Tumor and Stem Cell Biology

Decreased Expression and Androgen Regulation of the Tumor Suppressor Gene INPP4B in Prostate Cancer

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Abstract

Patients with metastatic prostate cancer who undergo androgen-ablation therapy invariably relapse and develop incurable castration-resistant disease. Activation of the prosurvival Akt pathway accompanies androgen ablation. We discovered that the androgen receptor induces the expression of the tumor suppressor inositol polyphosphate 4-phosphatase type II (INPP4B) but not PTEN in prostate cancer cells. Optimal induction of INPP4B by an androgen receptor required the expression of the transcriptional coactivator NCoR. INPP4B dephosphorylates phosphatidylinositol-3, 4-bisphosphate, which leads to reduced phosphorylation and activity of Akt. In support of a key role for INPP4B in Akt control, INPP4B depletion activated Akt and increased cellular proliferation. The clinical significance of INPP4B in androgen-dependent prostate cancers was determined in normal or primary tumor prostate tissues derived from radical prostatectomy specimens. In primary tumors, the expression of both INPP4B and PTEN was substantially reduced compared with normal tissue. Further, the decreased expression of INPP4B reduced the time to biochemical recurrence. Thus, androgen ablation can activate the Akt pathway via INPP4B downregulation, thereby mitigating the antitumor effects of androgen ablation. Our findings reinforce the concept that patients undergoing androgen ablation may benefit from Akt-targeting therapies. Cancer Res; 71(2); 572–82. ©2011 AACR.

Introduction

Androgen-ablation therapy, through suppression of testicular androgen production or treatment with an androgen receptor (AR) antagonist, remains the cornerstone of systemic prostate cancer treatment. Although initially successful at controlling advanced tumors, the disease inevitably progresses to a more aggressive state that is termed hormone-refractory, castration-resistant, or androgen-independent prostate cancer. However, castration-resistant tumors retain AR and select AR-regulated gene expression in the absence of or with low levels of circulating androgens, demonstrating that AR signaling continues to play a significant role in patients with castration-resistant disease (1–3).

In the normal mature prostate, AR is functionally dichotomous: supporting proliferative epithelial renewal while maintaining the terminal differentiation of secretory epithelium. Prostate-specific deletion of the AR leads to dedifferentiation and increased proliferation of luminal epithelial cells (4). In addition, androgens regulate the expression of growth-suppressing genes, such as Nkx3.1 and AS3 (5–8). Hence, androgens regulate the growth of prostate epithelial cells through the regulation of a select subset of AR target genes. Although AR plays a pivotal role in maintaining cellular quiescence and the terminal differentiation of the normal prostate epithelium, during the development and progression of prostate cancer, there is a gradual shift in AR function, making it predominantly proliferative. The expression of differentiation markers, such as prostate specific antigen (PSA), demonstrates that the AR retains some differentiating function in prostate cancer. The functional shift in AR activity is potentially mediated through the altered expression of multiple AR coregulatory proteins and the activation of extracellular signaling pathways (9). Coregulators of the AR, such as the p160 family of coactivators, potentiate AR function and show increased expression in prostate cancer that correlates with poor patient outcome (10–12). Promoter-specific modulation of AR function by coregulators may account for the selective reactivation of AR signaling pathways that favor growth in advanced prostate cancers (9).

Androgen-ablation therapies routinely utilized in the treatment of advanced prostate cancers and androgen-independent tumors are associated with increased Akt signaling (13, 14). Furthermore, androgen starvation of prostate cancer cells leads to increased P38/Akt activity, which supports survival and androgen-independent growth that can be suppressed by dihydrotestosterone (DHT; refs. 15, 16). Androgens, therefore, control the proliferation of prostate epithelial cells, in part, through the downregulation of Akt.
signaling. Activated-Akt signaling stimulates cellular proliferation, cell survival, cell cycle progression, growth, migration, and angiogenesis (17). As an example, proapoptotic Forkhead transcription factor class-O family (FOXO) members are phosphorylated by Akt, which targets them for degradation (18). Deregulation of Akt signaling is associated with numerous human cancers, including prostate cancer. The expression of activated Akt is elevated in prostate cancer compared with normal tissue and is associated with reduced time to biochemical recurrence (19).

