The androgen receptor (AR) is a steroid hormone receptor playing a pivotal role in normal prostate homeostasis and in the initiation/progression of prostate cancer through androgen-induced signaling (1). The AR is composed of an N-terminal domain, a central DNA-binding domain, a hinge region, and a C-terminal ligand-binding domain (2). In the absence of ligand, the AR is sequestered to the cytoplasm by heat-shock proteins, which mask a nuclear localization sequence of the AR. Upon ligand binding, the AR is translocated into the nucleus to activate androgen-responsive genes by binding to the androgen-responsive elements of the genes. The regulation of AR transcription activity is achieved via interaction with other coactivators and repressors, some of which are involved in posttranslational modifications of the AR including phosphorylation, acetylation, sumoylation and ubiquitination, leading to the modulation of protein stability, interaction with other proteins, cellular localization, and structure conformation of the AR (2).

Ubiquitination is one of the most important protein modifications in eukaryotic cells by which the cell regulates the turnover of proteins as well as functions as a signaling moiety to affect signaling pathways (3). Similar to other transcription factors, the AR is subject to regulation by ubiquitination (2). As the first E3 ligase identified for the AR, mouse double minute 2 induces polyubiquitination of the AR, resulting in AR degradation and attenuation of AR transactivity as well as the inhibition of androgen-mediated cell growth (4). Recently, other E3 ligases including carboxy terminus of HSP70-interacting protein (CHIP), ring finger protein 6 (RNF6), and Siah E3 ubiquitin protein ligase 2 (Siah2) have been identified in the control of AR stability and activity (5–7). The CHIP E3 ligase can promote AR degradation via ubiquitination, which is implicated for modulating mitotic arrest in prostate cancer cells (8). The RNF6 E3 ligase can induce poly-ubiquitination of AR as well as mono-ubiquitination of AR, resulting in promoting AR degradation and attenuation of AR transactivity (6). The Siah2 E3 ligase targets the AR for degradation, promoting expression of select AR target genes (6). It appears that whether upregulation or downregulation of AR transcription activity occurs depends on the type or status of ubiquitination.
The von Hippel-Lindau (VHL) gene (pVHL) is a classic tumor suppressor, and its inactivation is linked to the development of clear-cell renal cell carcinomas, hemangioblastomas, pheochromocytomas, and tumors in other organs (9, 10). The best-characterized function of pVHL is acting as a substrate recognition subunit of a multiprotein E3 ubiquitin ligase complex that targets the proline hydroxylated hypoxia-induced factor (HIF)-1α for degradation under normoxic conditions (11–16). In pVHL-deficient cells, the stabilized HIF transcription complexes induce the expression of a diverse set of target genes involved in angiogenesis, glycolysis, glucose transport, and survival (17), many of which are clearly linked to tumorigenesis (18).

As a component of the VHL-Elongin B/C E3 ligase complex, other non-HIF-α targets of pVHL have been identified, including estrogen receptor-α (19), Kruppel-like factor 4 (20), and ERK5 (21), in which pVHL regulates protein turnover. In addition, pVHL can also target protein for K63-linked ubiquitination, which does not induce protein degradation (16). Overall, apart from HIF-α, the mechanisms and the consequences of pVHL-induced ubiquitination of its targets are still largely unknown.

Moreover, pVHL also has multiple ubiquitin E3 ligase-independent functions, including the maintenance of microtubule stability (15), regulation of the extracellular matrix (10), Akt-mTOR signaling (22), fibroblast growth factor receptor signaling (23), regulation of atypical protein kinase C activity (24), and stabilization of p53 (25). Although the contribution of these activities to inhibition of tumorigenesis remains unclear, they are likely linked to many of the other types of VHL disease (26).

In this study, we demonstrated that the AR is a novel target of pVHL and that pVHL binding inhibits AR transcription activity. As a result, pVHL suppresses androgen-induced cell proliferation. However, surprisingly, pVHL induces de-ubiquitination instead of ubiquitination of the AR, contrary to its E3 ligase activity. These data suggest a novel function for pVHL on its binding partner and connect pVHL to an androgen-induced signaling pathway.

