TNIK swaths AR to WNT pathway and drives Castration-Resistant Prostate Cancer

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Abstract

Background: The development of CRPCa was driven by complex genetic and epigenetic mechanisms that remained poorly understood. TNIK (Traf2 and Nck-interacting kinase) has been reported to be a serine/threonine kinase and associated with tumor cell proliferation or unfavorable cancer behavior. The present study was conducted to investigate the TNIK gene expressions in CRPCa.

Methods: Using a microarray approach, we identified higher expression of TNIK in CRPCa. The interaction between AR and H3K27me3 upon TNIK depression was determined through molecular and cell biological methods. Co-immunoprecipitations assays were Performed to confirm that TNIK interacted and phosphorylated with β-catenin in CRPCa cell.

Results: Specifically we found AR repressed TNIK gene transcription via forming complex with H3K27me3. TNIK was recruited to promote transcription of Wnt target genes in a β-catenin-dependent manner in C4-2 cells. In vitro binding showed that TNIK directly band and phosphorylated β-catenin. Depletion or mutant of TNIK kinase abrogated β-catenin transcription, highlighting the essential function of TNIK kinase activity in Wnt target gene activation.

Conclusions: Our findings revealed a regulatory role of AR in TNIK repressor, TNIK interacted with β-catenin and phosphorylated activaing Wnt pathway to promote CRPCa progression. TNIK may present an attractive candidate for drug targeting in CRPCa.

Background

Castration resistant prostate cancer (CRPCa) remains leading cause in male patients deaths worldwide[1–2]. The development of CRPCa was driven by complex genetic and epigenetic mechanisms that remained poorly understood. A few studies have reported androgen-repressed genes, some hinted to indirect mechanisms involving into progression of CRPCa[3–6]. There is a urgent need to identify new targets to advance the development of CRPCa.

TNIK(Traf2 and Nck-interacting kinase) was originally identified as germinal center kinases (GCKs) and played a pivotal role in the regulation of a number of fundamental cellular actions by phosphorylating its downstream substrates[7]. TNIK was a proto-oncoprotein that was overexpressed in various types of cancer, including prostate cancer, multiple myeloma, pancreatic cancer, hepatocellular carcinoma and gastric cancer[8–15]. As a kinase, TNIK phosphorylated TCF4 and mediated the activation of Wnt target genes in colorectal cancer growth[8]. Furthermore, Tnik-deficient mice showed reduced expression of Myc and Cd44[16]. TNIK was shown to be involved into the NF-κB and SMAD signaling pathways [7, 17].

Emerging evidence has shown an important role of the Wnt signaling pathways in prostate regulatory mechanisms in prostate tumorigenesis, Wnt responsive cells appeared to possess stem/progenitor cell properties in the mouse prostate[18–20]. Castration could elevate Wnt signaling and promoted cell survival in the mouse prostate[21]. Overexpression of active β-catenin was found to be associated with

high-grade intraepithelial neoplasia and resistance to castration[22]. β-catenin was a key effector of Wnt signaling, however, a potential function for TNIK in β-catenin active regulation has never been documented.

In this study, using a genearray approach, we identified TNIK as a potential CRPCa driver gene interacting with β-catenin in CRPCa. Our evidence demonstrated that AR directly repressed TNIK transcription through the AR-H3K27me3 complex. Furthermore, following siAR or MDV3100 treatment, TNIK gene expression was switched on, TNIK interacted and phosphorylated activating β-catenin in Ser675 site. Finally, TNIK also enhanced β-catenin-mediated, PTCH1-induced CRPCa cell proliferation. Pharmacological inhibitors of TNIK (NCB-0846) inhibited the growth of CRPCa cell xenograft. This kinase may present an attractive candidate for drug targeting in CRPC.

Material And Methods

Clinical samples. Tissue samples were obtained from patients who underwent radical prostatectomy or transurethral resection of the prostate at the Tianjin Medical University Hospital (Tianjin, China) and inspected by a certified pathologist for Gleason grading.

Cell culture. The PCa cell line LNCaP, C4-2 ,22RV1 and PC3 were obtained from ATCC. Cells were maintained in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% glutamine. DHT was obtained from Amersham (Braunschweig, Germany).

siRNA. Validated siRNAs were from GenePharma (ShangHai, China). Transfections were performed using the lipo2000 (Thermo Fisher) according to the manufacturer's instructions.

Co-Immunoprecipitation and Western blotting.

LNCaP and C4-2 cells were harvested and lysed in lysis buffer (150 mM KCl,75 mM Hepes, pH 7.5, 1.5 mM EGTA, 1.5 mM MgCl2, 10% glycerol, and 0.075% NP-40 supplemented with protease inhibitor cocktail[Roche,USA]. Extract proteins were precleared using a mixture of protein A–Sepharose (CL-4B; GE Healthcare) and antibody for overnight hr at 4˚C. Immunoprecipitates were washed with lysis buffer and resuspended in sample buffer, boiled and analyzed by SDS-PAGE. Individual samples (40 μg of protein) were separated on 8% SDS polyacrylamide gel and transferred to PVDF membranes (Millipore, Billerica, MA). Membranes were blocked in a PBS-Tween 20 solution with 5% fat-free milk for 1 h at room temperature, and then the membranes were incubated with appropriate dilutions of specific primary AR or TNIK antibodies overnight at 4 °C. After washing, the blots were incubated with HRP conjugated anti-rabbit or anti-mouse IgG for 1 h. The blots were developed in ECL mixture(Vector Laboratories, Burlingame, CA) and visualized by Imager.

Chromatin Immunoprecipitation

LNCaP cells were grown in 1640 (Invitrogen) supplemented with 10% charcoal-stripped FBS (CSF, HyClone, USA) for 12 h. DNA cross-linking was performed by adding 1% formaldehyde into the cell cultures at room temperature for 10 min, and glycine was then added (0.125 M final concentration) for 5 min to stop the cross-linking reaction. Cells were lysed with a lysis buffer with protease inhibitors and sonicated to shear genomic DNA to lengths between 200 and 1000 bp. One-tenth of the cell lysate was used for input control, and the rest was used for immunoprecipitation using AR or H3K27me3 antibody. After collecting the immunoprecipitates using protein G-agarose columns, protein-DNA complexes were eluted and heated at 65 °C to reverse the cross-linking. After digestion with proteinase K, DNA fragments were purified using spin columns and analyzed using PCR for 35 cycles in a sequence of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. Specific primer sets were designed to amplify a target sequence within the human TNIK promoter as table 2. PCR products were electrophoresed in a 1.5% agarose gel with ethidium bromide and visualized under ultraviolet light.