Akt activity is dependent on the availability of phosphati- 
dylinositol-3,4,5-trisphosphate [PI(3,4,5)P3] and phosphatidy- 
linositol-3,4-bisphosphate [PI(3,4)P2]. The signaling lipids 
PI(3,4,5)P3 and PI(3,4)P2 are generated through P3K (phos- 
phoinositide 3-kinase) activity and are degraded by PTEN 
(phosphatase and tensin homologue deleted on chromosome 
10) and INPP4B (inositol polyphosphate 4-phosphatase 
type II), respectively (20). The PTEN substrate PI(3,4)P3 
contributes predominantly to Thr308 phosphorylation and 
membrane-associated activation of Akt, whereas PI(3,4)P2, 
INPP4B’s substrate, contributes mostly to Ser473 phosphory- 
ation and cytoplasmic activation of Akt (21). PTEN is a dual- 
specificity phosphatase that dephosphorylates PI(3,4,5)P3 as 
well as serine, threonine, and tyrosine residues in vitro and 
focus adhesion kinase in vivo. PTEN loss of function or 
expression is frequently observed in human cancers, and loss 
of PTEN in mice results in the development of a number of 
different tumor types (22–24). Homozygous gene deletions, 
loss of heterozygosity (LOH), and inactivating mutations of 
PTEN in prostate cancers show that PTEN activity is lost in 
the majority of prostate cancers and likely contributes to 
prostate cancer progression (25, 26).

INPP4B is a class II phosphatase that preferentially hydro-
lyzes the 4 position of PI(3,4,5)P2. Silenced INPP4B expression 
in malignant proerythroblast was associated with increased 
avtivated-Akt levels that could be alleviated by the re- 
expression of INPP4B (27). In a nonbiased RNAi-based 
genetic screen, the loss of INPP4B was shown to facilitate 
the anchorage–independent growth of human mammary 
epithelial cells (28). Significantly, INPP4B was recently sug-
gested to be a tumor suppressor gene in breast and ovarian 
cancers, which suppresses P13K/AKT signaling. Reduced 
INPP4B mRNA levels were identified in BRCA1 and basal-
cancers, which suppresses PI3K/AKT signaling. Reduced 
INPP4B protein was also observed in breast and ovarian 
cancer correlated with decreased survival as determined by tissue microarray expression 
analysis (29).

Given that activated Akt is elevated in human prostate 
cancers and is associated with castration resistance, we 
investigated the regulation of INPP4B and its possible impli- 
cation in prostate cancer. Significantly, we found that AR 
directly regulates INPP4B but not PTEN expression in prostate 
cancer cells. We show that INPP4B regulates Akt activation 
and cellular proliferation in prostate cancer cells. Using 
prostate cancer tissue microarrays, we observed the decreased 
expression of both PTEN and INPP4B in prostate cancer 
compared with benign tissue. Our data indicate that the 
decreased expression of INPP4B has similar predictive value 
to PTEN for prostate cancer recurrence and is, therefore, 
potentially equally as important as PTEN in the etiology of 
prostate cancer.

Materials and Methods

Cell culture

LNCaP and VCaP prostate cancer cells were purchased 
from ATCC and were maintained in RPMI 1640 or DMEM 
media, respectively, and supplemented with 10% FBS, accord-
ing to ATCC guidelines. All media were purchased from 
Invitrogen (Carlsbad, CA), and FBS and charcoal-stripped 
serum (chs) were purchased from Sigma-Aldrich (St. Louis, 
MO). R1881 was purchased from Perkin Elmer (Waltham, MA); 
bicalutamide was purchased from LKT Laboratories (St. Paul, 
MN); cycloheximide was purchased from Sigma-Aldrich; and 
epidermal growth factor (EGF) was purchased from Becton 
Dickinson.