Materials and Methods

Cell lines and plasmid constructions

HEK293T and LNCaP were originally obtained from the American Type Culture Collection. C4–2, a subline of LNCaP, was originally developed by Thalmann et al (27). HEK293T was maintained in DMEM (HyClone) supplemented with 10% fetal bovine serum (HyClone). LNCaP and C4–2 were maintained in RPMI 1640 (HyClone) supplemented with 10% fetal bovine serum. All cells were grown at 37°C in a humidified incubator containing 5% CO2.

The Probasin-LUC, AR-MMTV-LUC, and PSA-LUC reporters were kindly provided by Dr Roland Schüle (28, 29). The RNF6 construct was kindly provided by Dr Yun Qiu (6). Human AR and human pVHL were subcloned into the vectors pCMV-tag-2C, pCMV-Myc, pGEX-2T, pHAGE, and pET32-α. For pVHL overexpression, a lentivirus expressing pVHL was made by pHAGE vector. For pVHL knockdown, pSUPER vector (30) was used to make 2 pVHL short hairpin RNA (shRNA) constructs, pSUPER-pVHL1 and pSUPER-pVHL2, which target the sequences 5′-GGAGCGGCTGAGAGAGA-3′ (pSUPER-pVHL1) and 5′-AGACCTGGACGCGGCTGACA-3′ (pSUPER-pVHL2), respectively. pSUPER-GFP was used as a control. In addition, a lentivirus expressing pVHL shRNA was made using the lentivirus vector, Lentilox3.7 (pL3.7-shRNA-pVHL), which targets the sequence 5′-GGAGCGGATGCGACATCA-3′. A lentivirus expressing luciferase shRNA was used as a control.

The ubiquitin mutants KO, K6, K11, K27, K29, K33, K48, and K63 and AR mutant K845S/S474T were made by PCR and verified by sequencing. Human androgen receptor activator 54 (ARA54) was amplified form a human cDNA pool by PCR and subcloned into pCMV-HA vector.

Luciferase reporter assay

LNCaP or C4–2 cells were grown in phenol red-free RPMI 1640 (Gibco) with 10% double charcoal-stripped fetal bovine serum (Biological Industries). The cells were transfected with the indicated plasmids for 12 hours and then treated with DHT (10nM) (Sigma; dissolved in ethanol) for 12 hours. Luciferase activity was assayed using the dual-luciferase reporter assay system following the protocol provided by the manufacturer (Promega). Data were normalized to Renilla luciferase. Data are reported as mean ± SEM of 3 independent experiments performed in triplicate. The statistical analysis was performed using GraphPad Prism version 5 (unpaired t test) (GraphPad Software Inc).

Semiquantitative real-time RT-PCR

Total RNA was extracted by Trizol reagent (Invitrogen), and cDNA synthesis was carried out using a first-strand cDNA synthesis kit (Fermentas). Primer sequences were PSA, 5′-TCTGCGGCGGTGGTGTCTC-3′ and 5′-GCCGACGACGGCAAGAGC-3′; TMPRSS2, 5′-GGACGGGTTGAGCCTCAAGAG-3′ and 5′-TCCACGAGGAAAGTCCC-3′; and β-actin, 5′-GCATTCCGGCTGTATATC-3′ and 5′-GGATCGACGAGACGCACTCCGTTCC-3′. Data are reported as mean ± SEM of 3 independent experiments performed in triplicate. The statistical analysis was performed using GraphPad Prism version 5 (unpaired t test).

Western blot and immunoprecipitation assay

The following antibodies were used for Western blot or immunoprecipitation analysis: AR (N20; Santa Cruz), prostate-specific antigen (PSA) (C19; Santa Cruz), Myc (9E10, Santa Cruz), α-tubulin (Upstate), gynecaldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz), histone H3 (Cell Signaling Technology), Flag (M2; Sigma), and pVHL (Abclonal). LNCaP or C4–2 cells were grown in phenol red-free RPMI 1640 supplemented with 10% double charcoal-stripped fetal bovine se-
rum on 6-well plates. The cells were treated in the presence or absence of DHT (10nM) for 16 hours after transfection for 12 hours. The procedures for Western blot and immunoprecipitation assays were described previously (31). The Fuji Film LAS4000 mini luminescent image analyzer was used to photograph the blots. Multi Gauge version 3.0 was used for quantifying the protein levels based on the band density obtained in the Western blot analysis.

