Identification of the PTEN-ARID4B-PI3K pathway reveals the dependency on ARID4B by PTEN-deficient prostate cancer

Ray-Chang Wu1, In-Chi Young1, Yu-Fang Chen1, Sung-Ting Chuang1, Antoun Toubaji2 & Mei-Yi Wu1,3

PTEN is frequently mutated in prostate cancer. The tumor suppressor function of PTEN is attributed to its lipid phosphatase activity that counters PI3K action. Here, we report a PTEN-ARID4B-PI3K axis in which PTEN inhibits expression of ARID4B, while ARID4B is a transcriptional activator of the PI3K subunit genes PIK3CA and PIK3R2 that are crucial for activation of the PI3K/AKT pathway. Reciprocal binding of ARID4B and histone H1 to the PIK3CA and PIK3R2 promoters modulates chromatin condensation, suggesting a mechanism by which ARID4B activates these promoters. Functional analyses reveals that ARID4B is required for prostate tumorigenesis when PTEN is deficient. The biological significance is further substantiated by the existence of a PTEN/ARID4B/PIK3CA three-gene signature that improves the predictive power for prostate cancer recurrence in patients. In summary, we identify ARID4B as a master regulator in the PTEN-PI3K pathway, thus providing a potential therapeutic target for prostate cancer carrying PTEN mutations.
Prostate cancer is the second leading cause of male cancer-related death worldwide. Loss of the tumor suppressor gene PTEN is a frequent genetic alteration in prostate cancers, and many late-stage prostate cancers showed altered activity in the PTEN-PI3K pathway[1,2]. To date, most targeted anticancer drugs inhibit oncoproteins that acquire gain-of-function mutations in cancer. However, treatment of cancers that harbor loss-of-function mutations on tumor suppressor genes, such as PTEN, remains a significant challenge because there are no direct targets amenable to pharmaceutical intervention. The emerging concept of synthetic essentiality in cancer describes cancers with specific tumor suppressor deficiencies that depend on expression of synthetic-essential genes. Targeting such genes may be a promising alternative treatment approach for cancers that acquire loss-of-function mutations on tumor suppressor genes3. Therefore, significant efforts have been devoted to identifying synthetic-essential genes in PTEN-deficient prostate cancers4,5. PI3K phosphorylates phosphatidylinositol (PtdIns)-4,5-bisphosphate (PIP2) to generate PtdIns-3,4,5-trisphosphate (PIP3), which activates the AKT signaling pathway to mediate cellular events, including cell survival, growth, proliferation, and cell migration6,7. Hyperactivation of the PI3K-AKT signaling pathway is associated with prostate cancer progression8. The biological effects of PTEN are dominated by its ability to dephosphorylate PIP3 back into PIP2, leading to suppression of the proto-oncogenic PI3K-AKT signaling pathway9. Three classes of PI3Ks (class I, class II, and class III) have been identified in mammals. Class I PI3Ks are heterodimeric enzymes that consist of a catalytic subunit p110α, p110β, or p110δ (encoded by PIK3CA, PIK3CB, PIK3CG, or PIK3CD, respectively) and a regulatory subunit p85α, p55α, p50α (all encoded by PIK3R1), p85β (encoded by PIK3R2), p55γ (encoded by PIK3R3), p150 (encoded by PIK3R4), or p101 (encoded by PIK3R5)10,11. PIK3CA is frequently mutated in prostate cancers, and many of these mutations result in activation of PI3K9. In addition, the Pik3ca gain-of-function mutation accelerates cancer progression of PTEN-deficient prostate in mice10. Although alterations of the PTEN-PI3K-AKT pathway are strongly implicated in prostate cancer pathogenesis, their therapeutic targeting has not improved clinical efficacy9. Therefore, identification of effectors of this important pathway to target PTEN-deficient prostate cancers is urgently needed.

ARID4B belongs to the ARID family and is a chromatin remodeling factor11. ARID4B was previously identified as a component of the HDACI/SIN3A chromatin remodeling complex12. Although ARID4B is a chromatin remodeling protein, it lacks methyltransferase or acetyltransferase activities. To date, how ARID4B modulates chromatin modifications to regulate gene expression is not well understood. However, ARID4B contains a Tudor domain and a chromo domain, and these domains may function as adaptors to assemble chromatin remodeling complexes13,14. Expression of ARID4B is elevated in many types of tumors15–18, and ARID4B promotes mammary tumor growth and metastasis19. Despite these early findings, the role of ARID4B in prostate cancer remains unclear.

In the present study, we identify ARID4B as a regulator of the PTEN-PI3K pathway by functioning as a transcriptional activator of PIK3CA and PIK3R2. Importantly, we demonstrate that ARID4B is required for initiation and progression of PTEN-deficient prostate cancer, suggesting ARID4B is a potential therapeutic target for prostate cancer harboring PTEN mutations.

Results
ARID4B regulates the PI3K-AKT pathway. To determine genomic alterations of ARID4B in prostate cancer, we analyzed the Cancer Genome Atlas (TCGA) and other prostate cancer genomic datasets20–25. Amplification of ARID4B was detected in up to 20% of human prostate cancer cases (Supplementary Fig. 1a, left), while deletion and loss-of-function mutation of PTEN are frequent (Supplementary Fig. 1a, right). Using two prostate cancer gene expression datasets in the Oncomine database23,26, we found that expression of ARID4B was significantly increased in prostate carcinoma (PCa) compared to normal prostate glands (Supplementary Fig. 1b). In addition, the human prostate cancer cell lines PC3, DU145, LNCaP, and C4-2B expressed higher levels of ARID4B protein than the normal (nonmalignant) prostate epithelial cell lines RWPE-1, PZ-HP-7, and HPrEC (Supplementary Table 1 and Supplementary Fig. 1c). Furthermore, patients expressing higher levels of ARID4B had a significantly increased risk of prostate-specific antigen recurrence in two datasets27,28 (Supplementary Fig. 1d). These results indicate that expression of ARID4B positively correlates with prostate cancer development, and tumor recurrence is sensitive to higher levels of ARID4B expression.

To determine how ARID4B plays a role in prostate cancer, we performed whole transcriptome analysis (RNA-Seq) to compare gene expression profiles of PC3 cells with or without knockdown of ARID4B (Supplementary Data 1). Expression of the PI3K subunit genes PIK3CA and PIK3R2 was significantly reduced in PC3 cells with knockdown of ARID4B (Table 1 and Supplementary Data 1). Independent quantitative RT-PCR (qRT-PCR) analysis confirmed decreased expression of PIK3CA and PIK3R2 (Fig. 1a). In contrast, E-cadherin (CDH1), an invasion suppressor in many cancers, was upregulated (Table 1 and Fig. 1a). Furthermore, luciferase reporter gene assays showed that ARID4B activated the promoters of PIK3CA and PIK3R2 (Fig. 1b). Consistent with these results, we found a positive correlation between ARID4B and PIK3CA expression within the prostate cancer cohort in the TCGA database (Fig. 1c).