Whole-transcript expression array and microarray image processing

One μg/μl of total RNA was used as a starting material for making total RNA/Poly-A RNA controls, and was mixed with a GeneChip®Eukaryotic Poly-A Control Kit (Affymetrix, Inc., CA, USA). The majority of the rRNA was removed from the total RNA samples prior to target labeling, so as to increase sensitivity by RiboMinusTM Human Transcriptome Isolation Kit (Invitrogen, CA, USA); cDNA was synthesized using the GeneChip® WT Sense Target Labeling and Control Reagents Kit, as per the manufacturer's instructions (Affymetrix, Inc., CA, USA). The sense cDNA was then fragmented by UDG (uracil DNA glycosylase) and APE 1 (apurinic/apyrimidinic endonuclease 1), and biotin-labeled with TdT (terminal deoxynucleotidyl transferase) using a GeneChip® WT Terminal Labeling Kit. (Affymetrix, Inc., CA, USA). After the biotin-labeled sense target DNA was prepared, the sample was ready to hybridize to gene chip (The GeneChip® Human Exon 1.0 ST array). Hybridization was performed using 5 μg of biotinylated target, which was incubated with a GeneChip® Hybridization, Wash and Stain Kit and a GeneChip® Fluidics Station 450 (Affymetrix, Inc., CA, USA). The arrays were scanned using a GeneChip® Scanner 3000 7G (Affymetrix, Inc., CA, USA). Raw data were extracted from the scanned images and analyzed with GeneSpring GX software version 11.5 (Agilent Technologies, CA, USA).

Luciferase assays.

For the dual luciferase assay, C4-2 cells were plated in triplicate into 12-well plates and cotransfected with 1 μg of the reporter construct and 15 pmol of β-catenin luciferase together with TNIK wt or D171A of plasmids by using transfection reagent (Roche). Transfected cells were cultured and 24 h later, the supernatants were collected for luciferase assay using Dual Luminescence assay kit (GeneCopoeia MD) according to the manufacturer's instructions.

Animal studies

Four-week-old male Babl/c mice (HFK Bio-Technology Co. Ltd, Beijing) were injected subcutaneously with 2×10^6 C4-2 cells suspended in 0.1 mL of Matrigel (BD Biosciences), and were implanted subcutaneously into the dorsal flank on both sides of the mice. Once the tumors reached an stage about 100 mm^3, mice administered daily by oral gavage either with vehicle (10% DMSO in PBS) or NCB-0846 (80 mg/kg of body weight) for 10 days (n = 4 mice for each treatment). Tumor volume was recorded by digital caliper san estimate using the formula LW2/2, where L = length of tumor and W = width. At the end of the studies mice were killed and tumors extracted and weighed. All procedures involving mice were approved by the University Committee on Use and Care of Animals at the Tianjin Medical University and conform to all regulatory standards. Mice permit number is SYXK(Jin)2019-0004.

Immunohistochemistry.

Tissue sections were de-waxed in xylene and rehydrated in graded alcohol. Antigen retrieval was done under pressure for 5 min in citrate buffer (pH adjusted to 6.0). Endogenous peroxidase was blocked in 0.3% hydrogen peroxide for 10 min and blocked using 1.5% horse serum. Incubation with primary antibody was done in a humidified chamber overnight at 4 °C (anti-TNIK, 1:100 from sigma; anti-Ser675 1:100 from Cell Signaling Technology; Ki67 1:100 from Abcom). After applying Poly-HRP anti-rabbit IgG (30 min), secondary antibody detection was performed using the Ultraview DAB detection kit (Zhongshan Co, China). In the case of TNIK, Ser675 and Ki67, only nuclear staining was considered as positive and was scored. All immunostained sections were evaluated under Zeiss microscope (× 200). At least 10 high power fields around each of the malignant glands were evaluated and scored.

Results

TNIK was upregulated in castration resistant prostate cancer.

Previously, with LNCaP xenografts, we established a model that castration resistant LNCaP tumors (CR-LNCaP) and androgen sensitive tumors (HS-LNCaP)[6]. In order to identify an unrecognized molecular mechanisms of CRPCa between intact and castrated mice, we measured changes in human genes mRNA levels in HS- and CR-LNCAP tumors and identified upregulated 1884 genes and 588 genes repressed in CR-LNCaP as compared to HS-LNCaP tumors (fold > 1.5, FDR < 0.05) (Fig. 1A Table 1). GO analysis showed that the up-regulated differentially expressed genes were significantly enriched involved in mRNA processing and nuclear transport signaling pathway(Fig. 1B). We focused on TNIK serine/threonine kinase because of up-regulated expression of the gene in PCa is associated with Wnt signaling pathway. To verify microarray, We analyzed samples obtained from mice xenografts tumor with CR-LNCaP (4castrasion) and HS-LNCaP (4 uncastrasion) for expression of TNIK by QPCR (Fig. 1C). The mRNA expression of TNIK was higher in CR-LNCaP compared with HS-LNCaP. To investigate a potential role of TNIK, we analyzed prostatectomy samples obtained from patients with benign prostate hyperplasia (BPH 5cases), Hormone native(10cases) and CRPCa (7cases) for expression of TNIK by IHC (Fig. 1D). The expression of both TNIK was circumscribed to cell nuclear of the epithelial compartment, with TNIK showing a pattern of progressively, significantly increased expression in CRPCa. These data, as shown in

Fig. 1C,D and in good agreement with the Microarray data analysis, CRPCa cells exhibit increased mRNA and protein expression of TNIK.

Knockdown TNIK inhibited CRPCa Cell Proliferation

To determine the effect of TNIK on CRPCa cell proliferation, C4-2, 22RV1 and PC3 cells of CRPCa were transfected with siRNA of TNIK. We established efficient siRNA-mediated knockdowns of TNIK in CRPCa cell lines C4-2, 22RV1 and PC3 (Fig. 2A). The proliferation of C4-2, 22RV1 and PC3 cells was clearly inhibited following TNIK downregulation(Fig. 2B). In addition, the downregulation of TNIK in C4-2, 22RV1 and PC3 cells caused a significant reduction in their invasion activity in matrigel (Fig. 2C). Results showed that TNIK had an effect on cell proliferation, significantly inhibited cell growth depended on knockdown TNIK and TNIK should be enough to induce efficient proliferation. These data indicated that TNIK was a potent inhibitor for CRPC cell proliferation. In conclusion, TNIK had independent functions in androgen independent prostate cancer cells that may contribute to the growth and spread of castration resistant tumors.

