-regulation of PDGF D and β-PDGFR. Given that Akt is a downstream effector of PI3K, the expression levels of the three Akt isoforms were analyzed by RT-PCR and immunoblot analyses. The mRNA and protein levels of Akt1 were consistent regardless of the PTEN status, whereas Akt2 and Akt3 were increased in $PTEN^{-/-}$ cells compared with $PTEN^{+/+}$ and $PTEN^{+/-}$ cells (Figure 3, A and B). As expected, active pAkt was evident in $PTEN^{-/-}$ cells (Figure 3B). Treatment of $PTEN^{-/-}$ cells with Akt Inhibitor IV, a pan inhibitor of all Akt isoforms, reduced PDGF D and β-PDGFR mRNA levels in $PTEN^{-/-}$ cells, demonstrating that the PI3K/Akt pathway is critical for

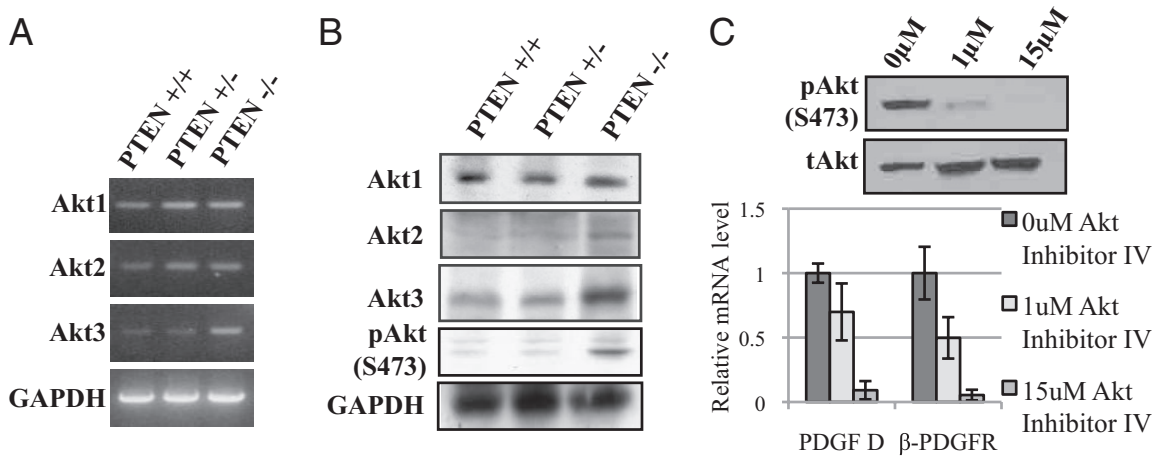

Figure 3. Loss of PTEN leads to increased Akt3 expression. Expression of Akt isoforms in PTEN$^{+/+}$, PTEN$^{+/-}$, and PTEN$^{-/-}$ cells was determined by RT-PCR (**A**) and immunoblotting (**B**). **C:** PTEN$^{-/-}$ cells were treated overnight with increasing concentrations of Akt Inhibitor IV. Cell lysates were collected and resolved on reducing SDS-PAGE and immunoblotted with antibodies directed against pAkt and total Akt (tAkt; **top panel**). mRNA expression levels of PDGF D and β-PDGF were analyzed by real-time RT-PCR analysis from two independent experiments performed in triplicate (**bottom panel**). Data are given as mean ± SD.

the maintenance of increased PDGF D and β-PDGFR mRNA expression in the context of PTEN loss (Figure 3C).

In Vitro *PTEN Knockout in Mouse Epithelial Cells Results in a PDGF Ligand Switch from PDGF B to PDGF D*

The previous results demonstrated increased PDGF D expression in PTEN$^{-/-}$ cells derived from prostate tumor of prostate-specific PTEN-null mice, whereas normal mouse prostate epithelial cells express PDGF B. Next, we asked whether the PDGF ligand switch was a direct effect of PTEN loss or an indirect effect due to genetic alterations that occurred during PCa progression initiated by PTEN loss *in vivo*. To address this question, mouse prostate epithelial cells were isolated from prostates of mice harboring floxed *PTEN* at the age of 8 weeks, as depicted in Figure 4A. After *in vitro* delivery of Cre recombinase by a lentiviral vector, deletion of *PTEN* exon 5 was confirmed by RT-PCR analysis (Figure 4B) and increased pAkt by immunoblot analysis (Figure 4C). Consistent with what was observed in PTEN$^{-/-}$ tumor cells, increased Akt2 and Akt3 protein levels were readily detected in normal mouse prostate epithelial cells on PTEN loss *in vitro* (Figure 4D). More important, *in vitro* PTEN knockout resulted in decreased expression of PDGF B expression and increased PDGF D, compared with the control PTEN$^{loxP/loxP}$ cells (Figure 4B). Treatment of these *in vitro* PTEN knockout cells with inhibitors of PI3K or Akt reduced PDGF D mRNA levels in a dose-dependent manner (Figure 4E). These results demonstrate that PTEN loss results in an immediate PDGF ligand switch from PDGF B to PDGF D and that PI3K/Akt plays a critical role for the up-regulation of PDGF D expression.