Furthermore, western blot analysis revealed that knockdown of ARID4B reduced protein levels of p110α (encoded by PIK3CA) and p85β (encoded by PIK3R2) in PC3 cells (Fig. 1d). Accordingly, phosphorylation of the PI3K downstream effector AKT at Ser473, required for the full activation of AKT29, was reduced in PC3 cells after knockdown of ARID4B (Fig. 1d). In addition, phosphorylation of mTOR at Ser2448 was also diminished (Fig. 1d), consistent with reports that phosphorylation of AKT at Ser473 is linked to phosphorylation of mTOR at Ser2448 and activation of the mTOR pathway30,31. Concomitantly, expression of downstream target genes in the PI3K/AKT signaling network, such as cyclin D1 (CCND1), was decreased (Table 1, Fig. 1a, d Supplementary Fig. 2, and Supplementary Table 2). Consistent with these results, knockdown of ARID4B in the DU145 and LNCaP human prostate cancer cell lines reduced expression or phosphorylation of the core regulators and downstream effectors of the PI3K-AKT pathway (Supplementary Fig. 3). Together, these results suggest that ARID4B positively regulates expression
of PIK3CA and PIK3R2 and is crucial for activation of the PI3K/AKT signaling pathway (Fig. 1e).

**PIK3CA and PIK3R2 are direct target genes of ARID4B.** Since RNA-Seq analysis identified genes directly or indirectly regulated by ARID4B (Supplementary Data 1), we performed ChIP using antibody against ARID4B followed by DNA sequencing (ChIP-Seq) analysis to globally identify direct binding targets for ARID4B in PC3 cells (Supplementary Data 2). By combining the results from RNA-Seq ($P$ value $< 0.05$, $|\text{Log2FC}| \geq 0.59$) and ChIP-Seq analyses, we identified potential genes directly regulated by ARID4B (Supplementary Data 3). ChIP-Seq analysis showed enrichment of ARID4B near the transcriptional start sites
(TSSs) of genes (Supplementary Fig. 4a, b), and identified PIK3CA and PIK3R2, whose promoters were associated with ARID4B (Fig. 2a, b, orange lines). The CRISPR-Cas9 gene editing system was used to achieve knockout of ARID4B (ARID4BKO) in PC3 cells (Supplementary Fig. 6a). ChiP-qPCR analysis using the antibody against ARID4B further confirmed recruitment of ARID4B to the PIK3CA and PIK3R2 promoters in control PC3 cells, but showed minimal ARID4B binding in ARID4BKO PC3 cells (Fig. 2c). In contrast, ARID4B was not recruited to the promoters of myogenic differentiation 1 (MYOD1), glycerolaldehyde 3-phosphate dehydrogenase (GAPDH), or ARID4B itself (Fig. 2c, Supplementary Fig. 4c, and Supplementary Data 2), indicating that recruitment of ARID4B to the PIK3CA and PIK3R2 promoters is specific. Together with the fact that ARID4B regulates promoter activities of PIK3CA and PIK3R2 (Fig. 1b) and controls their expression (Table 1 and Fig. 1a), these results indicate that PIK3CA and PIK3R2 are direct target genes of ARID4B.

ARID4B and histone H1 reciprocally bind to PIK3CA and PIK3R2. Histone H1 is depleted in the region surrounding the TSSs, and we showed that ARID4B is recruited to TSSs of genes (Supplementary Fig. 4a, b). Thus, we examined whether recruitment of ARID4B to the PIK3CA and PIK3R2 promoters affects binding of histone H1. ChiP-qPCR analyses showed that abolition of ARID4B increased binding of histone H1 to the PIK3CA and PIK3R2 promoters (Fig. 2d) without affecting histone H4 acetylation (H4Ac), H3 lysine 27 acetylation (H3K27ac), or H3 lysine 4 trimethylation (H3K4me3) (Supplementary Fig. 5). Consistent with no recruitment of ARID4B to the MYOD1, GAPDH, and ARID4B promoters (Fig. 2c and Supplementary Fig. 4c), ablation of ARID4B did not influence histone H1 binding to these promoters (Fig. 2d and Supplementary Fig. 4d).

ARID4B modulates chromatin condensation on PIK3CA and PIK3R2. Since histone H1 stabilizes the condensed chromatin structure that contributes to gene silencing, reciprocal binding of ARID4B and histone H1 to the PIK3CA and PIK3R2 promoters led us to examine whether ARID4B impacts chromatin structure on these promoters. Using micrococcal nuclease (MNase) that preferentially digests loosened chromatin followed by qPCR analysis, the promoter regions of PIK3CA and PIK3R2 (but not MYOD1) in control PC3 cells were more sensitive to MNase digestion than ARID4BKO PC3 cells (Fig. 2e), indicating that the chromatin was less condensed in the presence of ARID4B. Collectively, these results suggest that reciprocal binding of ARID4B and histone H1 to the PIK3CA and PIK3R2 promoters modulates chromatin condensation. Binding of ARID4B excludes histone H1 from the PIK3CA and PIK3R2 promoters, leading to relaxed chromatin architecture and gene activation (Fig. 2f). Upon ARID4B ablation, histone H1 binds to the promoters and condenses the chromatin structure to suppress gene expression (Fig. 2f). Together, these results suggest a mechanism by which ARID4B activates transcription of PIK3CA and PIK3R2.

ARID4B is required for PTEN-deficient prostate cancer. As a tumor suppressor, PTEN counters the actions of PI3K via its lipid phosphatase activity to govern cellular processes, including cell growth, proliferation, and cell migration. PTEN is frequently disrupted in prostate cancer. Loss of PTEN function leads to derepression of the PI3K-AKT pathway that dictates tumor susceptibility and favors cancer progression. To determine the functional significance of our findings that ARID4B is a transcriptional activator of the PI3K subunits PIK3CA and PIK3R2 (Table 1 and Fig. 1a, b), we investigated the role of ARID4B in the development of prostate cancer harboring PTEN deficiency. By analyzing the Cancer Genome Atlas (TCGA) and other prostate cancer genomic datasets, we identified that ARID4B is consistently retained in prostate carcinoma cohorts with deep deletion of PTEN, although deletion of ARID4B also occasionally occurs in prostate cancer (Fig. 3a).

Because there tends to be mutual exclusivity between ARID4B and PTEN deletions in prostate cancer genome (Supplementary Table 3), ARID4B might be a synthetic-essential gene for prostate tumorigenesis in the context of PTEN deficiency. Therefore, we examined whether inhibition of ARID4B impairs the tumor-promoting actions in prostate cancer cells driven by loss of PTEN. In PC3 cells, knockout of ARID4B not only efficiently constrained cell growth (Fig. 3b), but also significantly suppressed aggressiveness of cancer cells, including cell migration, cell invasion, and tumorsphere formation (Fig. 3c). In addition, ablation of ARID4B sufficiently inhibited cell proliferation and tumor formation in tumor xenograft experiments (Fig. 3d, e). On the other hand, knockout of ARID4B in the PTEN-intact prostate cancer cell line DU145 only moderately suppressed tumor growth and cell proliferation, and not to the same extent as in PC3 cells (Supplementary Fig. 6). Together, these results indicate that ARID4B is required for tumor growth of PTEN-null prostate cancer cells.