Androgen-AR signaling suppressed TNIK gene expression

Up-regulated expression of TNIK in CR-LNCaP vs HS-LNCaP tumors suggested that TNIK may be regulated by androgens. Therefore, we studied how dihydrotestosterone (DHT) (10 nM) treatment effects on the levels of TNIK. A time-course study of DHT incubation LNCaP cells showed that androgen treatment repressed the expression of TNIK (Fig. 3A). We found increased TNIK protein expression levels following AR knockdown in PCa cells (Fig. 3B) while treatment of LNCaP cells with AR antagonist MDV3100 also upregulated TNIK mRNA and protein levels and phosphorylation levels of TNIK significantly (Fig. 3C,D). In line with the upregulation of TNIK detected in CR-LNCaP, we also observed that inhibited androgen exposure increased the abundance of TNIK in cell nucleus with immunofluorescence imaging(Fig. 3E). We previously reported EZH2 and AR can cooperate for YAP1 transcriptional repression[23]. while EZH2 was the only identified methyltransferase with activity toward H3K27 and was responsible for all H3K27 methylation[24]. Therefore, we hypothesized that AR may recruit the H3K27me3 to repress TNIK expression in PCa cells. To verify this hypothesis, we first investigated the interaction between AR and H3K27me3 in LNCaP cells by co- immunoprecipitation (Co-IP) experiments. As shown in Fig. 3F, the AR formed a stable complex with H3K27me3 with each other in LNCaP cells. The interactions between the two proteins were specific because not any visible interaction was showed in IgG control. Moreover, AR and H3K27me3 were recruited to the TNIK gene promoter, but the ability of AR to form a complex was abolished by treatment with MDV3100 by Chromatin immunoprecipitation experiments(Fig. 3G). Furthermore, DZNep treatment with an EZH2 inhibitor also lead to a significant increase in the expression of TNIK (Fig. 3H). Likewise, the H3K27 demethylase activator GSK-J1 increased H3K27me3 levels but decreased TNIK levels. Overall, these experiments demonstrated that AR and H3K27me3 complex mediated the androgen-driven epigenetic repression of the TNIK.

TNIK interacts directly withβ-catenin

Gene set enrichment analysis (GSEA) was performed involvement of activating Wnt pathways where the profile of genes differentially expressed in LNCaP-CR vs LNCaP-HS (Table 1). While the TNIK activating wnt through TCF4 in the growth of colorectal cancer has been well documented[8], less is known about the role of TNIK in CRPCa. To confirm the function of TNIK in regulation of the Wnt target gene expression, we used the C4-2 cell line in which the Wnt pathway is present. We examined the interaction between TNIK and β-catenin in C4-2 cells. TNIK was immunoprecipitated from C4-2 lysates and probed for interaction with β-catenin by western blotting (Fig. 4A,B). As expected, β-catenin also displayed direct binding to TNIK. Next, we examined the effect of over TNIK or siRNA-mediated knockdown of TNIK on β-catenin phosphorylation, we fund over expressed TNIK active β-catenin phosphorylation at Ser675 and knockdown of TNIK attenuated β-catenin phosphorylation (Fig. 4C). We also observed that over expressed TNIK increased the abundance of TNIK and β-catenin in cell nucleus with immunofluorescence imaging (Fig. 4D). Mutant of TNIK catalytic activity at S171 resulted in specific suppression of β-catenin - dependent TOPFlash activity (Fig. 4E). QPCR data showed knockdown TNIK with siRNA down-mediated β-catenin downstream genes transcriptional activation(Fig. 4F). We concluded that TNIK was required for optimal β-catenin phosphorylation and transcriptional activation.

TNIK Inhibitor Inhibited proliferation and Invasion of CRPC Cell

The novel small molecule TNIK inhibitor NCB-0846 was confirmed effective on tumor cell[25–26]. We first analyzed the ability of NCB-0846 to suppress TNIK protein expression in C4-2 and PC3 cells. NCB-0846 robustly decreased TNIK protein level after 24 h of treatment with 1 μm or 10 μm (Fig. 5A). A decreased activity of β-catenin (Ser675) in cells treated with inhibitor following TNIK downregulation were also observed in C4-2 and PC3 cells with WB(Fig. 5A). C4-2 and PC3 cells were found to be sensitive to the NCB-0846 treatment with 10 μm (Fig. 5B). NCB-0846 treatment also inhibited cell invasion of C4-2 and PC3 cells in 10 μm (Fig. 5C). Overall, these experiments suggested TNIK as a novel potential therapeutic target in CRPCa.

Targeting TNIK Suppressed CRPCa Tumor Growth in vivo.

To investigate the effect of TNIK inhibitor on CRPCa xenograft tumor growth, 2×10^6C4-2 cells were implanted subcutaneously in Balb/c mice. When the tumors reached approximately 100 mm^3 in size, the mice were randomized and administered daily by oral gavage either with vehicle (10% DMSO in PBS) or NCB0846 (80 mg/kg of body weight) for 10 days (n = 4 mice for each treatment). Although DMSO-treated mice formed robust subcutaneous CRPCa tumors, tumor growth was observed smaller in the NCB-0846-treated group(Fig. 6A). A significant tumor growth inhibited was noticed in NCB-0846-treated mice compared with the DMSO-treated mice (Fig. 6B). We observed that Tumors treated by NCB-0846 expressed reduced levels of TNIK in parallel to diminished cell proliferation, and decreased expression of Ki67 and β-catenin S675 markers(Fig. 6C). Together, these results highlighted the potential of targeting the TNIK signaling to sensitize to CRPCa therapy.

Discussion

In the current study, we unveiled key elements of the crosstalk between the AR and the WNT signaling pathways through TNIK and targeted TNIK inhibiting β-catenin phosphorylation, blocked the growth of CRPCa.

The previous study was reported TNIK were frequently upregulated in high-grade tumors ovarian cancer and serous hepatocellular carcinoma[14]. Moreover, TNIK hyperactivity contributes to Human Lung Adenocarcinoma cell metastasis[13]. In this study, we firstly identified TNIK as a candidate biomarker of CRPCa from gene array files using mouse models. We observed not only TNIK higher expression in mice CR-LNCaP tumors, but also TNIK was higher in CRPCa than localized PCa and BPH in patient(Fig. 1D). It suggested us TNIK was relation to cancer aggressive behavior.