Constructs

Full-length human INPP4B was obtained from Open Bio-
systems. FLAG–INPP4B was generated by the PCR amplifica-
tion (Forward: aattaattagcggccgcgaaattaaagaggaaggggc, 
Reverse: aattaatgcggccgcttaggtctagcttttcutaagtc) of the 
INPP4B CDS and the insertion into the NotI site of 
p3xFLAG-CMV-10 (Sigma-Aldrich).

Transfection

LNCaP cells were transfected with siRNA using Lonza 
electroporation buffer R, as recommended by the manufac-
turer (Lonza). Briefly, 2 × 10⁶ cells were electroporated with 
800 pmol of the indicated siRNA. Cells were seeded onto poly-
D-lysine–coated plates, treated as described per experiment, 
and harvested for RNA and protein analysis. NCoR down-
regulation was done with siRNA, as previously described (30).
INPP4B downregulation was done with silencer siRNAs, and 
noncoding silencer siRNA was used as a control (Ambion). For 
the overexpression of INPP4B, LNCaP cells were seeded at 2.5 
× 10⁵ cells per well in 6 well plates. Cells were transfected with 
3 μg of FLAG–INPP4B, or empty vector per well, with Lipo-
fectamine, as described by the manufacturer (Invitrogen).

Western blot analysis

Protein was extracted with a buffer (20 mmol/L Tris-HCl, 
PH 7.5; 150 mmol/L NaCl; 1mmol/L EDTA; 1% Triton-X 100), 
including protease and phosphatase inhibitors (Roche and 
Calbiochem, respectively). For each sample, 50 μg of protein 
was resolved on 7.5% or 4%–15% PAGE and transferred 
to nitrocellulose membranes. Immunoblotting was done, as 
previously described (31), with antibodies against INPP4B 
(1:1,000), AR (1:1,000; Santa Cruz Biotechnology), total Akt 
(1:1,000), phospho-Akt Thr308 (1:1,000), phospho-Akt Ser473 
(1:1,000), FOXO3a (1:1,000), phospho-FOXO3a S253 (1:1,000; 
Cell Signaling), M2 FLAG epitope (1:1,000; Sigma-Aldrich), 
β-actin (1:5,000; Sigma-Aldrich), and β-tubulin (1:2,000; Milli-
pore). Luminescent signals were captured on a Gel Logic 2000 
imaging system with Kodak Molecular Imaging software 
(Kodak).

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Androgen Regulation of INPP4B

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Proliferation assay

Proliferation assays were done with a Roche DP real-time cell analyzer (RCTA) xCELLigence machine, as described by the manufacturer (Roche). Background impendence was determined by incubating E-Plates with 100 μL of RPMI 1640 with 10% FBS at room temperature for 30 minutes. LNCaP cells were electroporated with 800-pmol of control noncoding or INPP4B-specific siRNAs, and 2 x 10^6 cells were seeded per well. Cells were incubated at room temperature for 30 minutes prior to placement into the RTCA. Cells were grown for 50 hours, and impedance was measured every 15 minutes. Impedance is represented by cell index (CI) and was calculated as follows: CI = (Zt - Zo)/15 Ω, where Zo is the impedance at an individual time-point, and Zt is the background impendence. Average CI was calculated from a minimum of 3 wells per time-point and per experiment. Raw CI values were normalized to a time-point following cell adherence but prior to proliferation. Normalized cell index (NCIti) was calculated as the cell index CIi at a given time-point divided by the cell index CInml_time at the normalized time-point (nml_time)=(NCIti=CIti/CInml_time).

