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Wnt5a augments intracellular free cholesterol levels and promotes castration resistance in prostate cancer

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Abstract

Background Prostate cancer (PCa) is a leading cause of cancer-related mortality in men globally. While androgen deprivation therapy (ADT) can extend the asymptomatic phase and overall survival of patients with metastatic PCa, prolonged ADT often leads to the development of castration-resistant prostate cancer (CRPC) within 18–24 months. The mechanisms underlying CRPC remain incompletely understood, presenting a significant challenge in clinical prostate cancer treatment.

Methods In this study, we investigated the role of Wnt5a, a member of the Wnt family, in CRPC. Tumor tissues from CRPC patients were analyzed to assess the expression levels of Wnt5a. Prostate cancer cells were used to examine the impact of Wnt5a on androgen-dependent and -independent growth, as well as sensitivity to bicalutamide. RNA-seq analysis, qRT-PCR, intracellular cholesterol content and the activation of the androgen receptor (AR) signaling pathway were evaluated to elucidate the mechanistic role of Wnt5a in CRPC progression. Drug target Mendelian randomization analysis was performed to investigate the effect of PCSK9 inhibitor on prostate cancer.

Results Our study revealed a significant overexpression of Wnt5a in tissues from CRPC tumors. Wnt5a was found to enhance both androgen-dependent and -independent growth in prostate cancer cells while reducing their sensitivity to bicalutamide. Mechanistically, Wnt5a was shown to upregulate intracellular free cholesterol content and activate the AR signaling pathway, contributing to hormone therapy resistance in CRPC. PCSK9 inhibitor significantly reduced the risk of PCa.

Conclusions The findings of this study highlight a novel molecular mechanism underlying endocrine therapy resistance in CRPC mediated by Wnt5a. Targeting Wnt5a or reducing cholesterol level would be a promising

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therapeutic strategy for the treatment of CRPC, providing new insights into potential avenues for combating this challenging form of prostate cancer.

Keywords Prostate cancer, Castration resistance, Cholesterol, Androgen receptor signaling

Introduction

Prostate cancer accounts for the second highest incidence of cancer in men. As the global population ages, the prevalence of prostate cancer will rise even more dramatically. The estimated number of new prostate cancer cases in the United States by gender in 2023 will be 288,300, accounting for about 29% of all new cancer cases in men, and the estimated number of deaths will be 34,700, accounting for about 11% of all new cancer deaths in men [1].

Prostate cancer is driven by androgen receptor (AR), which belongs to the nuclear receptor family of ligand-dependent transcription factors [2]. Androgens, including testosterone, androstenedione and dihydrotestosterone (DHT) are all ligands for the AR, with DHT being the most potent binding agent. Currently, androgen deprivation therapy (ADT) is the standard of care for primary or recurrent metastatic prostate cancer, which can be achieved through surgery or by using drugs such as luteinizing hormone-releasing hormone (LHRH) antagonists, LHRH agonists, androgen synthesis inhibitors and androgen receptor antagonists. As a mainstream clinical treatment for prostate cancer, ADT has been shown to improve the quality of life of patients [3], but castration-resistant prostate cancer (CRPC) has emerged with the prolongation of androgen deprivation time [4, 5]. In this case, although serum testosterone levels have reached castrated levels (< 20ng/dL (0.7nmol/L)) [6, 7], the disease continues to deteriorate, prostate specific antigen (PSA) levels continue to rise and patients survive for only 16–18 months after recurrence [8, 9]. Clarifying the mechanism of castration resistance of prostate cancer can provide potential molecular markers or therapeutic targets for clinical treatment and prognosis assessment. So far, the specific mechanism for the occurrence of CRPC is still not fully understood.