Knockout of Arid4b compromises prostate cancer progression. To demonstrate the effects of ARID4B ablation on prostate tumorigenesis driven by PTEN deficiency in mice, we used the well-characterized prostate cancer mouse model that carries prostate-specific deletion of Pten (PtenPC−/−), and generated mice carrying prostate-specific deletions of both Pten and Arid4b (PtenPC−/−Arid4bPC−/−) (Supplementary Fig. 7a). While deletion of Pten resulted in enlarged prostates in the PtenPC−/− mice, this effect was reduced in the PtenPC−/−Arid4bPC−/− mice (Fig. 4a and Supplementary Fig. 7b). The PtenPC−/− mice develop low-grade prostatic intraepithelial neoplasia (PIN) that may progress to high-grade PIN or adenocarcinoma, which faithfully mimics prostate cancer progression in humans. Consistent with the earlier report, we showed that most PtenPC−/− mice developed high-grade PIN or adenocarcinoma at 5 months of age (Fig. 4b, c and Supplementary Fig. 7c). In contrast, tumor development in prostates of most of the 5-month-old PtenPC−/−Arid4bPC−/− mice was arrested at the normal, hyperplastic, or low-grade PIN stages (Fig. 4b, c and Supplementary Fig. 7c), suggesting that ablation of ARID4B attenuated prostate cancer progression. Correspondingly, there were fewer Ki67-labeled nuclei (a marker of proliferation) in prostates of the PtenPC−/−Arid4bPC−/− mice (Fig. 4d), suggesting that ablation of ARID4B compromised the proliferation advantage elicited by loss of PTEN. These effects were not due to defects in prostate development since the Arid4bPC−/− mice had prostates of normal size and histology (Supplementary Fig. 8a, b).

Consistent with the results from PC3 cells with ablation of ARID4B (Fig. 1d), expression or phosphorylation of the core regulators and downstream effectors of the PI3K-AKT pathway were reduced in prostates of PtenPC−/−Arid4bPC−/− mice (Fig. 4e), suggesting a mechanism by which ARID4B is required for executing actions that promote prostate cancer in the context of PTEN deficiency.

To analyze whether ablation of ARID4B delays the onset of prostate cancer caused by PTEN deficiency, we then examined the PtenPC−/−Arid4bPC−/− mice at 9 months of age. While the PtenPC−/− mice exhibited enlarged prostates, prostate size was still comparable in the PtenPC−/−Arid4bPC−/− and control mice.
(Supplementary Fig. 9a). Similar to the results from 5-month-old mice, only normal/hyperplasia and PIN were found in the Pten<sup>PC<sup>-/-</sup>Arid4b<sup>PC<sup>-/-</sup> mice (Supplementary Fig. 9b, c). These results suggest that in the Pten<sup>PC<sup>-/-</sup>Arid4b<sup>PC<sup>-/-</sup> mice, PIN did not progress to adenocarcinoma over time. Collectively, these data demonstrate a synthetic essentiality of ARID4B in prostate cancer elicited by PTEN deficiency.

**PTEN deficiency increases expression of ARID4B.** To better understand the inner workings of the synthetic-essential
relationship between ARID4B and PTEN, we queried the dataset of transcriptome comparison between wild-type and Pten-/- prostates at 15 weeks of age36, and found that expression of Arid4b was elevated in prostates of the Pten-/- mice (fold change: 4.859; P = 0.0094; statistical analysis: t test). We confirmed the increase of Arid4b mRNA in the Pten-/- prostates by qRT-PCR analysis (Fig. 5a). Immunohistochemistry and western blot analyses verified a robust increase of ARID4B protein levels in prostates of the Pten-/- mice (Fig. 5b, c). Consistent with these results, expression of ARID4B was significantly increased in prostate cancer patients harboring PTEN deletion compared to those with unaltered PTEN in the TCGA provisional dataset (Fig. 5d). These results suggest that deficiency of PTEN increases expression of ARID4B.

On the other hand, re-expression of PTEN in PC3 cells (PTEN-deficient) decreased expression of ARID4B (Fig. 5e), accompanied by lessened abundance of p110α and p85β (encoded by PIK3CA and PIK3R2, respectively) and inactivation of the PI3K-AKT signaling pathway (Fig. 5e and Supplementary Fig. 10a). Furthermore, ARID4B promoter-driven luciferase reporter gene assays showed that knockdown of PTEN activated the ARID4B promoter in the PTEN-intact prostate cancer cell line DU145 and cervical cancer cell line HeLa (Fig. 5f and Supplementary Fig. 10b), whereas overexpression of PTEN suppressed the ARID4B promoter activity (Fig. 5g). Together, these results suggest that PTEN negatively regulates expression of ARID4B.

PTEN lipid phosphatase activity dephosphorylates PIP3 into PIP2 to negatively regulate the PI3K signaling cascade. Thus, loss of PTEN promotes PI3K-AKT signaling67. To demonstrate that PTEN deficiency correlates with increased ARID4B expression via activation of the PI3K-AKT pathway, we showed that knockdown of AKT in the PTEN-deficient prostate cell line PC3 decreased expression of ARID4B (Fig. 5h). Consistent with this result, adding an AKT inhibitor MK-2206 downregulated ARID4B in PC3 cells (Fig. 5i). Furthermore, the ARID4B promoter-driven luciferase reporter gene assay showed that a constitutively active form of AKT (CA-AKT) activated the promoter of ARID4B (Fig. 5j). These results indicate that AKT positively regulates expression of ARID4B. Together with the fact that ARID4B is required for activation of the PI3K-AKT signaling pathway (Fig. 1), our results suggest an AKT-ARID4B-positive feedback loop (Fig. 5k).

**Functional interactions between PTEN, ARID4B, and PIK3CA.** To investigate the clinical relevance of the PTEN-ARID4B-PI3K axis in prostate cancer, we performed immunohistochemical staining with antibodies against ARID4B, PTEN, and p110α on tissue microarrays (TMAs) consisting of 118 prostate adenocarcinoma samples from 85 patients and 73 prostate hyperplasia samples from 36 patients. Results revealed that expression of ARID4B was higher in prostate adenocarcinoma compared to prostate hyperplasia (Fig. 6a, left), shown as an increase in the number of ARID4B-positive cells in prostate adenocarcinoma samples (Fig. 6c, left and Supplementary Fig. 11a, left). This is consistent with results from our Oncomine database analysis, showing increased mRNA levels of ARID4B in human prostate carcinoma compared to normal prostate glands (Supplementary Fig. 1b). In prostate adenocarcinoma, PTEN-positive cells were diminished (Fig. 6a, c, middle and Supplementary Fig. 11a, middle), whereas the number of p110α-positive cells was increased (Fig. 6a, c, right and Supplementary Fig. 11a, right). Furthermore, expression of ARID4B and PTEN was negatively correlated in human prostate adenocarcinoma (Fig. 6b, c and Supplementary Fig. 11a), while expression of ARID4B and p110α was positively correlated (Fig. 6b, c and Supplementary Fig. 11a).