ChIP assays

ChIP assays were done exactly as previously described (31). Briefly, LNCaP cells were grown in a medium supplemented with 10% css for 36 hours, cross-linked, sonicated, and immunoprecipitated with either 5 μg of AR antibody or 5 μg of rabbit IgG. Cross-linking was reversed overnight, and immunoprecipitated DNA was examined by real-time quantitative PCR with the Roche Universal Probe library. The primers and probe sets that were used to detect AR recruitment were as follows: PSA enhancer (31), INPP4B ARE1 (Forward: aggtagctacacgaagaga, Reverse: tcgtgataactcatgtagttgggaa, Probe 46), INPP4B ARE2 (Forward: atttgtgcttcaatataa, Reverse: gcaagagaaaaagataaa, Probe 24), and negative region (Forward: atgcgctctagctaatatcaacc, Reverse: cctataagcctcctca-tcccagatgattctttaacaggtagc, Probe 48).

Real-time polymerase chain reaction analysis

RNA was prepared from cell lines using Trizol reagent, as described by the manufacturer (Invitrogen). First strand cDNA was synthesized using the Superscript III kit (Invitrogen). The PSA primer and probe set was previously described (11). The Roche Universal Probe library and primers were used to amplify the following genes: INPP4B (Forward: gaaagcttc-gtagaaga, Probe 59), PMEPA1 (Forward: ggttaatgcatgctagaaacaca, Reverse: agatggttgagcagctttcg, Probe 48), and INPP4B ARE2 ( Forward: attggtggctcaaaatccaa, Reverse: tcgtgataactcatgtagttgggaa, Probe 46). INPP4B ARE1 (Forward: aggtagctacacgaagaga, Reverse: tcgtgataactcatgtagttgggaa, Probe 46), and INPP4B ARE2 (Forward: atttgtgcttcaatataa, Reverse: gcaagagaaaaagataaa, Probe 24), and negative region (Forward: atgcgctctagctaatatcaacc, Reverse: cctataagcctcctca-tcccagatgattctttaacaggtagc, Probe 48).

Immunohistochemical analysis of human tissue microarrays

Tissue microarrays that were used in this study were described previously (32). Samples were procured from radical prostatectomies of 640 patients who received no adjuvant therapy. Immunohistochemical analysis for INPP4B was done with an INPP4B goat polyclonal antibody (Santa Cruz) exactly as previously described (29). Samples were scanned using a Bliss automated slide scanner to generate high-resolution digital images. Staining was evaluated in normal luminal epithelial cytoplasm in normal samples or epithelial tumor cells of prostate cancer samples. Staining index was calculated as a product of average staining intensity (0–3) and the average extent of staining (0–3), yielding a staining index of 0–9, as described previously. PTEN and Ki67-staining and quantitation have been reported previously (33, 34).

Statistical analysis

Corresponding independent samples of t-tests were used after testing the equal variances assumption for INPP4B protein levels in in vitro experiments. Spearman correlation coefficients were used to evaluate the relationships between INPP4B and clinicopathologic variables. Comparisons of levels of INPP4B and PTEN between normal and tumor tissues were done with a Wilcoxon Signed Ranks test. A Mann–Whitney test was used to compare INPP4B and PTEN levels among Gleason-grade groups. Boxplots were used for the illustration of these results. Kaplan–Meier recurrence-free survival curves for different levels of INPP4B, INPP4B/Kit67, PTEN, and PTEN/ Kit67 combinations were plotted. The minimum p-value method was used to divide the patient population into low and high expressing recurrence-free groups. The Cox proportional-hazard regression modeling of biochemical recurrence was used to compare groups and to develop multivariate survival models. For quantitative PCR analysis, statistical significance was determined by Student’s t-test.