Wnt5a is a member of the Wnt gene family, which consists of structurally related genes encoding secreted signaling proteins that are implicated in tumorigenesis and certain developmental processes. Wnt5a amplification is seen in approximately 29% of cases in CRPC, with upregulation observed in 6% of these instances. Notably, Wnt5a expression is typically low in low-grade prostate cancer but elevated in cases of metastatic prostate cancer, where heightened Wnt5a levels correlate with a poorer prognosis [10]. In the context of prostate cancer skeletal metastases, Wnt5a has been identified as a key factor in inducing and sustaining cancer cell dormancy and inhibiting bone metastasis [11]. Moreover, the expression of Wnt non-classical pathway was found to be significantly upregulated in prostate cancer samples with high Gleason score [12–15]. Additionally, a study highlighted that Wnt5a mRNA expression level was increased in androgen-independent prostate cancer cells, and knockdown of Wnt5a could suppress growth of the androgen-independent prostate cancer cells [16]. As of now, the precise role and underlying mechanism of Wnt5a in CRPC remain inadequately understood.

In the present study, we assessed the impact of Wnt5a on the hormone-independent growth potential and sensitivity to bicalutamide in prostate cancer cells. We also endeavored to identify the possible mechanism by which Wnt5a promotes CRPC. Our study proposed the potential value of Wnt5a as a therapeutic target for clinical CRPC.

Materials and methods

Cell culture

Androgen-dependent human prostate cancer cells LNCaP are kept in our laboratory. Androgen-insensitive human prostate cancer cell line 22RV1 was purchased from Shanghai Genechem Co.,LTD. Charcoal-dextrose stripped fetal bovine serum were purchased from Biological Industries (C3830-0500). LNCaP and 22RV1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Bicalutamide-resistant LNCaP cells (LNCaP-BicR) were developed by long-term culturing LNCaP cells in the presence of bicalutamide, and routinely cultured in RPMI 1640 medium supplemented with 5μM Bicalutamide (Sigma).

Establishment of stable cell lines

The full-length sequence of Wnt5a cDNA was cloned into the eukaryotic expression plasmid GV362 and transfected into LNCaP cells to overexpress Wnt5a. The cell line with stable Wnt5a overexpression was then obtained by geneticin (G418) selection. For achieving a stable knockdown of Wnt5a in the 22RV1 cell line, denoted as 22RV1-Wnt5a-KD, CRISPR/Cas9 technology was utilized. The sgRNA sequence targeting Wnt5a (CAGGACC ACATGCAGTACAT) were incorporated into a CRISPR/Cas9 single vector system, which was subsequently packaged into lentiviral particles. Following infection of the 22RV1 cells with the lentiviral particles, the infected cells were then subjected to selection using puromycin at a concentration of 2 μg/mL for a duration of 2 weeks. Both

the lentivirus and plasmids utilized in this study were procured from Shanghai Genechem Co., LTD.

MTS assay

The CellTiter 96 AQueous One Solution Cell Proliferation Assay kit was procured from Promega for the experiment. A total of 1000 cells in 100 μL per well were seeded into a 96-well plate and cultured for a duration of 1 to 4 days. During this incubation period, 20 μL of MTS solution reagent was added into the medium. After a 2-hour incubation, the absorbance at 490 nm was measured. The growth curve was plotted based on the absorbance values.

Cytotoxicity assay

The cytotoxicity assay was used to test the sensitivity of the cells to bicalutamide. 5000 cells/ well were seed into 96-well plate. The next day, bicalutamide were added into the cell at a final concentration gradient of 10, 20, 30, 40, 50, 60, 70, 80 and 90 μM. 72 h later, 20 μL of MTS solution were added into the well and incubated for 2 h. The OD value at 490 nm was measured to test cell viability.

Colony formation assay

1000 cells per well were plated in a 6-well plate and incubated for approximately two weeks. When approximately 50 clumpy colonies were observed under a microscope, the cells were fixed with ice-cold methanol for 20 min and stained with 0.05% crystal violet for 30 min. The number of cell colonies in each well was photographed and counted.

Filipin cholesterol fluorescence assay

5000 cells per well were plated in 96-well plates and cultured for three days under hormone deprivation conditions. The cells were then rinsed three times with PBS and fixed with 4% paraformaldehyde for 1 h at room temperature. Following this, cells were washed three times with PBS, and 200 μL of 1.5 mg/mL glycine was added at room temperature for 10 min. Subsequently, 80 μL of Filipin working solution (0.1 mg/mL) was added to the well and stained for 2 h. Afterward, the Filipin staining solution was aspirated, the cells were washed three times with PBS, and observed under fluorescence microscopy.