To complement our TMA results, we determined how expression of these three genes correlates with prostate cancer recurrence in patients. A multivariate Cox proportional hazards model was used to combine expression levels of PTEN, ARID4B, and PIK3CA with time to tumor recurrence from the dataset GSE4072228 into an integrated risk score model. We found that the PTEN/ARID4B/PIK3CA three-gene signature had an increased predictive power for prostate cancer recurrence compared to any individual gene alone or two genes combined (Table 2 and Supplementary Table 4). This three-gene signature reliably stratified patients into high- and low-risk groups for tumor recurrence (Fig. 6d). The enhanced prognostic value of the three-gene signature for recurrent prostate cancer was similarly confirmed by analysis of another two independent datasets GSE21032 and TCGA provisional (Supplementary Tables 5, 6 and Supplementary Fig. 11b, c). Together, these data support the importance of functional interactions between PTEN, ARID4B, and PIK3CA in human prostate cancer.

**Discussion**

Although ARID4B does not have intrinsic methyltransferase or acetyltransferase activities, it contains a Tudor domain and a chromo domain11. These two domains may promote assembly of chromatin remodeling complexes13. ARID4B is a component of the SIN3A/HDAC1 repressor complex that inhibits expression of target genes12. In this report, we showed that ARID4B was recruited to the promoters of PIK3CA and PIK3R2 and activated their expression. These results are consistent with our previous findings that ARID4B functions as coactivator (not a co-repressor) for androgen receptor in the testis and is essential for testis development37.

In addition, while knockdown of ARID4B decreased expression of p110α and p85β in the prostate cancer cell lines PC3, DU145, and LNCaP (Fig. 1d and Supplementary Fig. 3), knockdown of HDAC1 increased expression of p110α and p85β (Supplementary Fig. 12). These results indicate that ARID4B and HDAC1 have opposite effects on regulation of PIK3CA and PIK3R2 expression. As a repressor, HDAC1 deacytelates histones H4 and H3 to inhibit transcriptionally active states of the gene promoters. While HDAC1 suppresses expression of PIK3CA and PIK3R2, ablation of ARID4B in PC3 cells did not affect levels of H4.
acetylation on the promoters of PIK3CA and PIK3R2 (Supplementary Fig. 5a), suggesting that ARID4B does not mediate HDAC1 activity on these two promoters. Together, our results suggest that ARID4B and HDAC1 are likely to function independently to regulate PIK3CA and PIK3R2 in prostate cancer cells. Therefore, the SIN3A/HDAC1 repressor complex does not contribute to activation of PIK3CA and PIK3R2 by ARID4B. The function of ARID4B in transcriptional regulation may depend on the gene promoter context and the proteins that associate with ARID4B, which could dictate its function as an activator.
Fig. 3 ARID4B is required for tumor growth of PTEN-deficient prostate cancer. a Genomic alterations of ARID4B and PTEN in prostate adenocarcinoma from five prostate cancer genomic datasets. b Proliferation of control and ARID4BKO PC3 cells was measured by MTT assays. c Comparisons of cell migration, cell invasion, and tumorsphere formation between control and ARID4BKO PC3 cells. d Representative images of excised tumors (left) and measurement of tumor growth (right) from xenografts in mice injected with control or ARID4BKO PC3 cells. Data are means ± SEM. * Comparisons of cell migration (right) of immunohistochemical staining with Ki67 (cellular marker of proliferation) on excised tumors from mice injected with control or ARID4BKO PC3 cells (n = 3 for each group). Scale bar, 50 mm. Data are means ± SEM from three experiments performed in triplicate (b, c, e). ***P < 0.01 (b, d, e); **P < 0.001 (c). Statistical analysis: t test

or a repressor. Identifying ARID4B-associated proteins could provide mechanistic insight into how ARID4B regulates gene transcription.

Analysis of five prostate cancer genome datasets using the cBioportal data source (http://www.cbioportal.org/) suggest that ARID4B and PTEN deletions tend to be mutually exclusive in the prostate cancer genome, although the relationship did not reach statistical significance (P > 0.05) (Supplementary Table 3). In most cohorts, the ARID4B gene alteration rate is less than 5%, which makes it difficult to accrue enough samples for a significant P value. Similar to our findings, others have reported a tendency of mutual exclusivity between CHD1 and PTEN deletions in prostate cancer without reaching statistical significance. In addition, although the mutual exclusivity between ARID1B and KRA8 is not statistically significant in any individual type of cancer, statistical significance for mutual exclusivity was achieved when multiple cancer types were combined to increase the sample size. The tendency of ARID4B-PTEN to be mutually exclusive...
Fig. 5 PTEN deficiency increases expression of ARID4B via an AKT-ARID4B feedback loop. a Levels of Arid4b mRNA in anterior prostate from 3-month-old control and PtenPC−/− mice were measured by qRT-PCR analysis. b, c Levels of ARID4B, p110α, p85β, HSP70, and actin in control and PtenPC−/− anterior prostate at 3 months of age were determined by western blot analysis (b) or immunohistochemical staining (c). Scale bar, 50 μm. d Relative ARID4B expression levels in human prostate cancers with unaltered or deleted PTEN from the TCGA dataset (provisional). Data are means ± SEM. e Levels of PTEN, ARID4B, p110α, p85β, and actin in PC3 cells with or without PTEN re-expression were determined by western blot analysis. f, g Luciferase reporter assays using the ARID4B promoter-driven luciferase reporter were performed in DU145 cells with or without knockdown of PTEN by two different short interfering RNAs SiPTEN 1 or SiPTEN 2 (f), and in HeLa cells with or without PTEN overexpression (g). h, i Western blot analyses determined the levels of ARID4B and AKT in PC3 cells with or without knockdown of AKT by short interfering RNAs SiAKT 1 or SiAKT 2 (h), or in PC3 cells untreated or treated with an AKT inhibitor MK-2206 (i). j Luciferase reporter assay using the ARID4B promoter-driven luciferase reporter was performed in HeLa cells with or without overexpression of CA-AKT (constitutively active AKT). Data are means ± SEM from three experiments performed in triplicate (a, f, g, j). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; Statistical analysis: t test (a, d, f, g, j). k Genetically engineered mouse models demonstrate that ARID4B is a key factor in the PTEN/PI3K pathway to promote tumorigenesis in prostate with PTEN deficiency. In normal prostate (left), PTEN opposes the PI3K/AKT signaling pathway and constrains prostate tumorigenesis. In PtenPC−/− mice (middle), PTEN ablation results in activation of the PI3K/AKT pathway and the AKT-ARID4B positive feedback loop, leading to prostate tumorigenesis. In PtenPC−/− Arid4bPC−/− mice (right), knockout of ARID4B suppresses PI3K expression to compromise activation of the PI3K/AKT pathway, thus preventing prostate tumorigenesis elicited by PTEN deficiency.
led us to further evaluate the relationship between these two genes. Extensive in vitro and in vivo data, including those from depletion of ARID4B in the PTEN-deficient prostate cancer cell line PC3 and the prostate-specific Arid4b and Pten double knockout mouse model, demonstrated that PTEN-deficient prostate cancer depends on ARID4B. In both PC3 and DU145 cells, depletion of ARID4B resulted in reduced expression or phosphorylation of the core regulators and downstream effectors of the PI3K-AKT pathway, while expression of PTEN in DU145 cells was unchanged (Supplementary Fig. 3a). We showed that depletion of ARID4B inhibited colony formation in PC3 and DU145 cells (Supplementary Fig. 13a, b), and re-expression of ARID4B in the ARID4B KO PC3 and ARID4BKO DU145 cells increased colony formation (Supplementary Fig. 13c). Depletion of ARID4B in another PTEN-deficient prostate cancer cell line, LNCaP, significantly suppressed cell growth (Supplementary Fig. 14). In the PC3 (PTEN-deficient) cells, knockout of ARID4B efficiently inhibited cancer cell growth and tumor formation in xenografts, suggesting that PTEN-deficient prostate cancer cells depend on ARID4B. In the DU145 (PTEN-intact) cells, knockout of ARID4B also suppressed cell growth and tumor formation in xenografts, but less than in PC3 cells. PTEN-deficient PC3 cells may depend more on activation of the PI3K-AKT pathway for tumor growth than the PTEN-intact DU145 cells. This would explain why the AKT inhibitor MK-2206 inhibited cell growth more effectively in PC3 cells than