Results

INPP4B is a primary androgen-receptor target gene

In the absence of androgens, Akt and phospho-Akt protein levels are increased in LNCaP cells, and this is reversed by treatment with DHT (16). LNCaP cells lack functional PTEN because of a frameshift mutation (35, 36); thus, we sought to determine whether INPP4B expression was responsive to androgens and potentially mediated androgen regulation of Akt signaling. To determine whether INPP4B was an androgen-responsive gene, LNCaP cells were cultured in the absence of androgens and subsequently treated with the synthetic androgen R1881, and INPP4B expression was evaluated by quantitative RT-PCR. Significantly, INPP4B demonstrated both dose- and time-dependent regulation by R1881 in LNCaP prostate cancer cells (Fig. 1A). Pretreatment with cycloheximide to inhibit de novo protein synthesis did not reduce the R1881 induction of primary AR target gene PMEPA1 (37) or of INPP4B (Fig. 1A), indicating that INPP4B is regulated by androgens at the level of transcription. INPP4B mRNA expression was induced in both LNCaP and VCaP AR-expressing prostate cancer cells. VCaP expressed functional PTEN and, although LNCaP cells lack functional PTEN, they retained mRNA expression (38,39). No induction of PTEN transcription was observed in LNCaP or VCaP cells (Fig. 1B). PSA and the
TMPRSS2–ERG fusion gene were evaluated in LNCaP and VCaP cells, respectively, as known direct AR target genes and controls for hormone induction. Examination of a data set reported by Wang et al. indicated the presence of 2 AR-binding regions in the INPP4B locus in LNCaP cells (Fig. 1C; 40). Direct recruitment of AR to both AR-binding regions in the INPP4B locus was evaluated by ChIP analysis (Fig. 1C). As expected, AR was recruited to the PSA-enhancer region (41). The androgen
stimulation of LNCaP cells substantially enhanced AR recruitment to both binding regions of the INPP4B locus, which further confirmed INPP4B as a direct AR target gene. No recruitment to a negative control region 15 kb upstream of the INPP4B transcription initiation start site (Fig. 1C) was observed. In agreement with our INPP4B expression analysis, culturing LNCaP cells in the absence of androgens led to decreased INPP4B expression and the elevation of activated Akt (Fig. 1D). In addition, androgen starvation of VCaP cells, which are PTEN positive, led to decreased expression of INPP4B and elevated levels of activated Akt (Fig. 1D). Thus, INPP4B contributes to AR-driven suppression of Akt activation.

Depletion of INPP4B activates Akt and stimulates proliferation

Gewinner et al. previously reported that INPP4B depletion in breast cancer cells increased proliferation (29). Since LNCaP cells lack functional PTEN, we were interested to know whether depleting INPP4B could further activate PI3K/Akt signaling and cellular proliferation. LNCaP cells treated with 2 independent siRNAs specifically targeting INPP4B showed substantially reduced INPP4B levels after 48 hours, without appearing to affect AR or total Akt steady state levels (Fig. 2A). In agreement with Figure 1D, depletion of INPP4B in LNCaP cells increased the levels of activated Akt (Fig. 2A). Further, depletion of INPP4B