Determination of intracellular total and free cholesterol content

Intracellular total and free cholesterol content were determined using commercial kits (E1015, E1016, Applygen Technologies, Beijing, China). Cells were lysed using the lysis buffer provided in the kits. The cell lysate was then centrifuged, and the supernatant was carefully transferred to a new centrifuge tube for enzymatic identification. For the cholesterol assay, 10 μL of standard and samples were added to a 96-well plate in triplicates. Mixed working solution (R1:R2 = 4:1) was added, and after 20 min incubation at 37 °C, absorbance value were taken at 550 nm. A standard curve was used to calculate sample cholesterol concentration.Additionally, BCA protein concentration determination was carried out using the lysate to calibrate the cholesterol content.

Steroid hormone determination

The LNCaP cells were cultured for three days under hormone deprivation conditions. Cells were collected and sent to Shanghai Baiqu Biomedical Technology Co., Ltd. for determination of steroid hormones. Metabolites of the cells were extracted, and standard solutions were prepared and tested. Chromatographic separation of target compound was performed on a Waters XBridge® HPLC BEH C8 (100 × 2.1 mm, 1.7 μm, Waters) LC Column. The A phase of the liquid chromatography was an aqueous solution containing 0.5 mmol/L ammonium fluoride, and the B phase was methanol. The column heater temperature was 50 °C, the sample tray was set to 10 °C, and the injection volume was 10 μL. Mass spectrometry analysis was performed in multiple reaction monitoring (MRM) mode using a Waters Xevo TQ-S Triple Quadrupole Mass Spectrometer. Water MassLynx V4.1 and Skyline Software were used for mass spectrometry data acquisition and quantification of target compounds. The calibration curve was plotted, the calibration solution was diluted 2-fold sequentially and then analyzed by UHPLC-MRM-MS, and the limit of detection and quantification of the method were calculated by its signal-to-noise ratio. The final measured concentration of the sample is the directly measured concentration multiplied by the dilution factor. The concentration of the target metabolite in the sample is equal to the final measured concentration of the sample multiplied by the sample processing volume divided by the total protein content in the sample.

RNA-seq and RT-qPCR

Total RNA of the cells was extracted using TRIzol reagent (Thermo, USA). RNA-seq was performed and analyzed by LC Bio (Hangzhou, China). For RT-qPCR, the RNA samples were reverse transcribed using the PrimeScript™ RT Master Mix kit (RR036A; TaKaRa, Japan). The SYBR® Premix Ex Taq™ II kit (RR820A; TaKaRa, Japan) was used to perform qPCR. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ formula. The primer sequences used for qPCR are shown in Supplementary Table 1.

Western blot analysis

Protein was extracted and quantified using the BCA method. Equal protein amounts were loaded for SDS-PAGE, followed by transfer to a PVDF membrane. The

membrane was then blocked with 5% skimmed milk and exposed to Wnt5a (1:1000, CST) or GAPDH (1:20,000, Proteintech) antibodies overnight at 4 °C. The next day, the membrane was washed with TBST, exposed to an HRP-conjugated secondary antibody, and visualized using ECL luminescence.

Drug target Mendelian randomization analysis

A Drug target Mendelian randomization (MR) analysis were conducted to explore the therapeutic implications of genetically mimicking a lipid-lowering drug target, PCSK9, on prostate cancer. We used PCa as the results of the drug target MR analysis, of which coronary heart disease (CHD) was a positive control dataset. The datasets were all from the European population. For details and download links for all data, please refer to Supplementary Table 2.