Inverse Correlation between PTEN and PDGF Expression in Human PCa

Next, we wanted to examine whether the PTEN pathway regulates PDGF expression in a similar fashion in human prostate epithelial cells. To this end, the PTEN-deficient human PCa cell line PC3 and the wild-type PTEN-expressing human PCa cell line DU145 were analyzed for PDGF expression. Loss of PTEN expression and increased pAkt in PC3 were confirmed by RT-PCR and immunoblot analyses (Figure 5, A and B). Consistent with results obtained from the mouse models of PTEN loss, increased PDGF D expression and decreased PDGF B expression were detected in PC3 cells compared with DU145 cells (Figure 5A). In addition, expression of Akt3 was evident in PTEN-deficient PC3 cells (Figure 5, A and B). Furthermore, inhibition of either PI3K or Akt resulted in decreased PDGF D mRNA expression in PC3 cells (Figure 5, C and D).

Our previous IHC analysis of human PCa tissues showed increased PDGF D expression in human PCa tissues.[23] To our knowledge, there are no published histological data concerning the relationship between PTEN status and PDGF D expression in human prostate tumor tissues. To address this, adjacent slides from PCa specimens used in our previous study were probed with anti-PTEN antibody. Representative images of IHC analysis demonstrated an inverse relationship between PTEN and PDGF D, as shown in Figure 5E. Areas of prostate carcinoma that exhibit loss of PTEN also exhibited a concomitant increase in PDGF D expression, whereas areas that showed PTEN expression often displayed PDGF D expression at a low level.

AMPK Regulates PDGF B Expression

The previous results showed that inhibition of mTOR, MAPK, or PI3K pathways failed to reverse PDGF B expression in PTEN$^{-/-}$ cells. To determine the signaling