Figure 2. INPP4B regulates proliferation and Akt phosphorylation in prostate cancer cells. A, two million LNCaP cells were electroporated with either a noncoding control (C) or 2 independent INPP4B-specific siRNAs (INPP-1 or INPP-2; Ambion). One million cells were plated per 10-cm dish for each siRNA. Cells were grown for 48 hours in medium supplemented with 10% FBS. Cellular protein extracts were prepared and analyzed by Western blot for INPP4B, AR, phospho-Akt (Thr308 and Ser473), total Akt, and β-actin (top). LNCaP cells were electroporated as described above and cultured for 48 hours posttransfection. Cellular protein extracts were prepared from cells and analyzed by Western blot for INPP4B, phospho-FOXO3a (Ser253), total FOXO3a, and β-tubulin (bottom) B, LNCaP cells were transfected with 3× FLAG-INPP4B or empty vector and cells grown in medium supplemented with 10% css. Protein extracts were prepared 48 hours posttransfection and analyzed by Western blot for INPP4B, AR, phospho-Akt (Thr308 and Ser473), total Akt, and β-actin (top). LNCaP cells were transfected as described above, grown for 48 hours, and prior to protein extraction, cells were treated with 100 ng/mL EGF for 30 minutes. Protein was extracted and analyzed by Western blot for INPP4B, phospho-FOXO3a (Ser253), total FOXO3a, and β-tubulin (bottom). C, LNCaP cells were electroporated as in A and seeded at 2× 10^4 cells per well of an E-Plate 16, and cellular impedance, as a measure of proliferation, was monitored continuously for 50 hours (left). Quantification of the proliferation slopes (right).
increased phosphorylation of FOXO3a (Ser253), a direct Akt substrate (Fig. 2A). Correspondingly, overexpression of FLAG–INPP4B in LNCaP cells cultured in 10% CSS decreased the levels of activated Akt without significantly altering AR and total Akt (Fig. 2B). In order to demonstrate INPP4B regulation of Akt downstream targets, we overexpressed INPP4B in LNCaP cells and measured phosphorylation of FOXO3a following a 30-minute stimulation with EGF prior to protein extraction. Exogenous expression of INPP4B clearly impeded phosphorylation of FOXO3a in LNCaP cells that were stimulated with EGF (Fig. 2B). Depletion of INPP4B in LNCaP cells substantially increased the rate of proliferation of LNCaP cells, as measured by cellular index (Fig. 2C). Cellular index is a measure of electrical impedance, which is proportional to the number of adherent cells on the electrode grid integrated into the bottom of the plate. The average slope of the impedance curve was calculated between 9 and 50 hours posttransfection to allow for the depletion of INPP4B protein. INPP4B depletion routinely decreased the doubling time of LNCaP cells by 25%–30% (data not shown).

INPP4B and PTEN are reduced in prostate cancer and are associated with reduced time to biochemical recurrence

To determine whether INPP4B is expressed in normal human prostate tissue and whether its expression was lost during the development and progression of prostate cancer, we screened a prostate tissue microarray. We observed specific staining for INPP4B in luminal epithelial cells in normal prostate specimens and in cancer cells (Fig. 3A–C). Examination of clinical prostate specimens showed a significant decrease in INPP4B expression in human prostate cancers compared with normal tissue (P < 0.0001; Fig. 3D). We observed quite consistent staining for INPP4B in normal tissue, with all but 5 samples showing expression with a staining index of 6, whereas half of the tumor samples scored less than 6 (Fig. 3D). In the previously reported tissue microarray analysis of breast and ovarian cancer, INPP4B also demonstrated epithelial compartment expression (27). Consistent with previous reports, we found that PTEN expression is decreased in prostate cancer specimens compared with normal tissue (Fig. 3D; P < 0.0001).
Since we observed a decrease in INPP4B and PTEN in prostate cancer, we sought to further identify correlations of these 2 proteins that regulate the Akt pathway with clinical variables. Significantly, patients with low INPP4B expression \((P = 0.0312)\) recurred earlier compared with patients with higher INPP4B expression \((P = 0.0312; \text{Fig. 4A})\). Patients who lost PTEN expression showed a similar decrease in recurrence-free survival \((P = 0.0261; \text{Fig. 4B})\).

Because prostate cancer is a heterogeneous disease ranging from an indolent to aggressively proliferating malignancy, INPP4B and PTEN expression were correlated with the recurrence rate of patients with high and low expression levels of the proliferation marker Ki67, which was determined previously in this same array. Our data suggest that, in rapidly proliferating tumors, the loss of INPP4B coincides with accelerated recurrence \((P = 0.0312; \text{Fig. 4C})\). The loss of INPP4B in more slowly proliferating cancers did not significantly alter the time to biochemical recurrence. Interestingly, the loss of PTEN in combination with Ki67 showed no correlation with aggressively growing prostate cancers but tended to separate slower-growing cancer patients into a higher risk group \((P = 0.0567).\)