The principal findings of our study were that AR assembled a repressive complex with H3K27me3 at the TNIK promoter to suppress TNIK transcription(Fig. 3). Consequently, androgen deprivation therapy could induce TNIK mRNA expression which, in turn, regulated β-catenin phosphorylation to switch on WNT/β-catenin signaling pathway and contributed to castration resistant prostate cancer growth. The transcriptional activity of AR was regulated by interacting coactivators that positively modulated receptor function. Conversely, AR inhibited target gene expression by “corepressors” such as Alien, SMRT, NCoR[27–29]. A few reports have studied AR inhibition of transcription. Some hinted to indirect mechanisms- DNA methylation or protein phosphorylation[23, 30] while others hinted to direct mechanisms in involving the epigenetic silencing complex[24, 31]. Using TNIK as a model, we found that AR can also inhibited gene expression by hormone induced recruitment of H3K27me3 to the AR/ H3K27me3 complex to directly inhibite transcription. Consequently, ADT restored TNIK expression due to the loss of AR/ H3K27me3 association to the TNIK promoter.

WNT/β-catenin signaling pathway also plays an important role in CRPCa. Evidence has accumulated that Approximately 24% of metastatic tumors from CRPCa patients were reported to be positive for nuclear localization of β-catenin[32]. Sequencing of CRPCa tumors has revealed more significant genomic alterations in multiple components of the Wnt pathway than in hormone treatment naïve prostate cancer[33–34]. Our results also suggested that castration resistance may be induced reciprocal interaction between TNIK and the WNT/β-catenin signaling pathway, and TNIK was a major inducer of the expression, nuclear translocation, and activation of β-catenin(Fig. 3C). Phosphorylation cascades that were dependent and independent of Wnt signaling played a critical role on β-catenin stability, intracellular distribution and transcriptional activity. Targeting β-catenin in N-terminal domain facilitated proteosomal degradation, whereas β-catenin non-canonical phosphorylation at Ser675 promoted its nuclear translocation and transcriptional activity, and promoted its interaction with transcriptional co-activators, including TCF4[35–36]. In this study, we found that upregulation of TNIK band and phosphorylated β-catenin at Ser675, promoted β-catenin nuclear translocation and transcription activation. It was reported that TNIK inhibitor decreased DU145 and 22RV1 cell viability in ERG positive PCa cells[24]. Our current data further revealed that TNIK band to β-catenin, resulting in phosphorylation of β-catenin and target

genes transcription activation and target TNIK reduced the proliferation and invasion of CRPC cells(Fig. 5,6).

Conclusions

Here, our mechanisms have shown that AR functions as a transcriptional repressor in TNIK by binding H3K27me3 complex. TNIK interacted with β-catenin and phosphorylated activating wnt pathway to escape from castration treatment and promote CRPCa progression. It explored novel therapeutic strategies to simultaneously target AR and TNIK pathways in PCa treatment.

Abbreviations

CRPC
Castration resistant prostate cancer; TNIK:Traf2 and Nck-interacting kinase;
AR
Androgen receptor; COIP:Co-Immunoprecipitation; CHIP:Chromatin Immunoprecipitation;
PCa
prostate cancer;

Declarations

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Authors' contributions
Jiang Ning and Niu yuanjie conceived of the study and Ning Jiang carried out its design. Guo tao, WMM and LYH performed the experiments. WYD and MFL collected clinical samples. Amilcar Flores-Morales and Jiang Ning analyzed the data and Jiang Ning wrote the paper. All authors read and approved the final manuscript.
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Availability of data and materials
All data generated or analyzed during this study are included either in this article.
Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the Research Ethics Committee of The Second Hospital of Tianjin Medical University and with the 1964 Helsinki declaration and its later amendments. ALL written informed consent to participate in the study was obtained from prostate cancer patients for samples to be collected from them.
Consent for publication
All subjects have written informed consent
Conflict of Interest

None of the authors have any relevant conflicts of interest pertaining to the studies and data in this manuscript.