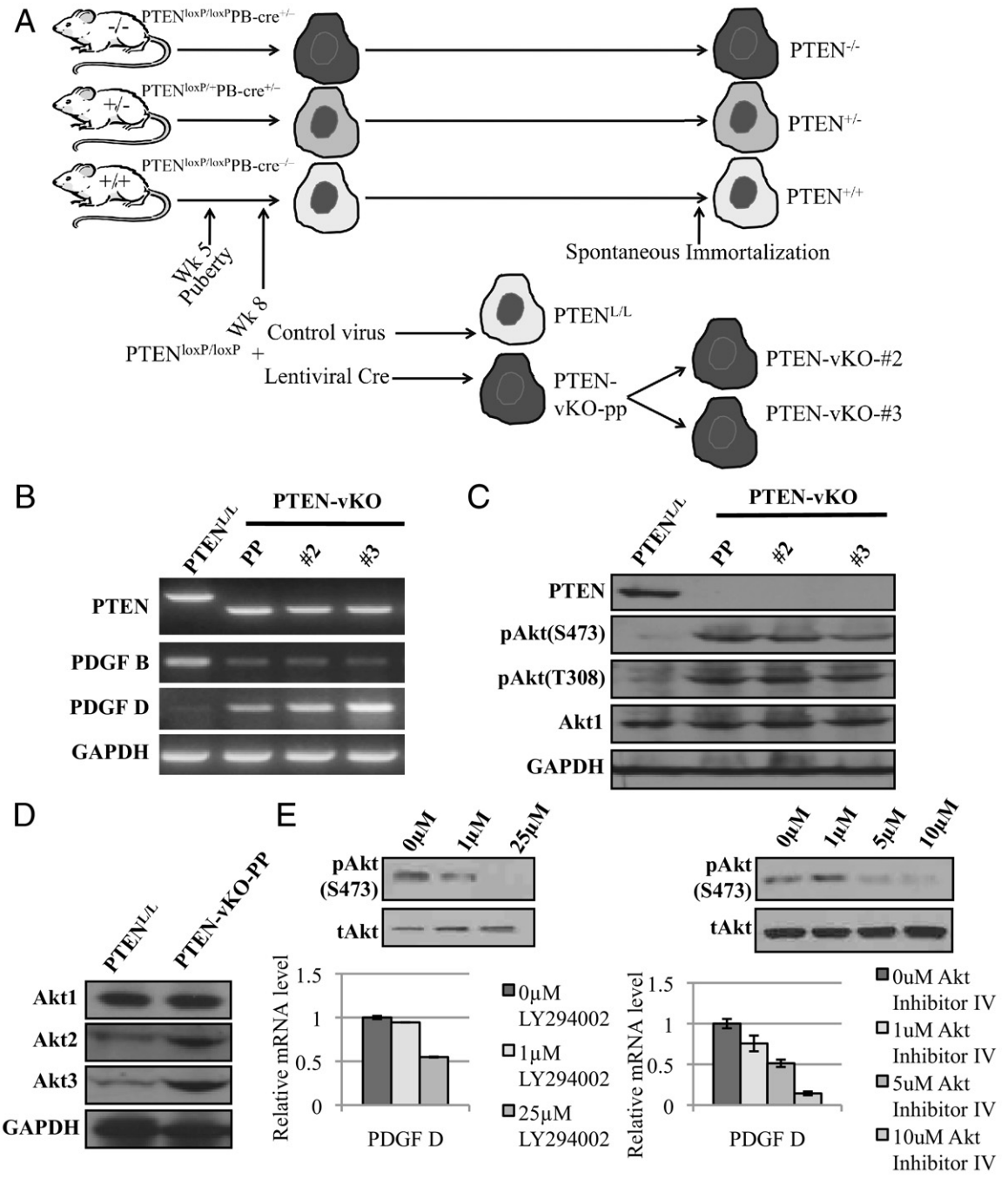

Figure 4. Direct effects of *in vitro* loss of PTEN on PDGF expression. **A:** The diagram depicts establishment of cell lines with *in vivo* knockout of PTEN ($PTEN^{-/-}$) or viral knockout of PTEN (PTEN-vKO). $PTEN^{+/+}$, $PTEN^{+/-}$, and $PTEN^{-/-}$ mouse prostate epithelial cells were isolated from prostates of corresponding mice at the age of 8 weeks. Cell lines were established after spontaneous immortalization. $PTEN^{loxP/loxP}$ cells were isolated from the prostates of 8-week-old mice. *In vitro* deletion of PTEN was achieved by expression of the lentiviral Cre recombinase vector. PTEN-knockout clones (PTEN-vKO-#2 and PTEN-vKO-#3) were then selected from the pooled population (PTEN-vKO-pp). A pooled population of wild-type PTEN control cells was also established by infection with a control lentivirus ($PTEN^{L/L}$). **B:** mRNA expression levels of PTEN and β-PDGFR and its ligands, PDGF B and PDGF D, in wild-type $PTEN^{L/L}$ and viral knockout PTEN-vKO cells were analyzed by RT-PCR. **C** and **D:** Expression levels of pAkt (**C**) and Akt (**D**) isoforms in wild-type $PTEN^{L/L}$ and knockout PTEN-vKO cells were determined by immunoblotting. **E:** PTEN-vKO-#2 cells were treated overnight with increasing concentrations of LY294002 (**left panel**) or Akt Inhibitor IV (**right panel**). Cell lysates were collected and resolved on reducing SDS-PAGE and immunoblotted with antibodies directed against pAkt and total Akt (**top panel**). PDGF D mRNA expression levels were analyzed by real-time RT-PCR analysis from two independent experiments performed in triplicate (**bottom panel**). Data are given as mean ± SD.

pathways altered by PTEN loss, which, in turn, down-regulate PDGF B, we further examined potential signaling pathways in relation to PTEN status, including AMPK. AMPK is a central component of a highly conserved cellular energy sensing system that functions to maintain cellular ATP levels.[28] AMPK was chosen in this study because increasing evidence suggests its critical role for tumorigenesis and regulation of growth factor expression, such as vascular endothelial growth factor.[29–32] As shown in Figure 6A, PTEN loss was associated with an