**Fig. 6** Prognostic potential of the PTEN/ARID4B/PIK3CA signature in human prostate cancer. a Expression of ARID4B, PTEN, and p110α between human prostate hyperplasia and prostate adenocarcinoma in tumor microarrays was compared. Seventy-three prostate hyperplasia samples from 36 patients and 118 prostate adenocarcinoma samples from 85 patients were analyzed. The expression levels of ARID4B, PTEN, and p110α were presented as the numbers of positive cells (×1000) per mm². b Regression plots show expression correlations of ARID4B with PTEN and p110α in prostate adenocarcinoma from tumor microarray results. In all, 118 prostate adenocarcinoma samples from 85 patients were analyzed. The expression levels of ARID4B, PTEN, and p110α were presented as the numbers of positive cells (×1,000) per mm². c Representative immunohistochemical staining of ARID4B, PTEN, and p110α from one patient with prostate hyperplasia (top) and one with prostate adenocarcinoma (bottom) in tumor microarrays. Scale bar, 200 μm. d Kaplan–Meier plot of prostate cancer recurrence-free survival in patients stratified by the PTEN/ARID4B/PIK3CA signature. Data are from the GSE40272 dataset. Statistical analysis: log-rank test.
DU145 cells (Supplementary Fig. 15a, b). In PTEN-deficient PC3 cells, ablation of PTEN activates the PI3K/AKT pathway, leading to tumorigenesis (Supplementary Fig. 15c, left). In ARID4BK0 PC3 cells, knockout of ARID4B compromises activation of the PI3K/AKT pathway, thus efficiently inhibiting tumorigenesis elicited by PTEN deficiency (Supplementary Fig. 15c, right). In PTEN-intact DU145 cells, PTEN dephosphorylates PI3P into PI2P, which opposes the PI3K/AKT signaling pathway (Supplementary Fig. 15d, left), suggesting tumorigenesis of DU145 cells is less dependent on the PI3K-AKT signaling than PC3 cells. Therefore, knockout of ARID4B that inactivates PI3K/AKT pathway only moderately suppresses tumorigenesis of DU145 cells (Supplementary Fig. 15d, right).

In addition, ARID4B may promote cancer cell activity through additional pathways unrelated to PTEN tumor suppressor function. For example, our immunohistochemical analyses detected increased numbers of cells positive for cleaved caspase 3, an apoptosis marker, in prostate of the Ptenfl/fl−/−Arid4bPC−/− mice compared to the Ptenfl/fl−/− mice, suggesting that ablation of ARID4B promoted apoptosis (Supplementary Fig. 16a, b). Consistent with this result, expression levels of the apoptosis regulators p53 and Fas were elevated in prostate of the Ptenfl/fl−/−Arid4bPC−/− mice compared to the Ptenfl/fl−/− mice (Supplementary Fig. 16c). For its oncogenic function, ARID4B might directly or indirectly inhibit the apoptosis pathway mediated by the p53 tumor suppressor.

Similar to the Pten/Arid4b/Pik3ca three-gene signature, PTEN together with SMAD, CCND1, and SPP1 form a four-gene signature that predicts recurrence and lethal metastasis in human prostate cancer.36 SMA4D is a key regulator of the TGFβ pathway and a suppressor of prostate tumor progression, whereas cyclin D1 and SPP1 act as mediators of the prostate cancer processes. Inactivation of PTEN and SMA4D and activation of cyclin D1 and SPP1 promote prostate cancer. The combination of PTEN/ SMA4D does not predict risk for prostate cancer recurrence, but the PTEN/SMA4D/CCND1/SPP1 four-gene signature does. This four-gene model distinguished patient groups at high versus low risk for prostate cancer recurrence by Kaplan–Meier analysis [hazard ratio (HR) = 2.6, P = 0.012].36 In addition, the PTEN/SMA4D/CCND1/SPP1 four-gene signature involves the PTEN and TGFβ pathways, which converges to regulate prostate cancer progression.36 Similarly, we showed that the PTEN/ARID4B/PIK3CA signature has greater predictive power for tumor recurrence than any individual gene alone or two genes combined. This PTEN/ARID4B/PIK3CA signature robustly stratified patients into those at high versus low risk for tumor recurrence (HR = 4.58, P = 0.006 in the GSE40272 dataset; HR = 3.23, P = 0.002 in the GSE21032 dataset; HR = 1.51, P = 0.025 in the TCGA dataset; Statistical analysis: log-rank test). Thus, we identified that the PTEN-ARID4B-PI3K regulatory axis underlies the predictive power of the PTEN/ARID4B/PIK3CA signature for tumor recurrence in prostate cancer patients.

In this study, our data suggest that ARID4B, by directly impinging on the PTEN-PI3K signaling pathway, functions as a synthetic–essential effector for prostate cancer initiation and progression elicited by loss of PTEN. Our study reveals the PTEN-ARID4B-PI3K axis and an AKT-ARID4B-positive feedback loop whereby PTEN antagonizes the PI3K-AKT signaling pathway. In normal prostate, PTEN is attributed to its lipid phosphatase activity that directly counters PI3K actions, whereas ARID4B is a transcriptional activator of the PI3K subunit genes PIK3CA and PIK3R2, and expression of ARID4B itself is regulated by the AKT-ARID4B-positive feedback loop (Fig. 5k, left). Deficiency of PTEN activates the PI3K-AKT pathway and the AKT-ARID4B-positive feedback loop, leading to prostate tumorigenesis (Fig. 5k, middle). In contrast, ablation of ARID4B inhibits the PI3K-AKT pathway, and compromises cancer progression in prostate harboring PTEN deficiency (Fig. 5k, right). Therefore, our study demonstrates the synthetic–essential nature of ARID4B in PTEN-deficient prostate cancer and provides the underlying mechanism by which ARID4B is required for executing prostate cancer-promoting actions in the context of PTEN deficiency. Together, our findings reveal insights into how PTEN controls prostate tumorigenesis, and support the concept that ARID4B is a potential target for interventions in patients with prostate cancer carrying PTEN mutations.