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Figures

Figure1

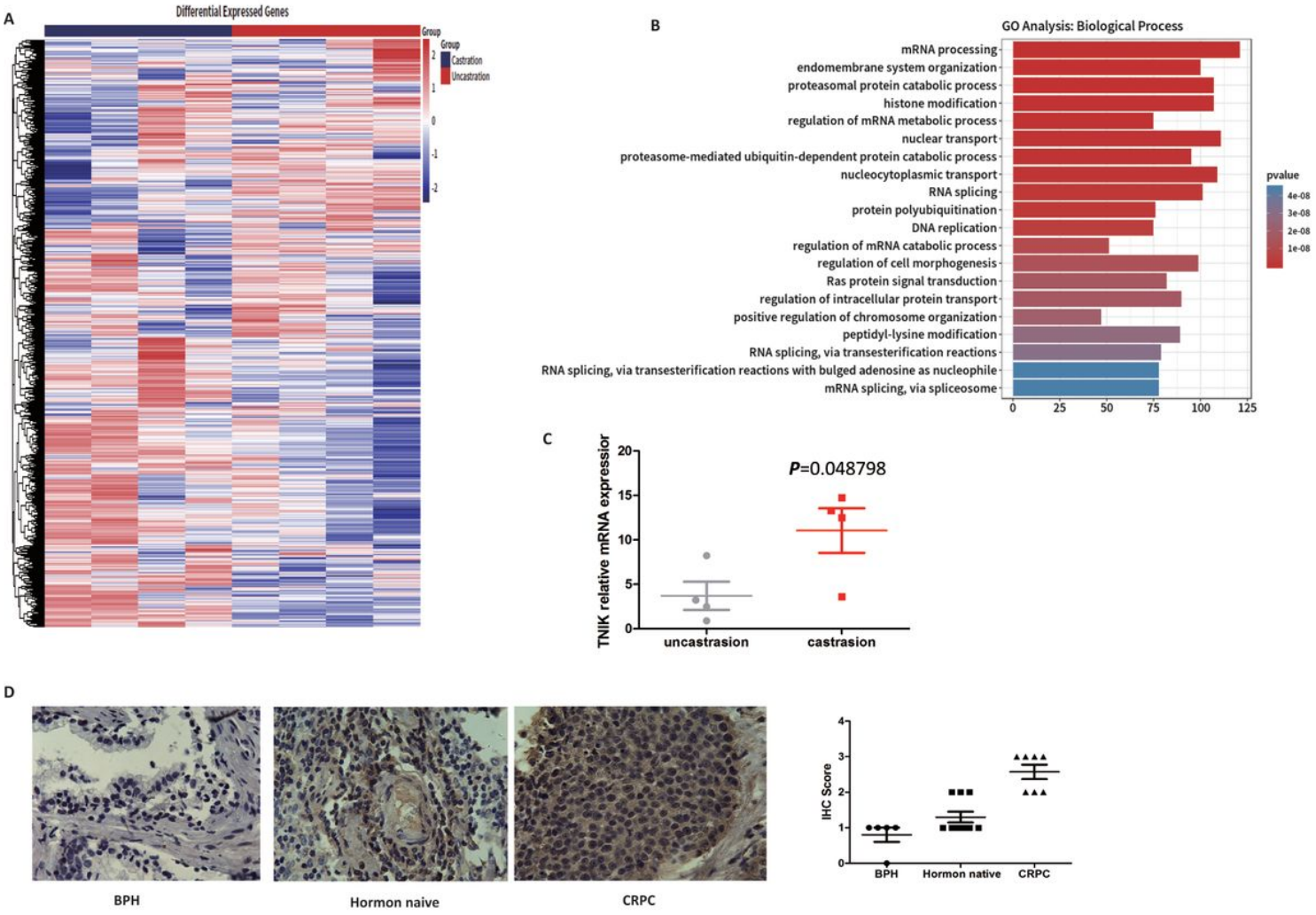

Figure 1

TNIK was upregulated in castration resistant prostate cancer. (A)Hotmap of the genome-wide transcript profile between (CR-LNCaP) castration tumors and (HS-LNCaP) uncastrasion tumors. (B)GO analysis of transcript profile. (C)QPCR detect mRNA expression of TNIK in (CR-LNCaP) castration tumors and (HS-LNCaP) uncastrasion tumors. (D)Clinical specimens of benign prostate hyperplasia (BPH), Hormon native and CRPC were analyzed by IHC for TNIK expression. The IHC was scored according to the strength of the expression and statistical analysis was performed in order to determine significance. Only significant p-values ($p<0.05$) are indicated.

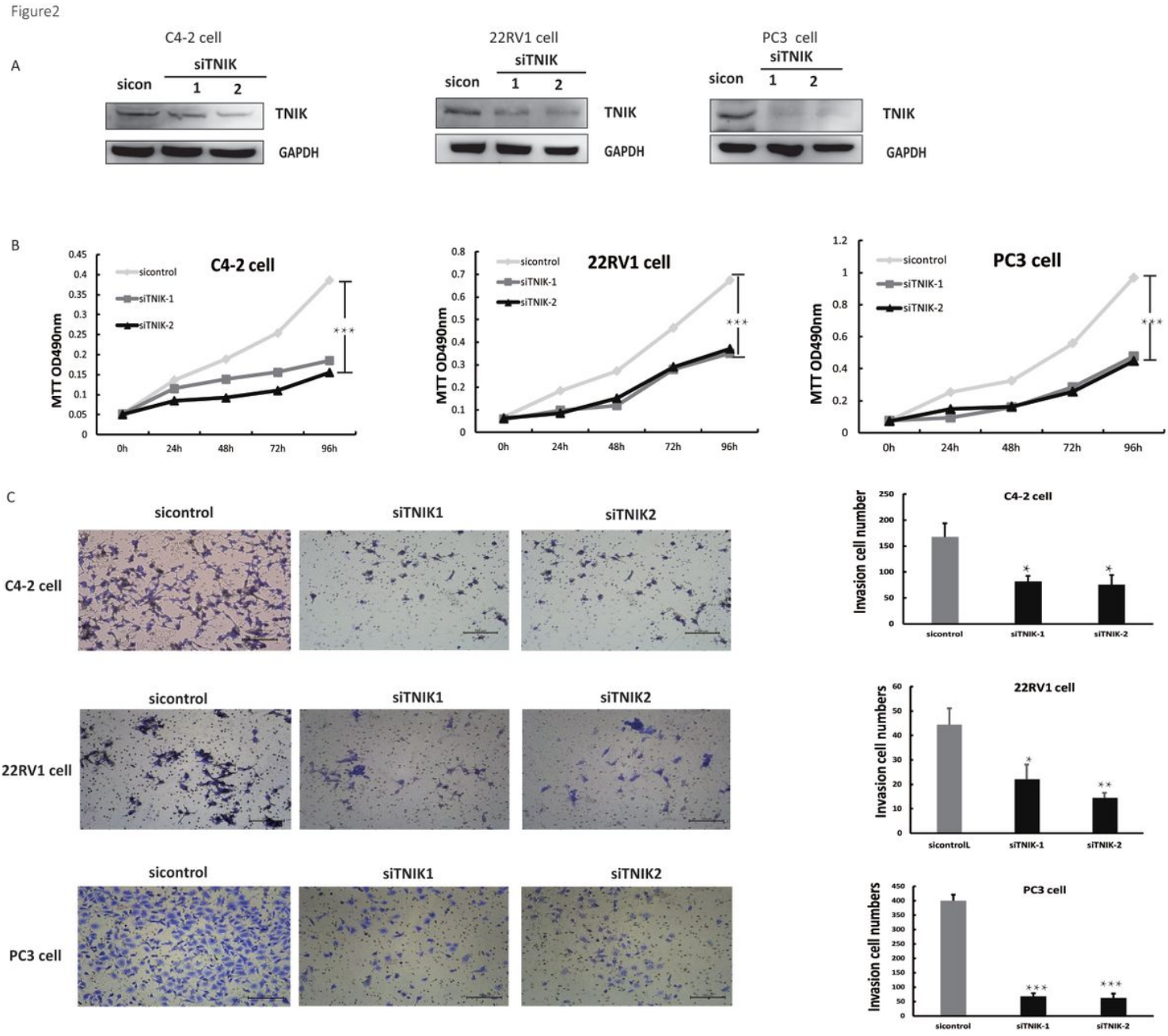

Figure 2

Knockdown TNIK inhibited CRPC Cell Proliferation. (A)C4-2, 22RV1and PC3 cells were transfected with TNIK siRNA and lysed 48 h post transfection. Whole cell extracts were analyzed by western blotting with the indicated antibodies. (B) C4-2, 22RV1and PC3 cells were transfected with sicontrol or siTNIK, MTT detected cell viability.(C) C4-2, 22RV1and PC3 cells were transfected with sicontrol or siTNIK, transwell detected cell invasion. Significance was determined by a two-tailed t test: *P < 0.05.