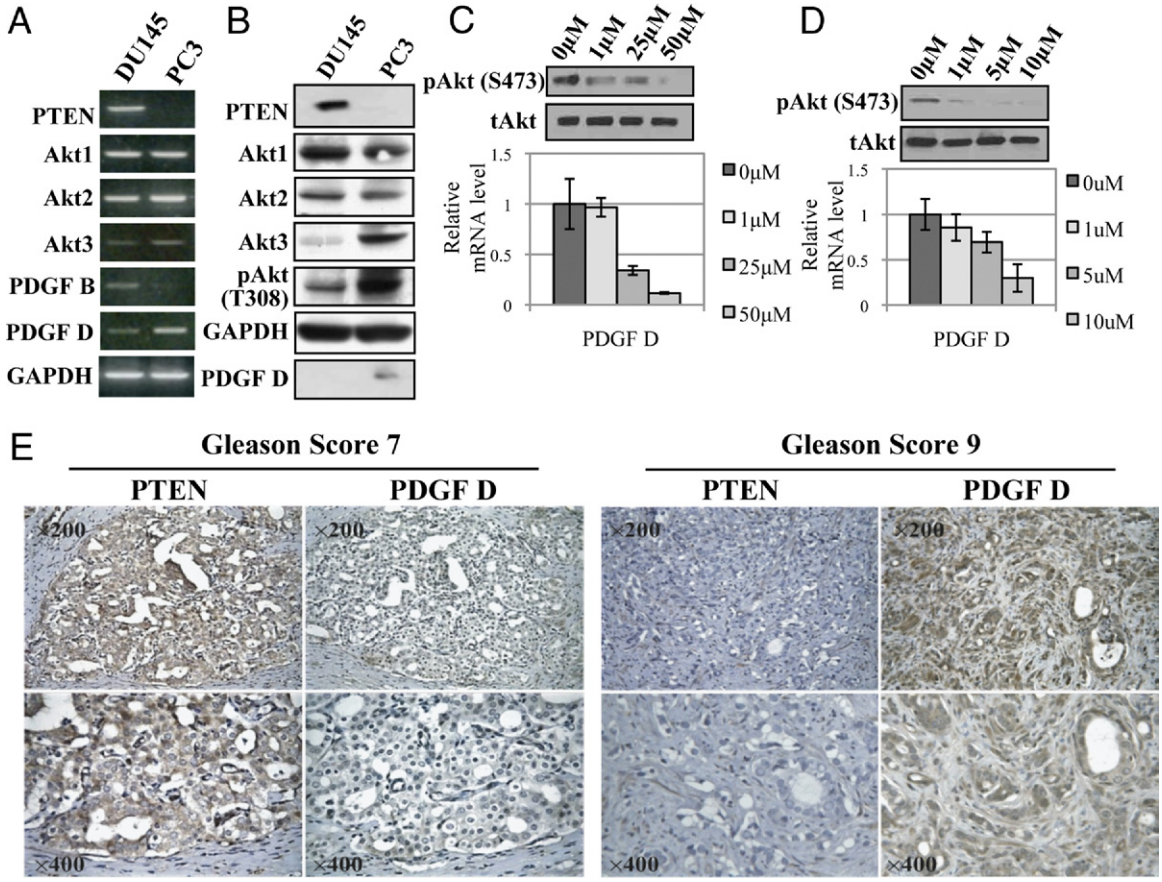

Figure 5. Relationship between PTEN and PDGF expression in human PCa. **A:** mRNA expression levels of PTEN, Akt isoforms, PDGF B, and PDGF D were analyzed by RT-PCR in human PCa cell lines DU145 and PC3. **B:** DU145 and PC3 cell lysates were collected and resolved on reducing SDS-PAGE, and immunoblotted with antibodies directed against PTEN, Akt isoforms, pAkt, and GAPDH. Conditioned media collected from DU145 and PC3 cells were resolved on reducing SDS-PAGE, and immunoblotted with anti-PDGF D Ab. **C** and **D:** PC3 cells were treated overnight with increasing concentrations of LY294002 (**C**) or Akt Inhibitor IV (**D**). Cell lysates were collected and resolved on reducing SDS-PAGE, and immunoblotted with antibodies directed against pAkt and total Akt (**top panel**). mRNA expression levels of PDGF D were analyzed by real-time RT-PCR from two independent experiments performed in triplicate (**bottom panel**). Data are given as mean±SD. **E:** IHC analysis of PTEN and PDGF D was performed on adjacent sections of prostate adenocarcinoma samples using anti-PTEN monoclonal Ab (1:250 dilution, clone 6H2.1; Cascade Bioscience) and anti-PDGF D polyclonal Ab (8D2, 1:50 dilution), as previously described.[11] **Left panel:** Gleason score, 7 (3 + 4); tumor stage, T2. **Right panel:** Gleason score, 9 (4 + 5); tumor stage, T3a.

increase in the level of phosphorylated (active) form of AMPK, as detected with an antibody directed against phosphorylated AMPKα (Thr172), whereas total AMPKα protein levels were comparable regardless of PTEN status. To examine whether increased AMPK activity is associated with PDGF B down-regulation, PTEN$^{+/+}$ cells were treated with the AMPK activator, AICAR, or the AMPK inhibitor, compound C, and their effects on PDGF B mRNA expression were evaluated by real-time PCR analysis. On pharmacological modulation, PDGF B mRNA levels were inversely correlated with AMPK activity (Figure 6B). Similar to mouse PTEN$^{+/+}$ prostate epithelial cells, PDGF B mRNA expression was inversely correlated with AMPK activity in human prostate carcinoma DU145 cells that express wild-type PTEN (Figure 6C). Unlike PDGF B, PDGF D expression was undetectable in mouse PTEN$^{+/+}$ or DU145 cells on AMPK modulation. Interestingly, mRNA levels of β-PDGFR were positively correlated with AMPK activity in both mouse prostate PTEN$^{+/+}$ and human prostate DU145 epithelial cells (Figure 6, D and E).

Taken together, we propose that activation of the PI3K/Akt pathway, possibly involving Akt3, potentiates the PDGF D signaling axis, the AMPK pathway regulates PDGF B expression, and both PTEN/PI3K/Akt and AMPK pathways are involved in β-PDGFR expression in prostate epithelial cells expressing PTEN.

Discussion

Increasing evidence indicates a critical role for β-PDGFR signaling in PCa progression and bone metastasis. For instance, PDGFR was identified as the most commonly amplified transcript from pooled aspirate specimens of PCa bone metastases.[33] Clinically, β-PDGFR is significantly up-regulated in 88% of primary and 80% of bone metastatic PCa.[9] In fact, bone marrow aspirates from patients exhibiting bone metastatic disease show frequent activation of β-PDGFR (50%) compared with α-PDGFR (17%).[34] β-PDGFR is activated by PDGF B and PDGF D[2]; however, detection of PDGF B in benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and high-grade adenocarcinoma has been unsuccessful.[35,36] Our previous findings demonstrated significant