Genetic information related to PCSK9 were leveraged from the MRC IEU OpenGWAS Project, a reputable source offering extensive Genome-Wide Association Study (GWAS) data for genomic epidemiology and causal inference analyses. We meticulously selected genetic variants significantly associated with the exposure variable (P-value $< 5 \times 10^{-8}$) for drug-target MR analysis, ensuring the robustness of instrumental variables. The genomic coordinates of the PCSK9 gene were pinpointed using NCBI, specifically in version GRCh37.p13 at 55505221.55530525. Instrumental variables, represented by single nucleotide polymorphisms (SNPs) within ± 100 kb of PCSK9 loci and linked to LDL-C levels, were carefully chosen. To minimize the influence of strong linkage disequilibrium (LD) on our findings, we set a stringent LD threshold ($r^2 < 0.3$). Ultimately, 12 significant SNPs associated with PCSK9 were retained for further analysis (refer to Supplementary Table 3).

The analytical approach encompassed a variety of MR methods, including MR Egger, weighted median, IVW, simple mode, weighted mode, and MR-PRESSO, with IVW being the predominant method [17]. Heterogeneity and pleiotropy assessments were performed using MR Egger and IVW, with *Cochrane's Q* statistic indicating no significant heterogeneity when $p > 0.05$. The MR Egger regression equation was employed to evaluate horizontal pleiotropy, with $p > 0.05$ denoting its absence [18]. Additionally, leave-one-out plots were utilized to gauge the influence of individual SNPs on the MR analysis results.

All analyses were conducted using the "MRPRESSO" "TwoSampleMR" "foreach" and "ggplot2" packages in R version 4.3.1 for comprehensive data visualization and interpretation [18, 19].

Statistical analysis

Data analysis was conducted using GraphPad Prism 8.0. The data are presented as mean ± standard deviation (SD) from at least three independent biological experiments or samples. A *t*-test for two independent samples was employed to compare means between two independent groups, with statistical significance set at $P \leq 0.05$.

Results

Wnt5a is associated with castration resistance in prostate cancer

Using public databases including GEO, we analyzed and compared the changes in expression of Wnt5a in prostate cancer tissue and normal prostate tissue. The results showed that Wnt5a mRNA levels were significantly higher in cancer tissues than in normal tissues, particularly in metastatic cases (Fig. 1A). Patients with a Gleason score greater than 8 displayed higher Wnt5a expression levels compared to those with a Gleason score of 7 or less (Fig. 1B). Notably, prostate tumor tissues resistant to castration exhibited highest Wnt5a expression level (Fig. 1C). In mouse models, post-castration tumor tissues displayed increased Wnt5a expression, indicating a role of Wnt5a in castration resistance (Fig. 1D), and androgen deprivation led to heightened Wnt5a mRNA levels in LNCaP cells (Fig. 1E). Collectively, these findings suggest that Wnt5a plays a role in castration therapy resistance and the progression of prostate cancer.

Wnt5a promotes androgen-dependent and -independent growth of prostate cancer cells

To investigate the role of Wnt5a in prostate cancer, we stably overexpressed Wnt5a in LNCaP cells (Fig. 1F), and knocked down the expression of Wnt5a in 22RV1 cells (Fig. 1G). And then, we assessed the effect of Wnt5a on the proliferative capacity of the cells. As shown in Fig. 1H & I, LNCaP cells with Wnt5a overexpression formed more and larger cell colonies, and had enhanced growth capacity compared with the control cells; whereas, knockdown of Wnt5a significantly inhibited proliferation ability of 22RV1 cells compared with control cells. These data suggest Wnt5a promotes the growth of prostate cancer cells.

As Wnt5a highly expressed in tumor tissues formed by LNCaP prostate cancer cells in mice with surgical castration and LNCaP cells cultured in hormone-deprived medium (Fig. 1D, E), we further assessed the impact of Wnt5a on androgen-independent growth of the cells. As shown in Fig. 1J & K, Wnt5a overexpression could remarkably enhance the proliferation of LNCaP cells under hormone-deprivation condition; while knockdown of Wnt5a substantially suppressed proliferation of 22RV1 cells, indicating that Wnt5a enhances the androgen-independent growth ability of PCa cells.