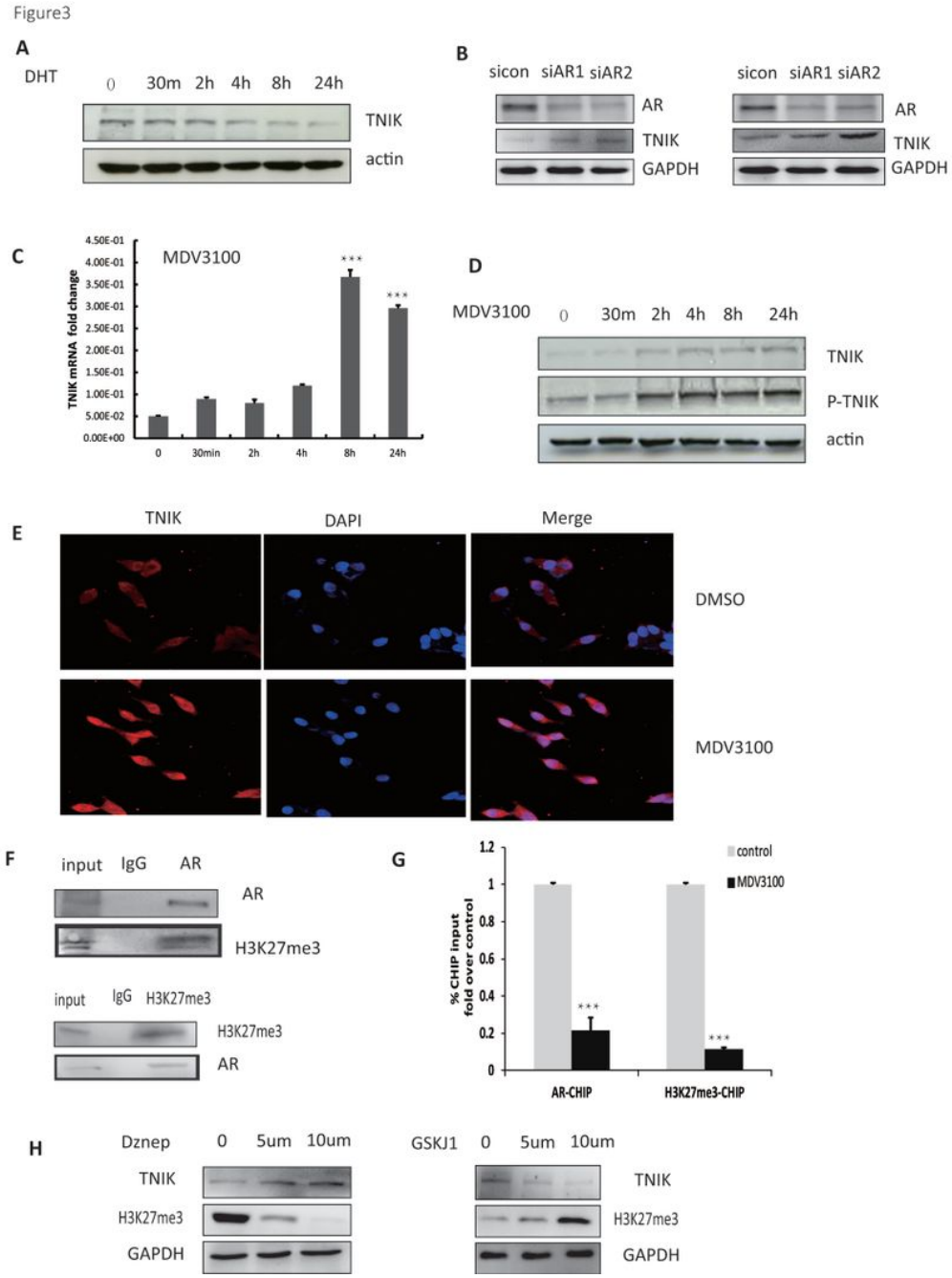

Figure 3

The essential role of the AR and H3K27me3 complex in the regulation of TNIK expression. (A) LNCaP cells were cultured in steroid depleted medium for 24 h and subsequently treated with DHT (10 nM) for the indicated time. Protein levels were analyzed by western blot. (B) The levels of TNIK protein were measured by WesternBlot in LNCaP and C4-2 cells upon AR knockdown. (C) QPCR analysis mRNA expression of TNIK after LNCaP cells were treated with MDV3100 for 24 h. (D) The levels of TNIK protein were measured in LNCaP cells treated for 24 h with the AR antagonist MDV3100 (enzalutamide, 100 nM). (E) Co-immunofluorescence (co-IF) analysis of TNIK proteins in LNCaP treated with MDV3100. (F) Immunoprecipitation of AR or H3K27me3 in LNCaP cells followed by immunoblot analysis of H3K27me3 or AR. IgG represents a control antibody used for IPs. (G) ChIP–PCR analysis of H3K27me3 and AR

binding to the TNIK gene promoter in LNCaP cells treated with MDV3100. ChIP–PCR primer in table2.(H) LNCaP cells were treated with DZNep (0,5,10 μM) and GSKJ1(0,5,10 μM) for 8 h, WB analysis of TNIK protein expression.