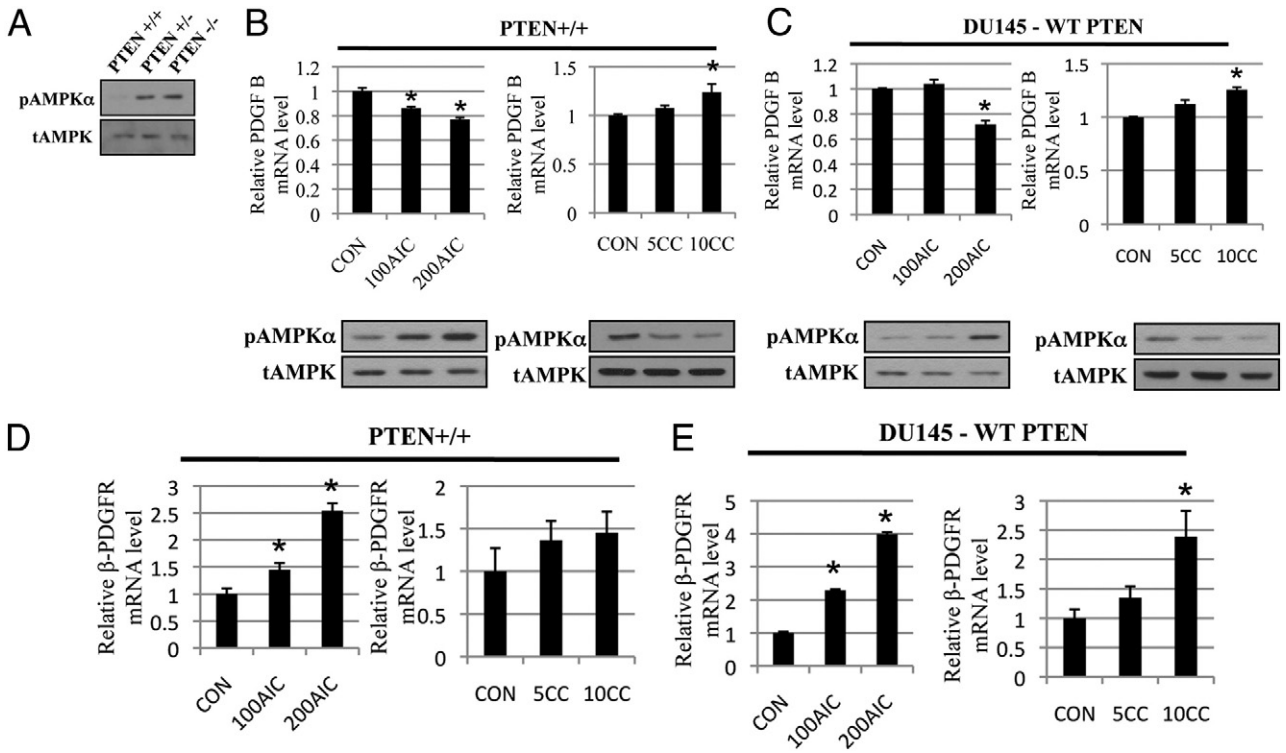

Figure 6. AMPK regulates PDGF B expression. **A:** Cell lysates collected from PTEN$^{+/+}$, PTEN$^{+/-}$, and PTEN$^{-/-}$ mouse prostate epithelial cells were resolved on reducing SDS-PAGE, and immunoblotted with antibodies directed against phosphorylated and total AMPK (pAMPK and tAMPK, respectively). **B–E:** PTEN$^{+/+}$ mouse prostate epithelial cells (**B** and **D**) and DU145 cells (**C** and **E**) were treated with increasing micromolar concentrations of AICAR (**left panel**) or Compound C (**right panel**), AMPK activator and inhibitor, respectively. Cell lysates were analyzed by immunoblot analysis for phosphorylated and total AMPK. mRNA expression levels of PDGF B (**B** and **C**) and β-PDGFR (**D** and **E**) were analyzed by real-time RT-PCR from two independent experiments performed in triplicate. Data are given as mean ± SD. $*P < 0.05$, relative to control (CON).

PDGF D overexpression with an increasing Gleason score, suggesting that PDGF D is the putative ligand responsible for β-PDGFR activation during PCa progression.[23] Although the PDGF A/α-PDGFR axis may play a role in PCa,[35,36] recent findings identified TMEFF2 as a PDGF A sequestering molecule abrogating its mitogenic potential.[37] More important, TMEFF2 expression is significantly up-regulated during PCa, positively correlating with PDGF A expression.[37] Taken together, these findings support greater clinical relevance of PDGF D–mediated activation of β-PDGFR in PCa progression.

Consistent with the potential oncogenic activity of PDGF signaling in PCa progression, preclinical studies[38–42] demonstrated the therapeutic potential of targeting the PDGFR axis in PCa. In a mouse model of PCa, treatment with the Bcr-Abl/PDGFR/c-Kit inhibitor Gleevec (STI571, imatinib mesylate) reduced tumor incidence and growth and increased apoptosis in the tumor cells and tumor-associated endothelial cells.[41] However, clinical trials with Gleevec were halted because of excessive adverse effects, such as diarrhea related to inhibition of c-kit in the intestines and cardiotoxicity associated with inhibition of c-abl in cardiac myocytes.[43–45] Therefore, more specific therapies that target PCa-specific PDGFR signaling with less toxicity may await understanding of molecular mechanisms underlying PCa-derived PDGF ligand expression.

In an effort to determine the mechanism resulting in increased PDGF signaling in PCa, we turned to the PTEN model because PTEN is a critical regulator of growth factor signaling and is frequently lost or mutated in PCa. By regulating the pathway of PI3K/Akt, a central intracellular signaling node, PTEN plays a critical role in the regulation of many cellular processes, including the cell cycle, cell motility/invasion, cell adhesion, protein synthesis, and glucose metabolism.[46] In addition, recent studies[47–51] unveiled new functions of PTEN as a protein phosphatase and as a nuclear protein. More important, evidence suggests that PTEN is critical for the maintenance of chromosomal integrity, a function attributed to its regulation of Chk1 for the control of DNA damage checkpoint, transcription of Rad51 involved in double-stranded DNA break repair, and its association with the centromere by docking onto a centromere-binding protein, CENP-C.[52,53] Thus, the loss of PTEN's ability to guard genomic integrity is thought to result in accumulation of genetic defects leading to the acquisition of aggressive phenotypes and metastasis. In this regard, the *in vitro* PTEN knockout mouse prostate epithelial cell lines established in this study provide a powerful tool to distinguish between direct effects of PTEN loss in the regulation of gene expression and *in vivo* accumulation of genetic alterations that resulted from genetic instability in PTEN-null tumor cells.

Herein, we found that PTEN loss results in a β-PDGFR ligand switch from PDGF B to PDGF D in prostate epithelial cells. PDGF D induction requires PI3K/Akt, possibly induction of Akt3, and, to a lesser extent, Akt2. PTEN