Glutathione S-transferase pull-down assay

Glutathione S-transferase (GST)-tagged AR and His-tagged VHL were expressed in bacteria and purified. After coimmunoprecipitation using GST agarose beads, the protein was separated by SDS-PAGE. The gel was stained with Coomassie blue or transferred into polyvinylidene difluoride (PVDF) membrane for detecting His-pVHL by Western blot analysis.

Cell growth assay

The LNCaP cells infected with lentivirus were grown in phenol red-free RPMI 1640 supplemented with 10% double charcoal-stripped fetal bovine serum for 72 hours. Cells (3 × 10^5) were seeded in 6-well plates with or without DHT (1nM). Cells were counted by an automated cell counter (Tc10; Bio-Rad) on days 1, 2, and 3. Data are reported as mean ± SEM of 3 independent experiments performed in triplicate. The statistical analysis was performed using GraphPad Prism version 5 (unpaired t test).

Results

pVHL interacts with AR in vitro and in vivo

We have previously shown that an androgen-upregulated gene, EAF2 (U19), can bind to and stabilize pVHL (32). To determine whether pVHL can interact with AR to modulate ligand-dependent transcriptional activity of AR, we performed coimmunoprecipitation assays. We cotransfected Myc-pVHL together with Flag-tagged AR or empty vector into HEK293T cells. Flag-AR could pull down Myc-pVHL efficiently (Figure 1A, left panel). In addition, Myc-pVHL could also pull down Flag-AR (Figure 1A, right panel). To confirm that pVHL interacts with AR endogenously, we conducted coimmunoprecipitation assays using a polyclonal anti-AR antibody in LNCaP cells. AR could indeed interact with endogenous pVHL as detected by a polyclonal anti-pVHL antibody, whereas this interaction was unaffected by adding ligand, DHT (10nM) (Figure 1B), suggesting that the ligand binding does not affect AR-pVHL interaction. The interaction between AR and pVHL appears to be direct, because bacterially expressed GST-tagged AR pulled down bacterially expressed His-pVHL in GST pull-down assays, but GST protein did not pull down His-pVHL (Figure 1C).
Next, we performed domain mapping to determine which region of pVHL can bind to AR. As shown in Figure 1D, the 114 to 154 region of the β-domain (1–154 amino acids [aa]) in pVHL was required for binding to AR. Moreover, we mapped pVHL-binding domain of AR. Full-length AR, as well as the ligand-binding domain (637–918, 637–870, 706–870, and 706–918 aa), associated with pVHL. In contrast, the AR truncated mutants lacking the ligand-binding domain (1–555 and 1–636 aa) did not interact with pVHL (Figure 1E), implying that pVHL may affect the activity of AR in response to androgen stimulation.

Taken together, these data show that pVHL interacts with AR in vitro and in vivo.

pVHL inhibits AR transactivity

To determine the functional importance of pVHL binding to AR, we performed transient transfection assays to examine whether pVHL modulates the transcription activity of AR. Overexpression of pVHL caused a strong ligand-dependent inhibition of a rat Probasin promoter-luciferase reporter as well as human PSA promoter-luciferase reporter in C4–2 cells (a castration-resistant subline of LNCaP cells) (27) (Figure 2, A and B). Consistently, the endogenous protein level of PSA was reduced when pVHL was overexpressed in C4–2 cells in the presence of ligand (10nM DHT) (Figure 2C).

Furthermore, in LNCaP cells, overexpression of pVHL also caused a ligand-dependent inhibition of a Probasin promoter-luciferase reporter, a PSA promoter-luciferase reporter, and a mouse mammary tumor virus (MMTV)-luciferase reporter (Figure 2, D–F). Moreover, semiquantitative RT-PCR analyses demonstrated that pVHL inhibited the androgen-induced expression of endogenous TMPRSS2 and PSA genes in LNCaP cells (7) (Figure 2, G