Figure4

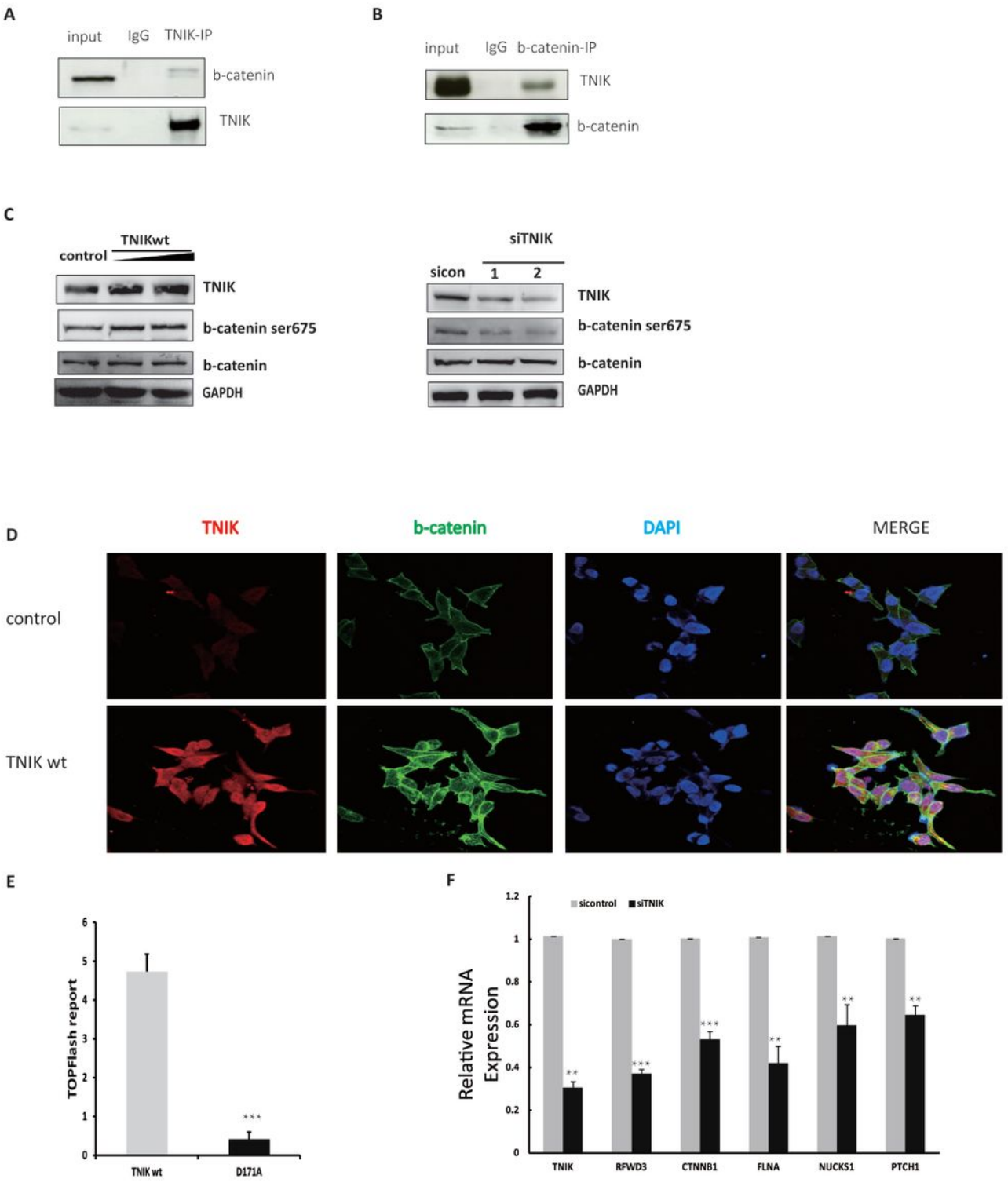

Figure 4

TNIK interacts directly withβ-catenin. (A) Immunoprecipitation of TNIK in C4-2 cells followed by immunoblot analysis ofβ-catenin or TNIK. (B) Immunoprecipitation of β-catenin in C4-2 cells followed by

immunoblot analysis ofβ-catenin or TNIK. (C) The levels ofβ-catenin and Ser675 protein were measured in C4-2 cells after transfected TNIK wildtype vector or siTNIK. (D)Co-immunofluorescence (co-IF) analysis of TNIK andβ-catenin proteins in C4-2 cell after transfected TNIK wildtype vector. (E) Expression of TNIK kinase mutants (D171A) abrogates β-catenin-driven transcription, whereas over expression of WT TNIK (last panel) specifically increases β-catenin driven transcription in c4-2 cell by TOPFlash activity. (F)C4-2 cells were transfected with siTNIK. qRT-PCR analysis was performed to measure the mRNA levels ofβ-catenin–induced target genes. Data are means ± SD.