### Methods

**Mice.** Mice with prostate-specific deletion of Pten (Ptenfl/fl−/−) contain the Pb-Cre (the Cre gene under control of the prostate-specific probasin promoter)39 and conditional knockout Ptenlox/lox−/− mice. The Ptenlox/lox−/− mice were obtained from Dr. Hong Wu at the University of California Los Angeles. The Pb-Cre transgenic mice were obtained from Dr. Fen Wang at the Institute of Bioscience and Technology, Texas A&M Health Science Center. The Ptenlox/lox−/− mice and Pb-Cre mice were backcrossed to generate the Ptenfl/fl−/− mice. The Ptenfl/fl−/− mice were further backcrossed with the Arid4bfl/fl−/− mice to generate the Ptenfl/fl−/−Arid4bfl/fl−/− mice that carry prostate-specific deletions of Pten and Arid4b containing the Pb-Cre39, Ptenlox/lox−/−, and Arid4bfl/fl−/− alleles. The Arid4bfl/fl−/− mice were previously generated by our laboratory.37 We have complied with all relevant ethical regulations for animal research. Protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the George Washington University (protocol number: A315). All mice were bred and maintained at the institution’s specific pathogen-free mouse facility. The facility is approved by the American Association for Accreditation of Laboratory Animal Care and operated in accordance with current regulations and standards of the US Department of Agriculture and the Department of Health and Human Services.

**Histology and immunohistochemistry.** The prostates were dissected from mice and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 24 h, processed, and embedded in paraffin. Histological analysis was performed on 5-μm-thick paraffin-embedded prostate sections in citric acid-based antigen unmasking solution (Vector Laboratories). Samples were blocked with blocking solution (5% donkey serum, 2% bovine serum albumin, and 0.02% Triton-X 100 in PBS), followed by incubation with primary antibodies in blocking solution. The primary antibodies used are anti-ARID4B (A302-233A, Bethyl Laboratories; dilution 1:1000), anti-PRC1021 and PRD961, included 118 prostate adenocarcinoma cores from 85 patients, 73 prostate hyperplasia cores from 36 patients, and 5 benign tumor cores. These TMA slides were baked at 60 °C for 30 min and then deparaffinized. After antigen retrieval, slides were incubated with primary antibodies: anti-ARID4B (A302-233A, Bethyl Laboratories; dilution 1:400), anti-PTEN (A2B1; sc-7974,

**Immunohistochemical staining of tissue microarrays (TMAs).** Immunohistochemical staining of two TMAs (PRC1021 and PRD961) and image scanning services were provided by Pantomics (Pantomics, Inc. USA). Two TMA slides, PRC1021 and PRD961, included 118 prostate adenocarcinoma cores from 85 patients, 73 prostate hyperplasia cores from 36 patients, and 5 benign tumor cores. These TMA slides were baked at 60 °C for 30 min and then deparaffinized. After antigen retrieval, slides were incubated with primary antibodies: anti-ARID4B (A302-233A, Bethyl Laboratories; dilution 1:400), anti-PTEN (A2B1; sc-7974,
Santa Cruz Biotechnology; dilution 1:100, and anti-p110α (C73F8; 4249, Cell Signaling Technology; dilution 1:100). Signal detection was carried out using DAB (3,3′-diaminobenzidine) substrate. Slides were counterstained with hematoxylin. The scanned images were analyzed with QuPath (v0.1.2).40 Expression of ARID4B, PTEN, and benzidine substrate. Slides were counterstained with hematoxylin. The scanned

**Western blot analysis.** PC3 and DU145 cells were lysed in buffer [20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5% Nonidet P-40, 2 mM EDTA, protease inhibitors [Complete Protease Inhibitor Cocktail, Roche], and phosphatase inhibitor mixture (PhosSTOP®), Roche] for 30 min, after centrifugation at 13,400 × g for 20 min at 4 °C to clear debris. Proteases were lysed in RIPA buffer [25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, protease inhibitor mixture (Complete Protease Inhibitor Cocktail, Roche), and phosphatase inhibitor mixture (PhosSTOP®, Roche)]. For western blot analysis, the samples were resolved by SDS/PAGE and transferred to nitrocellulose membranes (Bio-Rad). After blocking with 5% milk in TBST buffer [20 mM Tris-HCl, 150 mM NaCl (pH 7.5), 0.1% Tween 20], the primary antibodies diluted in TBST buffer with 5% milk were added to the membranes overnight at 4 °C, followed by incubation with the appropriate secondary horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch; dilution 1: 5000) for 1 h at room temperature. Signal detection was carried out by SuperSignal substrates (Supersignal West Pico Plus Luminol/Enhancer Solution, Thermo Scientific). Subsequent probing with different antibodies was made possible by stripping the membranes (20 min in 2× SDS/6 M urea/100 mM Tris, pH 6.8, 2× SDS, 100 mM β-mercaptoethanol) at 55 °C for 30 min. The primary antibodies used for western blotting were: anti-ARID4B (A302-233A, Bethyl Laboratories; dilution 1:2000), anti-PTEN (ab26053, Abcam; dilution 1:100), anti-H3K27Ac antibody (ab4729, Abcam; dilution 1:100), and anti-H3K4me3 antibody (07-473, EMD Millipore; dilution 1:100) antibodies. DNA from immunoprecipitated chromatin was analyzed by qPCR using the following primer sets: PIK3CA (PCR product 1)