loss-mediated differential up-regulation of Akt isoforms is of particular interest, because the PI3K/Akt axis involving Akt2/Akt3 recently emerged as a key signaling pathway for induction of cell motility and invasion, whereas Akt1 is a general regulator of cell survival.[54–57] Our efforts to determine the functional significance of Akt3 in PTEN loss-mediated PDGF D induction using a small-interfering RNA approach were unsuccessful. Small-interfering RNA–mediated Akt3 knockdown resulted in increased Akt1 and Akt2 expression (data not shown), possibly as the result of feedback signaling mechanisms, which complicated our analyses. Nonetheless, by using established pharmacological inhibitors, we demonstrated that the PI3K/Akt signaling pathway is critical for the maintenance of PDGF D up-regulation in prostate epithelial cells. Interestingly, none of the pharmacological inhibitors that target PTEN downstream signaling molecules, such as mTOR, JNK, MEK, PI3K, and Akt, were able to reverse PTEN loss-mediated PDGF B down-regulation. The present study shows that AMPK may be a key regulator for PDGF B expression. In $PTEN^{-/-}$ mouse prostate tumor cells, there is an increase in the level of active AMPK and a decrease in PDGF B expression compared with the $PTEN^{+/+}$ cells. This was somewhat unexpected given that AMPK is a substrate of the tumor suppressor serine-threonine kinase LKB1 and that LKB1, together with AMPK, mediates suppressor functions.[29,58,59] However, recent studies[60,61] showed that AMPK regulates gene expression and promotes PCa cell growth and survival, suggesting complex roles of AMPK for tumorigenesis in a context-dependent manner. Thus, AMPK regulation of growth factors, including PDGFs and their roles in human cancers, warrants further investigation.

At present, little is known about PDGF isoform-specific β-PDGFR signaling pathways and their cellular effects during cancer development and progression. PDGF D contains an N-terminal CUB domain, composed of approximately 110 amino acids, that shares sequence homology with the CUB domains of the complement subcomponents C1r/C1s and bone morphogenetic protein-1.[62] Proteolytic removal of the CUB domain is required for PDGF D activation of β-PDGFR. Evidence suggests that proteolytically activated PDGF D/β-PDGFR signaling differs from PDGF B/β-PDGFR or PDGF D growth factor domain/β-PDGFR signaling for the oncogenic effects.[63] In agreement, we recently found that PDGF D expression facilitates intraosseous PCa growth and leads to both osteolytic and osteoblastic responses. PDGF D, but neither PDGF B nor the growth factor domain of PDGF D, mediates osteoclast activation, suggesting PDGF D–specific signaling pathways. Taken together, we propose that PTEN loss-mediated PDGF ligand switch from PDGF B to PDGF D results in PCa-specific molecular signature of PDGF signaling and also functionally contributes to PCa progression.

References

1. Jemal A, Siegel R, Xu J, Ward E: Cancer statistics, 2010. CA Cancer J Clin 2010, 60:277–300
2. Yu J, Ustach C, Kim HR: Platelet-derived growth factor signaling and human cancer. J Biochem Mol Biol 2003, 36:49–59
3. Chaudhary LR, Hofmeister AM, Hruska KA: Differential growth factor control of bone formation through osteoprogenitor differentiation. Bone 2004, 34:402–411
4. Godwin SL, Soltoff SP: Extracellular calcium and platelet-derived growth factor promote receptor-mediated chemotaxis in osteoblasts through different signaling pathways. J Biol Chem 1997, 272:11307–11312
5. Mehrotra M, Krane SM, Walters K, Pilbeam C: Differential regulation of platelet-derived growth factor stimulated migration and proliferation in osteoblastic cells. J Cell Biochem 2004, 93:741–752
6. Kubota K, Sakikawa C, Katsumata M, Nakamura T, Wakabayashi K: Platelet-derived growth factor BB secreted from osteoclasts acts as an osteoblastogenesis inhibitory factor. J Bone Miner Res 2002, 17:257–265
7. Himeno M, Enomoto H, Liu W, Ishizeki K, Nomura S, Kitamura Y, Komori T: Impaired vascular invasion of Cbfa1-deficient cartilage engrafted in the spleen. J Bone Miner Res 2002, 17:1297–1305
8. Robbins JR, McGuire PG, Wehrle-Haller B, Rogers SL: Diminished matrix metalloproteinase 2 (MMP-2) in ectomesenchyme-derived tissues of the Patch mutant mouse: regulation of MMP-2 by PDGF and effects on mesenchymal cell migration. Dev Biol 1999, 212:255–263
9. Ko YJ, Small EJ, Kabbinavar F, Chachoua A, Taneja S, Reese D, DePaoli A, Hannah A, Balk SP, Bubley GJ: A multi-institutional phase II study of SU101, a platelet-derived growth factor receptor inhibitor, for patients with hormone-refractory prostate cancer. Clin Cancer Res 2001, 7:800–805
10. Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, Tamayo P, Renshaw AA, D'Amico AV, Richie JP, Lander ES, Loda M, Kantoff PW, Golub TR, Sellers WR: Gene expression correlates of clinical prostate cancer behavior. Cancer Cell 2002, 1:203–209
11. Ustach CV, Huang W, Conley-Lacomb MK, Lin CY, Che M, Abrams J, Kim HR: A novel signaling axis of matriptase/PDGF-D/β-PDGFR in human prostate cancer. Cancer Res 2010, 70:9631–9640
12. Ustach CV, Taube ME, Hurst NJ Jr, Bhagat S, Bonfil RD, Cher ML, Schuger L, Kim HR: A potential oncogenic activity of platelet-derived growth factor d in prostate cancer progression. Cancer Res 2004, 64:1722–1729
13. Maehama T, Dixon JE: The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 1998, 273:13375–13378
14. Salmena L, Carracedo A, Pandolfi PP: Tenets of PTEN tumor suppression. Cell 2008, 133:403–414
15. Gray IC, Stewart LM, Phillips SM, Hamilton JA, Gray NE, Watson GJ, Spurr NK, Snary D: Mutation and expression analysis of the putative prostate tumour-suppressor gene PTEN. Br J Cancer 1998, 78:1296–1300
16. Whang YE, Wu X, Suzuki H, Reiter RE, Tran C, Vessella RL, Said JW, Isaacs WB, Sawyers CL: Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc Natl Acad Sci U S A 1998, 95:5246–5250
17. Di Cristofano A, Pandolfi PP: The multiple roles of PTEN in tumor suppression. Cell 2000, 100:387–390
18. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy-Burman P, Nelson PS, Liu X, Wu H: Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 2003, 4:209–221
19. Berquin IM, Min Y, Wu R, Wu J, Perry D, Cline JM, Thomas MJ, Thornburg T, Kulik G, Smith A, Edwards IJ, D'Agostino R, Zhang H, Wu H, Kang JX, Chen YQ: Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. J Clin Invest 2007, 117:1866–1875
20. Barclay WW, Cramer SD: Culture of mouse prostatic epithelial cells from genetically engineered mice. Prostate 2005, 63:291–298
21. Barclay WW, Axanova LS, Chen W, Romero L, Maund SL, Soker S, Lees CJ, Cramer SD: Characterization of adult prostatic progenitor/stem cells exhibiting self-renewal and multilineage differentiation. Stem Cells 2008, 26:600–610
22. Ustach CV, Kim HR: Platelet-derived growth factor D is activated by urokinase plasminogen activator in prostate carcinoma cells. Mol Cell Biol 2005, 25:6279–6288
23. Ustach CV, Huang W, Conley-LaComb MK, Lin CY, Che M, Abrams J, Kim HR: A novel signaling axis of matriptase/PDGF-D/β-PDGFR in human prostate cancer. Cancer Res 2010, 70:9631–9640