**Figure 2.** Overexpression of pVHL inhibits AR transactivity. A, The Probasin promoter activity is suppressed by overexpression of pVHL in the presence of DHT (10nM) in C4–2 cells. B, The PSA promoter activity is suppressed by overexpression of pVHL in the presence of DHT (10nM) in C4–2 cells. C, The protein level of PSA is reduced when pVHL is overexpressed in C4–2 cells. D, The Probasin promoter activity is suppressed by overexpression of pVHL in the presence of DHT (10nM) in LNCaP cells. E, The PSA promoter activity is suppressed by overexpression of pVHL in the presence of DHT (10nM) in LNCaP cells. F, The MMTV-LUC reporter activity is suppressed by overexpression of pVHL in the presence of DHT (10nM) in LNCaP cells. G, The mRNA level of TMPRSS2 is reduced when pVHL is overexpressed in LNCaP cells in the presence of DHT (10nM). H, The mRNA level of PSA is reduced when pVHL is overexpressed in LNCaP cells in the presence of DHT (10nM). I, The protein level of PSA is reduced when pVHL is overexpressed in LNCaP cells in the presence of DHT.
and H). Western blot analyses indicated that the androgen-induced endogenous protein level of PSA was reduced by overexpression of pVHL as well (Figure 2I).

Next, we knocked down endogenous pVHL in LNCaP cells by 2 VHL shRNAs (pSUPER-pVHL1 and pSUPER-pVHL2) efficiently (data not shown). After pVHL was knocked down, a significant ligand-dependent increase of Probasin-LUC reporter activity as well as PSA-LUC reporter activity was observed in LNCaP cells (10nM DHT) (Figure 3, A and B). Moreover, upon knockdown of pVHL, the ligand-induced endogenous TMRSS2 and PSA mRNA levels were further increased as revealed by semi-quantitative RT-PCR analyses (Figure 3, C and D). Western blot analyses demonstrated that knockdown of pVHL also enhanced the ligand-induced endogenous PSA protein level (Figure 3E). The efficiency of pVHL1-shRNA-mediated knockdown of pVHL was also confirmed (Figure 3E, line 3). Taken together, these data suggest that pVHL inhibits ligand-dependent transcription activity of AR.

pVHL induces de-ubiquitination of AR

Notably, overexpression or knockdown of pVHL does not alter AR protein level in vitro and in vivo. Given the regulation of AR nuclear trafficking accounting for one of the mechanisms modulating AR activity (7), we initially examined whether the nuclear translocation of AR is affected by pVHL overexpression. However, as shown in Figure 4A, overexpression of pVHL in LNCaP cells did not change the distribution of endogenous AR between cytoplasm and nucleus in the presence or absence of ligand (10nM DHT), whereas addition of ligand (DHT) could indeed increase the protein level of AR in the nucleus (lines 7 and 8 in the first panel of Figure 4A).

Because pVHL is a well-defined substrate recognition subunit of a multiprotein E3 ubiquitin ligase complex, to determine the mechanism of pVHL’s suppressive function on AR transcription activity, we sought to determine whether overexpression of pVHL can change the status of AR ubiquitination. We transfected HEK293T cells with His6-tagged wild-type ubiquitin- and Flag-tagged AR along with Myc empty vector (a negative control) or Myc-tagged pVHL. At 24 hours after transfection, we lysed cells, incubated the lysates with nitrolotriacetic acid (NTA) beads, and performed pull-down assays. Surprisingly, as shown in Figure 4B, a decrease of poly-ubiquitinated AR was detected when pVHL was overexpressed. By contrast, knockdown of pVHL by pSuper-pVHL1 enhanced poly-ubiquitinated AR (Figure 4C). We further examined whether mono-ubiquitination of AR is also affected by pVHL overexpression. We transfected HEK293T cells with His6-tagged lysine-null ubiquitin (KO) and Flag-tagged AR along with Myc empty vector or Myc-tagged pVHL and then conducted Ni-NTA pull-down assays. As shown in Figure 4D, when pVHL is overexpressed, mono-ubiquitination...
tion of AR was also decreased (Figure 4D). These data show that pVHL induces de-ubiquitination of AR, contrary to its E3 ubiquitin ligase activity.