Figure5

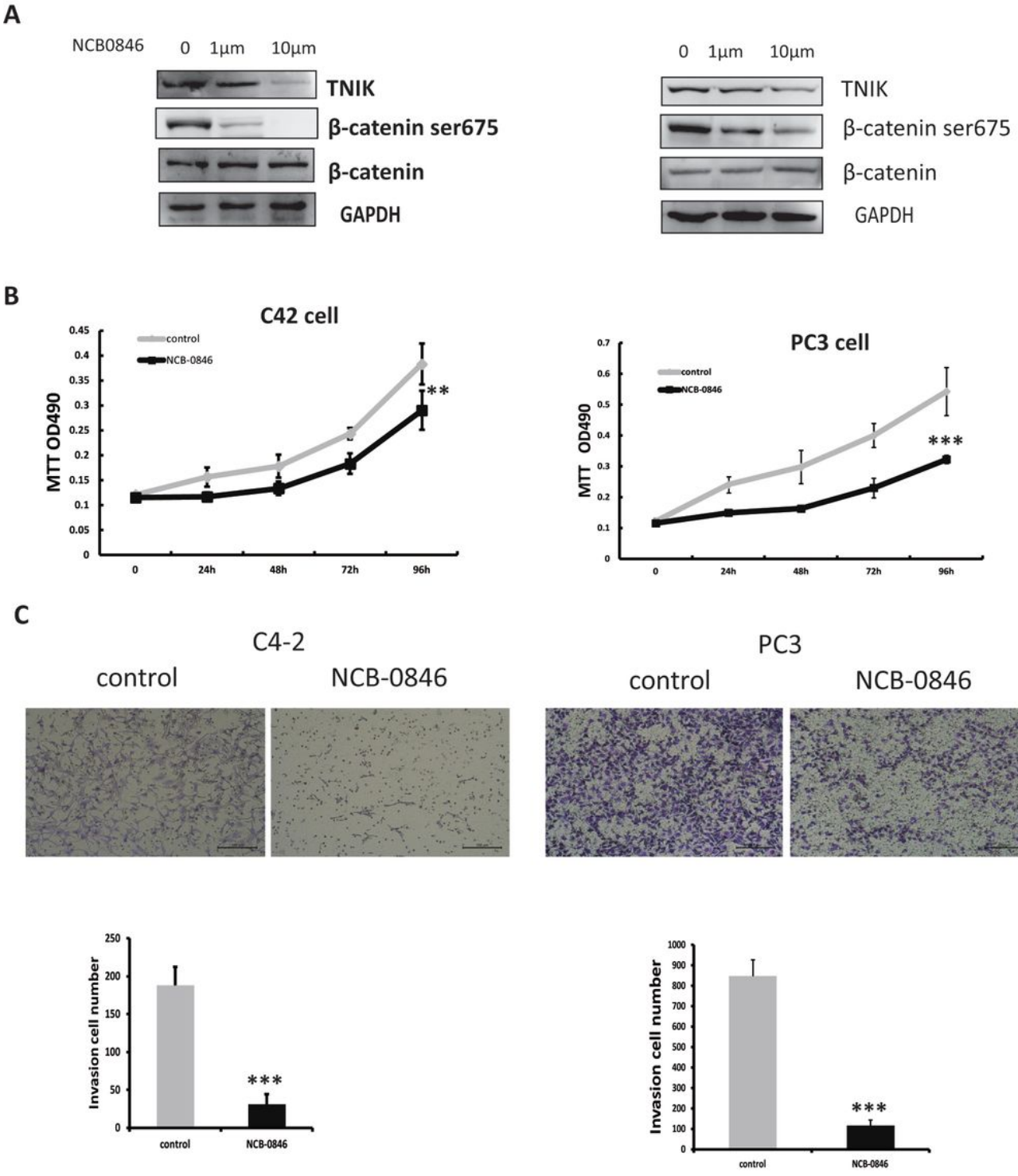

Figure 5

TNIK Inhibitor Inhibited proliferation and Invasion of CRPC Cell. (A)C4-2 and PC3 cells were treated with NCB-0846 24 h. Whole cell extracts were analyzed by western blotting with the indicated antibodies. (B) C4-2 and PC3 Cells were seeded into 96 well plates and treated with NCB-0846(10μm), MTT detected cell viability. (C) C4-2 and PC3 cells were treated with NCB-0846(10μm), transwell detected cell invasion ability.

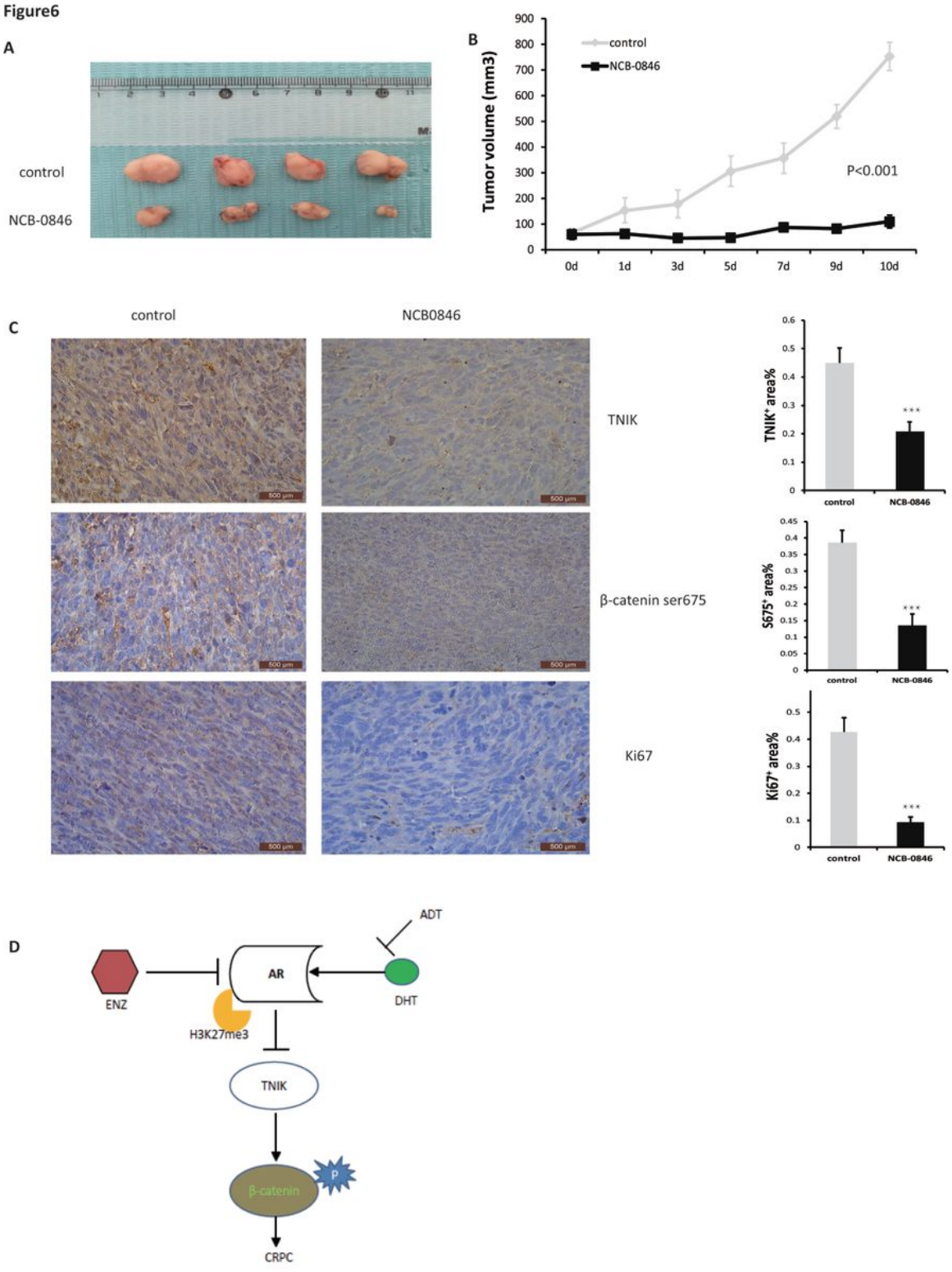

Figure 6

Targeting TNIK Suppresses CRPC Tumor Growth in vivo. (A)C4-2 cells were implanted subcutaneously in male Balb/c mice. When tumors became palpable, mice administered daily by oral gavage either with vehicle (10% DMSO in PBS) or NCB0846 (80 mg/kg of body weight) for 10 days (n = 4 mice for each treatment). Tumor volumes were measured with calipers. (B) tumor size of xenografts of the above represented the growth of tumor over 10 days (n = 4) in athymic nude mice. (C) Quantitation of Ki-67, TNIK and Ser675 expressions in c4-2 xenograft tumors from each group, specimens were got at 10 days post treatment. The IHC was scored according to number of cells expressing the indicated proteins and statistical analysis was performed (non-parametric Kruskal-Wallis test) in order to determine significance. see also Fig. 6 **p < 0.01, *p < 0.05. (D) Working model of TNIK repression by AR-h3k27me3 complex regulated and ADT result to TNIK active wnt to promote CRPC progression.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable2.xls
- Supplementarytable1a.xls