24. Zhou YJ, Xiong YX, Wu XT, Shi D, Fan W, Zhou T, Li YC, Huang X: Inactivation of PTEN is associated with increased angiogenesis and VEGF overexpression in gastric cancer. World J Gastroenterol 2004, 10:3225–3229
25. Zhao H, Dupont J, Yakar S, Karas M, LeRoith D: PTEN inhibits cell proliferation and induces apoptosis by downregulating cell surface IGF-IR expression in prostate cancer cells. Oncogene 2004, 23:786–794
26. Bergsten E, Uutela M, Li X, Pietras K, Ostman A, Heldin CH, Alitalo K, Eriksson U: PDGF-D is a specific, protease-activated ligand for the PDGF beta-receptor. Nat Cell Biol 2001, 3:512–516
27. LaRochelle WJ, Jeffers M, McDonald WF, Chillakuru RA, Giese NA, Lokker NA, Sullivan C, Boldog FL, Yang M, Vernet C, Burgess CE, Fernandes E, Deegler LL, Rittman B, Shimkets J, Shimkets RA, Rothberg JM, Lichenstein HS: PDGF-D, a new protease-activated growth factor. Nat Cell Biol 2001, 3:517–521
28. Hardie DG: AMPK: a key regulator of energy balance in the single cell and the whole organism. Int J Obes (Lond) 2008, 32(Suppl 4):S7–S12
29. Shackelford DB, Shaw RJ: The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nat Rev Cancer 2009, 9:563–575
30. Lee M, Hwang JT, Yun H, Kim EJ, Kim MJ, Kim SS, Ha J: Critical roles of AMP-activated protein kinase in the carcinogenic metal-induced expression of VEGF and HIF-1 proteins in DU145 prostate carcinoma. Biochem Pharmacol 2006, 72:91–103
31. Neurath KM, Keough MP, Mikkelsen T, Claffey KP: AMP-dependent protein kinase alpha 2 isoform promotes hypoxia-induced VEGF expression in human glioblastoma. Glia 2006, 53:733–743
32. Yun H, Lee M, Kim SS, Ha J: Glucose deprivation increases mRNA stability of vascular endothelial growth factor through activation of AMP-activated protein kinase in DU145 prostate carcinoma. J Biol Chem 2005, 280:9963–9972
33. Chott A, Sun Z, Morganstern D, Pan J, Li T, Susani M, Mosberger I, Upton MP, Bubley GJ, Balk SP: Tyrosine kinases expressed in vivo by human prostate cancer bone marrow metastases and loss of the type 1 insulin-like growth factor receptor. Am J Pathol 1999, 155:1271–1279
34. Mathew P, Thall PF, Bucana CD, Oh WK, Morris MJ, Jones DM, Johnson MM, Wen S, Pagliaro LC, Tannir NM, Tu SM, Meluch AA, Smith L, Cohen L, Kim SJ, Troncoso P, Fidler IJ, Logothetis CJ: Platelet-derived growth factor receptor inhibition and chemotherapy for castration-resistant prostate cancer with bone metastases. Clin Cancer Res 2007, 13:5816–5824
35. Fudge K, Bostwick DG, Stearns ME: Platelet-derived growth factor A and B chains and the alpha and beta receptors in prostatic intraepithelial neoplasia. Prostate 1996, 29:282–286
36. Fudge K, Wang CY, Stearns ME: Immunohistochemistry analysis of platelet-derived growth factor A and B chains and platelet-derived growth factor alpha and beta receptor expression in benign prostatic hyperplasias and Gleason-graded human prostate adenocarcinomas. Mod Pathol 1994, 7:549–554
37. Lin K, Taylor JR Jr, Wu TD, Gutierrez J, Elliott JM, Vernes JM, Koeppen H, Phillips HS, de Sauvage FJ, Meng YG: TMEFF2 is a PDGF-AA binding protein with methylation-associated gene silencing in multiple cancer types including glioma. PLoS One 2011, 6:e18608
38. Sintich SM, Lamm ML, Sensibar JA, Lee C: Transforming growth factor-beta1-induced proliferation of the prostate cancer cell line, TSU-Pr1: the role of platelet-derived growth factor. Endocrinology 1999, 140:3411–3415
39. Ng SS, MacPherson GR, Gutschow M, Eger K, Figg WD: Antitumor effects of thalidomide analogs in human prostate cancer xenografts implanted in immunodeficient mice. Clin Cancer Res 2004, 10:4192–4197
40. van der Poel HG: Smart drugs in prostate cancer. Eur Urol 2004, 45:1–17
41. Uehara H, Kim SJ, Karashima T, Shepherd DL, Fan D, Tsan R, Killion JJ, Logothetis C, Mathew P, Fidler IJ: Effects of blocking platelet-derived growth factor-receptor signaling in a mouse model of experimental prostate cancer bone metastases. J Natl Cancer Inst 2003, 95:458–470