RNF6 has been reported to enhance AR activity by promoting poly-ubiquitination of AR without affecting AR turnover (6). The action of pVHL appears to be contrary to that of RNF6, which inhibits AR activity by promoting de-ubiquitination of AR without affecting AR turnover. This phenomenon prompted us to hypothesize that pVHL might act by undoing the ubiquitination of AR triggered by RNF6. To test this hypothesis, we initially examined the effects of pVHL and RNF6 on poly-ubiquitination of AR. As expected, overexpression of pVHL reduced poly-ubiquitination of AR, but overexpression of RNF6 enhanced poly-ubiquitination of AR (Figure 5A). However, pVHL could still suppress the activity of the AR mutant (K845/847R) (Figure 5B), which cannot be poly-ubiquitinated by RNF6 (6). In addition, pVHL could de-ubiquitinate the AR mutant (K845/847R), but RNF6 almost lost its ability to poly-ubiquitinate this AR mutant (Figure 5C). As reported, RNF6 could enhance ARA54, an AR coactivator (33), and interaction with AR, resulting in enhancing AR activity (6). To further determine whether pVHL acts by undoing the ubiquitination of AR triggered by RNF6, we performed coimmunoprecipitation between AR and ARA54 in the absence or presence of pVHL. As shown in Figure 5D, pVHL had no effect on the interaction between AR and ARA54. These data suggest that pVHL might not act by undoing the ubiquitination of AR triggered by RNF6.

To further determine which lysine-linked poly-ubiquitination of AR is de-ubiquitinated by pVHL, we did poly-ubiquitination assays using 7 ubiquitin mutants (K6, K11, K27, K29, K33, K48, and K63). As shown in Figure 5E, pVHL dramatically de-ubiquitinated K6-linked poly-ubiquitination of AR and then K48-linked poly-ubiquitination of AR. K29-linked poly-ubiquitination of AR was merely de-ubiquitinated by pVHL (Figure 5E). However, K27-linked poly-ubiquitination of AR, one of the targets of RNF6, was not affected by pVHL, supporting the above notion that pVHL might not act by undoing the ubiquitination of AR triggered by RNF6.

Therefore, pVHL might inhibit AR activity mainly through de-ubiquitinating K6-linked poly-ubiquitination of AR.
poly-ubiquitination of AR. Of note, overexpression of wild-type ubiquitin had no obvious effect on AR activity, which might result from neutralization of an opposite effect of K6/K11 vs K33 lysine-linked ubiquitination of AR.

**pVHL inhibits androgen-induced cell proliferation**

We next evaluated the physiologic relevance of pVHL in the regulation of AR activity. To address whether pVHL governs androgen-dependent cell growth, we infected LNCaP cells with a lentivirus expressing pVHL (pHAGE-pVHL). Infection with pHAGE-pVHL caused overexpression of pVHL (Figure 6A, right panel). When compared with cells transduced with the pHAGE-control virus, androgen-induced proliferation (1 nM DHT) of LNCaP cells was inhibited by pHAGE-pVHL–mediated pVHL overexpression significantly (Figure 6A, left panel).

Moreover, we infected LNCaP cells with a lentivirus (pLL3.7-shRNA-pVHL) expressing small interfering RNA against pVHL. Infection with pLL3.7-shRNA-pVHL caused efficient and specific reduction of endogenous pVHL (Figure 6B, right panel). When compared with cells transduced with the pLL3.7-shRNA-control virus, androgen-induced proliferation (1 nM DHT) of LNCaP cells markedly enhanced by pLL3.7-shRNA-pVHL–mediated pVHL knockdown. Taken together, these results suggest that pVHL inhibits androgen-induced cell proliferation.

In addition, we also examined whether pVHL affects cell proliferation in the absence of hormone. As shown in Figure 6, C and D, even though the LNCaP cell proliferation rate without DHT treatment was lower than that with DHT treatment (compared with Figure 6, A and B), overexpression of pVHL still reduces poly-ubiquitination of AR linked through K6, K48, and K29. Therefore, pVHL can inhibit cell proliferation in the absence of hormone, which might reflect its general tumor suppressor function in prostate cancer cells.