**PIK3CA-PF1: 5′-CAGGAGCCTGACCATGCTC-3′
PIK3CA-PRI: 5′-GTGCTAACCAGGTGGTATTG-3′
PIK3CA (PCR product 2)

**PIK3CA-PF2: 5′-CTCTCACTGCGACAGGGCGG-3′
PIK3CA-PRI2: 5′-GCCATCTCTCAACCTACCCAG-3′
PIK3R2 (PCR product 3)

**PIK3R2-PF1: 5′-CTGTTTTGCTGACCTGGAACGC-3′
PIK3R2-PRI: 5′-GAATAAGGCGCAAcTCTTCTACG-3′
PIK3R2 (PCR product 2)

**PIK3R2-PF2: 5′-GTCAAGATACCCGAGCTTGGAC-3′
PIK3R2-PRI2: 5′-GTCACACAGACAGGCGACCTG-3′
PIK3R2 (PCR product 5)

**MYOD1 (PCR product 3)

**MYOD1-PF1: 5′-GTTCTATTGGCTCTGGACT-3′
MYOD1-PRI: 5′-CCCGCTTGTAGTACGAAACG-3′
GAPDH

**GAPDH-PF1: 5′-GGGCTCATAAGGGTTTAC-3′
GAPDH-PRI: 5′-GTCGAACAGAGGACGACAG-3′
ARID4B

**ARID4B-PF1: 5′-GACAAATGAAGGACGTATAGG-3′
ARID4B-PRI: 5′-GACTGCAGTCAGGGCCGATAC-3′

**Micrococcal nuclease (MNase) sensitivity assay.** Micrococcal nuclease assay was performed as described.37 Briefly, control and ARID4BKO PC3 cells were recovered in ice-cold lysis buffer (10 mM Tris-HCl (pH 7.4), 10 mM KCl, 3 mM MgCl2, 0.5% Nonidet P-40, 0.15 mM spermine and 0.5 mM spermidine), and the nuclei were pelleted. Nuclei were washed with MNase digestion buffer [10 mM Tris-HCl, 15 mM NaCl, 60 mM KCl, 0.15 mM spermine and 0.5 mM spermidine] and after centrifugation further resuspended in MNase digestion buffer containing 2 mM CaCl2. From the resuspended solutions, 100 µl aliquots were taken and incubated with or without MNase (100 units) (New England Biolabs) for 5 min. The reaction was stopped by adding 80 µl of MNase digestion buffer, 20 µl of MNase stop buffer (100 mM EDTA, 10 mM EGTA), and 3 µl of proteinase K (25 mg/ml) (Roche), and 10 µl of 20% SDS, and then incubated overnight at 37 °C. This was followed by phenol–chloroform extraction and ethanol pre-puriﬁcation. The puriﬁed DNA was subjected to qPCR analysis using the primer sets described for ChIP-qPCR analysis.

**Cell lines and culture conditions.** All cell lines were obtained from ATCC. PC3 and DU145 cells were cultured in F-12k medium and Eagle’s minimum essential medium supplemented with fetal bovine serum (FBS) to a ﬁnal concentration of 10%, and transfected. PC3 and DU145 cells were cultured in Dulbecco’s modiﬁed Eagle’s medium (DMEM/F12) (4:1) supplemented with heat-inactivated FBS (10%) and T-medium supplement (to 1x). To prepare 10×T-medium supplement in 100 ml 1% BSA (Sigma cat# A8022) in PBS, the following components were added: 50 mg insulin (Gibco cat# 12585-014), 136 ng triiodo-L-thyronine (Sigma cat# T2877), 50 mg transferrin (Sigma cat# T4382), 2.5 mg D-Biotin (Sigma cat# 47868), 250 mg adenine (Sigma cat# A3159), RWPE-1 and PZ-PV7-7 cells were maintained in keratinocyte serum free medium (K-SFM, Gibco 17005-042), supplemented with 0.05 mg/ml of bovine pituitary extract and 5 ng/ml of human recombinant epidermal growth factor. HPRC cells were maintained in prostate epithelial cell basal medium (ATCC, PCS-040-030), supplemented with prostate epithelial cell growth kit (ATCC, PCS-040-040). HeLa cells were cultured in DMEM supplemented with 5% FBS.

**Plasmids.** The mammalian expression plasmid for ARID4B was described previously.45 The mammalian expression plasmids for HA-PTEN and HA-PTEN G129R were purchased from Addgene. For luciferase reporter gene assays, the promoters of human ARID4B (2324 to +125 bp), PIK3CA (~4484 to +1099 bp), and PIK3R2 (~1303 to +1324 bp) were amplified by PCR using genomic DNA, prepared from PC3 cells and cloned into the pGL3-basic vector (Promega). For PCR ampliﬁcation of the promoters, the following primers were used: ARID4B-Koxl-PF2: 5′-GCCCTGCAGAGGAGCTATACCTGCTGACAGGTC-3′
ARID4B-HindIII-PRI: 5′-CCCAAAGCTTCTAAGGGCTGATTAGTCTCGG-3′
PIK3CA-SacI-PF4: 5′-CGACGTTCCACGACCAAAAGAAGAAAGACTCC-3′
PIK3CA-BglII-PRI: 5′-GGAAGATCTCGGAGAAGGAGCTTGGAC-3′
PIK3R2-KpnI-PF2: 5′-GGGGTACCTCCGAGTCAGTGCTACATAGCC-3′
PIK3R2-Nhel-PRI: 5′-CTAGTCAGACCCCTCATCTACGAGCAGACAG-3′

**Transfection and luciferase reporter gene assay.** Plasmid transfection into DU145 cells was carried out by FuGene HD (Promega) according to the

**RNA-Seq and qRT-PCR analyses.** Total RNA was puriﬁed from control (ScrControl) and ARID4B-Knockdown cells (ScrARID4B) PC3 cells using an RNeasy plus kit (Qiagen), and was treated by DNase I (Qiagen). Puriﬁed RNA was processed for RNA-Seq and initial data analysis (Q2 Solutions). For qRT-PCR analysis, 2 µg of total RNA was treated by DNase I (Qiagen), and was treated by DNase I (Qiagen). Puriﬁed RNA was processed for RNA-Seq and initial data analysis (Q2 Solutions). For qRT-PCR analysis, 2 µg of total RNA was treated by DNase I (Qiagen), and was treated by DNase I (Qiagen). Puriﬁed RNA was processed for RNA-Seq and initial data analysis (Q2 Solutions). For qRT-PCR analysis, 2 µg of total RNA was treated by DNase I (Qiagen), and was treated by DNase I (Qiagen). Puriﬁed RNA was processed for RNA-Seq and initial data analysis (Q2 Solutions). For qRT-PCR analysis, 2 µg of total RNA was treated by DNase I (Qiagen), and was treated by DNase I (Qiagen). Puriﬁed RNA was processed for RNA-Seq and initial data analysis (Q2 Solutions). For qRT-PCR analysis, 2 µg of total RNA was treated by DNase I (Qiagen), and was treated by DNase I (Qiagen). Puriﬁed RNA was processed for RNA-Seq and initial data analysis (Q2 Solutions).
manufacturer’s instructions. Plasmid transfection into HeLa cells was accomplished using Lipofectamine 2000™ Transfection Reagent (Invitrogen) according to the manufacturer’s instructions. Forty-eight hours after transfection, whole cell lysates were prepared and luciferase activity was determined by the luciferase assay system following the manufacturer’s instructions (Promega).

Transfection of SiRNA was carried out using Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen) according to the manufacturers’ instructions. Seventy-two hours after transfection, total RNA was purified for RNA-Seq and qRT-PCR analysis, or whole cell lysates were prepared for luciferase reporter gene assays. MISSION® Predesigned siRNA targeting human ARID4B (SASI_Hs01_00134230 and SASI_Hs02_00349566) and human PTEN (SASI_Hs01_00196478 and SASI_Hs01_00196479) were purchased from Sigma.
12. Fleischer, T. C., Yun, U. J. & Ayer, D. E. Identification and characterization of three new components of the mSin3A corepressor complex. *Mol. Cell. Biol.* 23, 3456–3467 (2003).