INPP4B is regulated by NCoR

The expression of AR target genes is regulated by numerous coactivators and corepressors. We and others have shown dynamic changes in coregulator expression between normal and tumor tissue in patients \((10, 11, 42–44)\). Investigating the role of the corepressor NCoR on the AR transcriptome in LNCaP cells (manuscript in preparation), we found that INPP4B was the most downregulated gene following NCoR depletion. Since it has previously been shown that NCoR modulates agonist-bound AR activity \((45, 46)\), we examined whether NCoR depletion affects INPP4B expression. To deplete NCoR in LNCaP cells, we used NCoR-specific siRNA (Fig. 5A). Note that while NCoR protein levels decreased, the levels of AR did not significantly change (Fig. 5A). As expected and in accordance with previous reports, PSA induction increased following NCoR depletion (Fig. 5B). Surprisingly, INPP4B expression requires NCoR for optimal expression both with and without androgen (Fig. 5C). Although AR does not lose the ability to induce INPP4B, basal INPP4B transcription is compromised. Similarly, while bicalutamide does not lose its ability to repress the transcription of INPP4B in full serum, overall expression is decreased (Fig. 5D).
may lead to androgen-driven proliferation and loss of differentiation.

In agreement with previous studies in breast cancer, knockdown of INPP4B in LNCaP cells enhanced proliferation. The observed increase in proliferation was associated with increased levels of activated Akt. However, unlike the study by Gewinner et al. (29), enhanced phosphorylation of Akt did not require added stimulation of cells with insulin or activation of other growth receptor pathways. This difference may be accounted for by the different cell types and/or reflects tissue specificity. Significantly, we observed INPP4B regulation of Akt activation in prostate cancer cell lines in both the presence and absence of functional PTEN. This further strengthens the association between androgen withdrawal, INPP4B depletion, and activation of Akt in prostate cancer. The proapoptotic transcription factor FOXO3a is phosphorylated by Akt, which leads to its cytoplasmic retention and degradation, thus impeding apoptosis (18). We found that INPP4B status was implicated in the extent of Akt-dependent phosphorylation of FOXO3a, confirming its regulatory function in Akt signaling.

Extracellular growth receptor pathways, including EGFR, IGF-1R, and HER2/Neu, have been implicated in prostate cancer and the development of castration-resistant disease (47–49). Significantly, the activation of these pathways induces PI3K/Akt signaling. Furthermore, growth receptor pathways and intracellular kinase signaling pathways modulate AR function, although predominantly through the modulation of AR coregulators (50–54). Hence, AR regulation of INPP4B may be an important mediator in the cross talk between extracellular growth signals and AR-regulated growth pathways. Significantly, PTEN has also been implicated in regulating AR turnover and activity (55, 56), and as such, tight regulation and coordination of AR and PI3K signaling appears to be crucial for the correct regulation of prostate epithelial proliferation and maintenance of cellular differentiation.

Importantly, immunohistochemical analysis of human prostate specimens showed luminal epithelial–specific staining of INPP4B, the site of prostate cancer initiation. In primary prostate cancers, INPP4B expression was significantly decreased. The occurrence of INPP4B mutations and the presence of splice variants and their correlation with clinical outcomes were not evaluated in the present study. Mutations of PI3K are rare in prostate cancer, with elevated PI3K signaling in prostate cancer previously being more commonly associated with inactivating mutations of PTEN (26). A recent publication by Taylor et al. identified alterations in the PI3K pathway in 42% of primary and 100% of metastatic prostate cancers (57). In the study by Taylor et al. INPP4B was decreased at the RNA level by an outlier analysis in 8% of clinically localized disease, which was twice the rate of PTEN (4%; ref. 57). Interestingly, INPP4B was decreased in 47% of metastatic samples (compared to 42% for PTEN). In combination with our findings, these data suggest an important role for INPP4B in prostate cancer. Our work confirms the RNA studies at the protein level in clinically localized disease using a much larger sample size. Mutational inactivation and differential isoform activities of