42. Kim SJ, Uehara H, Yazici S, Langley RR, He J, Tsan R, Fan D, Killion JJ, Fidler IJ: Simultaneous blockade of platelet-derived growth factor-receptor and epidermal growth factor-receptor signaling and systemic administration of paclitaxel as therapy for human prostate cancer metastasis in bone of nude mice. Cancer Res 2004, 64:4201–4208
43. Deininger MW, O'Brien SG, Ford JM, Druker BJ: Practical management of patients with chronic myeloid leukemia receiving imatinib. J Clin Oncol 2003, 21:1637–1647
44. Guilhot F: Indications for imatinib mesylate therapy and clinical management. Oncologist 2004, 9:271–281
45. Kerkela R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, Walters B, Shevtsov S, Pesant S, Clubb FJ, Rosenzweig A, Salomon RN, Van Etten RA, Alroy J, Durand JB, Force T: Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nat Med 2006, 12:908–916
46. Chalhoub N, Baker SJ: PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol 2009, 4:127–150
47. Gildea JJ, Herlevsen M, Harding MA, Gulding KM, Moskaluk CA, Frierson HF, Theodorescu D: PTEN can inhibit in vitro organotypic and in vivo orthotopic invasion of human bladder cancer cells even in the absence of its lipid phosphatase activity. Oncogene 2004, 23:6788–6797
48. Leslie NR, Yang X, Downes CP, Weijer CJ: PtdIns(3,4,5)P(3)-dependent and -independent roles for PTEN in the control of cell migration. Curr Biol 2007, 17:115–125
49. Gu J, Tamura M, Pankov R, Danen EH, Takino T, Matsumoto K, Yamada KM: Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. J Cell Biol 1999, 146:389–403
50. Liu JL, Sheng X, Hortobagyi ZK, Mao Z, Gallick GE, Yung WK: Nuclear PTEN-mediated growth suppression is independent of Akt down-regulation. Mol Cell Biol 2005, 25:6211–6224
51. Gimm O, Perren A, Weng LP, Marsh DJ, Yeh JJ, Ziebold U, Gil E, Hinze R, Delbridge L, Lees JA, Mutter GL, Robinson BG, Komminoth P, Dralle H, Eng C: Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. Am J Pathol 2000, 156:1693–1700
52. Puc J, Parsons R: PTEN loss inhibits CHK1 to cause double stranded-DNA breaks in cells. Cell Cycle 2005, 4:927–929
53. Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, Yin Y: Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell 2007, 128:157–170
54. Zhang B, Gu F, She C, Guo H, Li W, Niu R, Fu L, Zhang N, Ma Y: Reduction of Akt2 inhibits migration and invasion of glioma cells. Int J Cancer 2009, 125:585–595
55. Boyer B, Valles AM, Edme N: Induction and regulation of epithelial-mesenchymal transitions. Biochem Pharmacol 2000, 60:1091–1099
56. Grille SJ, Bellacosa A, Upson J, Klein-Szanto AJ, van Roy F, Lee-Kwon W, Donowitz M, Tsichlis PN, Larue L: The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res 2003, 63:2172–2178
57. Larue L, Bellacosa A: Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3′ kinase/AKT pathways. Oncogene 2005, 24:7443–7454
58. Zhou J, Huang W, Tao R, Ibaragi S, Lan F, Ido Y, Wu X, Alekseyev YO, Lenburg ME, Hu GF, Luo Z: Inactivation of AMPK alters gene expression and promotes growth of prostate cancer cells. Oncogene 2009, 28:1993–2002
59. Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, Tanti JF, Le Marchand-Brustel Y, Bost F: The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene 2008, 27:3576–3586
60. Park HU, Suy S, Danner M, Dailey V, Zhang Y, Li H, Hyduke DR, Collins BT, Gagnon G, Kallakury B, Kumar D, Brown ML, Fornace A, Dritschilo A, Collins SP: AMP-activated protein kinase promotes human prostate cancer cell growth and survival. Mol Cancer Ther 2009, 8:733–741
61. Chhipa RR, Wu Y, Ip C: AMPK-mediated autophagy is a survival mechanism in androgen-dependent prostate cancer cells subjected to androgen deprivation and hypoxia. Cell Signal 2011, 23:1466–1472
62. Bork P: Complement components C1r/C1s, bone morphogenic protein 1 and Xenopus laevis developmentally regulated protein UVS.2 share common repeats. FEBS Lett 1991, 282:9–12
63. Ehnman M, Li H, Fredriksson L, Pietras K, Eriksson U: The uPA/uPAR system regulates the bioavailability of PDGF-DD: implications for tumour growth. Oncogene 2009, 28:534–544