**Discussion**

Ubiquitination of AR has been implicated in the control of AR activity (2). Poly-ubiquitination of AR induced by MDM2, CHIP, and Siah2 has been shown to regulate the stability and turnover of AR (4, 5, 7). However, it appears that they act in an opposite fashion on the AR activity; whereas MDM2 and CHIP downregulate AR activity,
Siah2 upregulates AR activity through different mechanisms (4, 5, 7). Moreover, the RNF6-induced AR poly-ubiquitination has been demonstrated to be different from other E3 ligase-mediated ubiquitin modification (6). RNF6 has no impact on the stability and turnover of AR but enhances AR activity by promoting the assembly of K6- and K27-mediated poly-ubiquitination of AR, connecting poly-ubiquitination of AR to transcription activity enhancement without affecting AR turnover (6). In this study, we notice that K6-linked poly-ubiquitination of AR enhances AR activity dramatically, which might explain RNF6’s enhancement role on AR activity, at least in part. These reports also imply a complicated relationship between the E3 ligases and the regulation of AR activity. Here, we show that AR transcription activity is suppressed by pVHL-induced de-ubiquitination of AR, which is consistent with that RNF6-mediated ubiquitination of AR enhances AR transcriptional activity. However, we provide evidence to show that pVHL does not act by undoing the ubiquitination of AR triggered by RNF6. Given the enhancement role of K6-linked poly-ubiquitination on AR activity, the fact that pVHL de-ubiquitinates K6-linked poly-ubiquitination of AR might explain pVHL’s suppressive role on AR activity.

As a component of the E3 ligase complex, the E3 ligase-independent functions of pVHL have been extensively studied (10, 15, 22, 23); however, the de-ubiquitination enhancement of AR on its partners has never been noted. Although one protein that possesses de-ubiquitination and ubiquitin ligase domains has been reported, such as A20 (34), pVHL is a relatively shorter protein (213 aa); thus, it is hard to believe that pVHL possesses 2 opposite activities, ubiquitination and de-ubiquitination. However, in this study, we provide multiple lines of evidence to show that pVHL de-ubiquitinates K6- and K48-linked poly-ubiquitination, and even K29-linked poly-ubiquitination. One possible explanation for this noncanonical function of pVHL is that pVHL might mediate de-ubiquitination of its partners through indirect ways, such as recruiting other proteins possessing de-ubiquitination activity. Alternatively, pVHL may take advantage of its E3 ligase activity to induce ubiquitination of AR’s other specific E3 ligases, eventually resulting in a decrease of AR ubiquitination. Of course, we still cannot rule out that pVHL itself might possess de-ubiquitination activity through forming a complex, similar to its ubiquitination activity by forming a complex with Elongin B/C and Cullen-2. Although there is still a lack of convincing evidence to support the observation that pVHL has de-ubiquitination activity, further investigation of this feature of pVHL, specifically how pVHL specifically affects K6-, K48-, or K29-linked poly-ubiquitination, will provide mechanistic insight into the biological function of pVHL.

As a classic tumor suppressor, multiple lines of evidence show that dysfunction of pVHL is crucial for the
development of clear-cell renal cell carcinomas, hemangioblastomas, and pheochromocytomas (35). A lot of mutants of pVHL relating to these tumor development have been identified and investigated thoroughly (35). However, to date, none of pVHL mutants relating to prostate cancer have been screened; thus, whether or not dysfunction of pVHL has an impact on prostate cancer initiation and progression is still obscure. pVHL has been shown to be expressed in the epithelium of human prostate (36), implicating that pVHL might have some function in prostate homeostasis or prostate cancer development. Here, we show that pVHL interacts with AR to suppress AR transactivity and inhibits cell proliferation by inducing de-ubiquitination of AR, suggesting an important role of pVHL in androgen signaling. Given a critical role of AR in the development of prostate cancer (37), pVHL might have important impact on prostate cancer initiation and progression through affecting AR transactivation activity. Due to the lack of evidence showing that pVHL is mutated in prostate cancer, it is possible that dysfunction of pVHL in prostate cancer is caused by mechanisms other than mutagenesis, such as epigenetic regulation. To further explore the deregulation of pVHL in prostate cancer might shed new light on uncovering the cause of prostate cancer development, leading to novel therapeutic approaches for prostate cancer.

Acknowledgments

We are grateful to Drs Roland Schüle and Yun Qiu for the generous gifts of reagents.

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This work was supported by the “973” Grant 2010CB126306, National Natural Science Foundation of China Grant 31071212, 91019008, and Chinese Academy of Sciences Major Scientific and Technological Project XDA08010208 to W.X.

Disclosure Summary: The authors have nothing to disclose.

References


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