Discussion

Elevated PI3K/Akt signaling is routinely associated with androgen-ablation therapies and particularly with castration-resistant recurrent prostate cancers (13, 14). Here, we present evidence that INPP4B is an androgen-regulated gene in human prostate cancer cells, which suppresses Akt activation. INPP4B expression was induced by androgens in both a time- and dose-dependent manner in LNCaP cells. This is distinct from PTEN, the well-characterized tumor suppressor gene (TSG), which did not demonstrate androgen-regulated expression. PTEN is a global TSG, and INPP4B is an androgen-regulated TSG in prostate epithelium, which enables the AR to control proliferation through modulating Akt activity. Luminal epithelial–specific deletion of the AR in the mature prostate facilitates increased proliferation and loss of differentiation in the mouse (4). Therefore, INPP4B in association with genes such as Nkx3.1 forms a subset of androgen-regulated genes that control growth and maintain differentiation. Significantly, deregulation or loss of INPP4B in primary prostate cancers

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INPP4B may further contribute to the etiology of prostate cancer and explain the subgroup that has elevated mRNA levels of INPP4B. Androgen regulation of INPP4B suggests that its expression would be decreased or lost following androgen-ablation therapies. Prostatic tissue used in the current tissue microarray was obtained from patients who had not received hormone-ablation therapies prior to surgery. Hence, in future studies, it will be important to correlate INPP4B status with therapy response.

Our results suggest that decreased expression of either INPP4B or PTEN correlates with poor outcome for prostate cancer patients undergoing radical prostatectomy. In the present study, loss of PTEN expression correlated with reduced time to biochemical recurrence; however, only a decrease in INPP4B expression was sufficient for a correlation with an increased recurrence rate. This is significant since our data indicate that castration therapies targeting androgen signaling likely lead to further downregulation of INPP4B and subsequent upregulation of PI3K/Akt signaling and the progression of prostate cancer. Loss of expression and inactivating mutations of PTEN are more frequently associated with late-stage and metastatic prostate cancers (26). Recent data suggest that both PTEN and INPP4B likely play significant roles in prostate cancer metastasis (57). Hence, the loss of INPP4B potentially plays a significant role in the development of prostate cancer and the establishment of androgen independence and metastases. Our data indicate that INPP4B is an important TSG in prostatic epithelium and may serve an equally important role to PTEN in regulating cellular proliferation.

An interesting observation from this study was that knockdown of the corepressor NCoR in LNCaP cells suppressed basal expression and prevented full androgen induction of INPP4B. Steroid receptor coactivators potentiate the AR and, in addition to cell signaling pathways, regulate AR function in a promoter-specific manner (9). Elevated expression of coactivators and overactivation of growth receptor signaling pathways are likely involved in the functional switch of the AR to pro-proliferative. It has previously been shown that NCoR suppresses agonist-dependent AR transcriptional activity (58). Significantly, we found that NCoR expression is reduced in prostate cancer, which coincided with the decreased expression of INPP4B (manuscript in preparation). Furthermore, microarray analysis of LNCaP cells following NCoR depletion identified INPP4B as the most downregulated transcript in NCoR-depleted cells (manuscript in preparation). Therefore, as in LNCaP cells, NCoR likely regulates the expression of INPP4B in the human prostate either in a direct or indirect manner. The loss of NCoR and the associated loss of INPP4B further highlight the significant role of AR coregulators in normal prostate biology and prostate cancer etiology.

Inactivation of INPP4B in primary prostate cancers or preneoplastic lesions in association with other epigenetic and genetic alterations potentially facilitates the switch from androgen-regulated differentiation to proliferation. The loss of INPP4B may provide a marker for prostate cancer patients that would benefit from combined androgen-ablation therapy and PI3K/Akt inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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