13. Lu, R. & Wang, G. G. Tudor: a versatile family of histone methylation ‘readers’. *Trends Biochem. Sci.* 38, 546–555 (2013).

14. Nishibuchi, G. & Nakayama, J. Biochemical and structural properties of heterochromatin protein 1: understanding its role in chromatin assembly. *J. Biol. Chem.* 156, 1–20 (2014).

15. Cui, D. et al. Regression of gastric cancer by systemic injection of RNA nanoparticles carrying both ligand and siRNA. *Sci. Rep.* 5, 10726 (2015).

16. Luo, J. et al. mir-519b-3p promotes responsiveness to preoperative chemoradiotherapy in rectal cancer patients by targeting ARID4B. *Gene* 655, 84–90 (2018).

17. Tsai, W. C., Hueng, D. Y., Nieh, S. & Gao, H. W. ARID4B is a good biomarker to predict tumour behaviour and decide WHO grades in gliomas and meningiomas. *J. Clin. Pathol.* 70, 162–167 (2017).

18. Wang, R. et al. Overexpression of ARID4B predicts poor survival in patients with hepatocellular carcinoma. *Hum. Pathol.* 73, 114–121 (2018).

19. Winter, S. F., Lukes, L., Walker, R. C., Welch, D. R. & Hunter, K. W. Allelic variation and differential expression of the mSin3A histone deacetylase complex gene Arid4b promote mammary tumor growth and metastasis. *PLoS Genet.* 8, e1002735 (2012).

20. Armenia, J. et al. The long tail of oncogenic drivers in prostate cancer. *Nat. Genet.* 50, 645–651 (2018).

21. Baca, S. C. et al. Punctuated evolution of prostate cancer genomes. *Cell* 153, 666–677 (2013).

22. Beltran, H. et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat. Med.* 22, 298–305 (2016).

23. Grasso, C. S. et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature* 487, 239–243 (2012).

24. Kumar, A. et al. Substantial intradividual and limited intratradividual genomic diversity among tumors from men with metastatic prostate cancer. *Nat. Med.* 22, 369–378 (2016).

25. Robinson, D. et al. Integrative clinical genomics of advanced prostate cancer. *Cell* 161, 1215–1228 (2015).

26. Tomlins, S. A. et al. Integrative molecular concept modeling of prostate cancer progression. *Nat. Genet.* 39, 41–51 (2007).

27. Glinsky, G. V., Glinsky, A. B., Stephenson, A. J., Hoffman, R. M. & Gerald, W. L. Gene expression profiling predicts clinical outcome of prostate cancer. *J. Clin. Invest.* 113, 913–923 (2004).

28. Guzlar, Z. G., McKenney, J. K. & Brooks, J. D. Increased expression of NuSAP in recurrent prostate cancer is mediated by EZF1. *Oncogene* 32, 70–77 (2013).

29. Chan, C. H. et al. Posttranslational regulation of Akt in human cancer. *Cell Biosci.* 4, 59 (2014).

30. Nave, B. T., Ouwens, M., Withers, D. J., Alessi, D. R. & Shepherd, P. R. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino acid deficiency on protein translation. *Biochem. J.* 344 (Pt 2), 427–431 (1999).

31. Sekulic, A. et al. A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res.* 60, 3504–3513 (2000).

32. Krishnakumar, R. et al. Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes. *Science* 319, 819–821 (2008).

33. Hergeth, S. P. & Schneider, R. The H1 linker histones: multifunctional proteins beyond the nucleosomal core particle. *EMBO Rep.* 16, 1439–1453 (2015).

34. Cancer Genome Atlas Research N. The molecular taxonomy of primary prostate cancer. *Cell* 163, 1011–1025 (2015).

35. Wang, S. et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell* 4, 209–221 (2003).

36. Ding, Z. et al. SMAD4-dependent barrier constrains prostate cancer growth and metastatic progression. *Nature* 470, 269–273 (2011).

37. Wu, R. C., Zeng, Y., Pan, I. W. & Wu, M. Y. Androgen receptor coactivator ARID4B is required for the function of Sertoli cells in spermatogenesis. *Mol. Endocrinol.* 29, 1334–1346 (2015).

38. Kim, Y. A., Cho, D. Y., Dao, P. & Przytycka, T. M. MEMCover: integrated analysis of mutual exclusivity and functional network reveals dysregulated pathways across multiple cancer types. *Bioinformatics* 31, i284–i292 (2015).

39. Jin, C., McKeohan, K. & Wang, F. Transgenic mouse with high Cre recombinase activity in all prostate lobes, seminal vesicle, and ductus deferens. *Prostate* 57, 160–164 (2003).

40. Bankhead, P. et al. QuPath: open source software for digital pathology image analysis. *Sci. Rep.* 7, 16878 (2017).

41. Kumar, S. et al. NCOA3-mediated upregulation of mucin expression via transcriptional and post-translational changes during the development of pancreatic cancer. *Oncogene* 34, 4879–4889 (2015).

42. Wu, M. Y., Tsai, T. F. & Beaudet, A. L. Deficiency of Rbbp1/Arid4a and Rbbp11/Arid4b alters epigenetic modifications and suppresses an imprinting defect in the PWS/AS domain. *Genes Dev.* 20, 2859–2870 (2006).

43. Patel, S. R. & Dressler, G. R. The genetics and epigenetics of kidney development. *Semin. Nephrol.* 33, 314–326 (2013).

44. Cerami, E. et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2, 401–404 (2012).

45. Gao, J. et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* 6, p11 (2013).

46. Goswami, C. P. & Nakshatri, H. PROGeneV2: enhancements on the existing database. *Bmc Cancer* 14, 970 (2014).

47. Aguirre-Gamboa, R. et al. SurvExpress: an online biomarker validation tool and database for cancer gene expression data using survival analysis. *PLoS ONE* 8, e74250 (2013).

Acknowledgements

We thank Drs. Sophia Y. Tsai (Baylor College of Medicine) and Lopa Mishra (George Washington University) for comments and critical reading of the manuscript. This work was supported by grants R01CA188471 and R21CA187857 to R.-C.W. from the National Institutes of Health, and Katzen Cancer Research Center to R.-C.W., McCormick Genomic and Proteomic Center to R.-C.W. and M.-Y.W., and a startup fund to M.-Y.W. from George Washington University.

Author contributions

M.-Y.W., R.-C.W., and I.-C.Y. performed most of the experiments. I.-C.Y. and M.-Y.W. performed bioinformatics analyses from human prostate cancer datasets. Y.-F.C. performed a plasmid cloning experiment. S.-T.C. performed experiments on mouse samples. S.-T.C. performed a plasmid cloning experiment. A.T. analyzed histological data. M.-Y.W. and R.-C.W. provided guidance with experimental design and wrote the manuscript.

Additional information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41467-019-12184-8.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Peer review information *Nature Communications* thanks the anonymous reviewer(s) for their contribution to the peer review of this paper. Peer review reports are available.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.