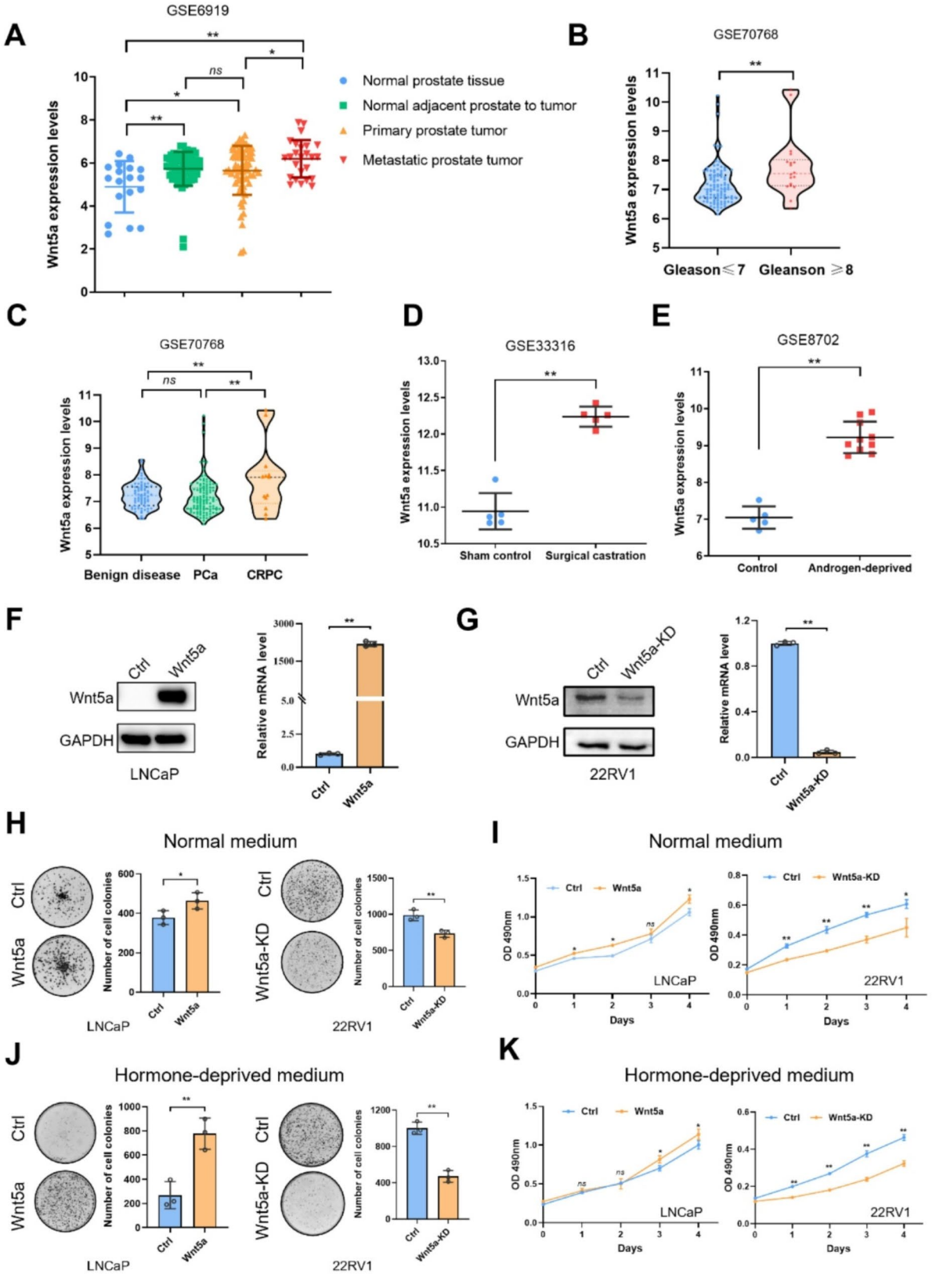

Fig. 1 (See legend on next page.)

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Fig. 1 Wnt5a is associated with castration resistance in prostate cancer and promotes androgen-dependent and -independent growth of PCa cells. (**A**) Comparison of mRNA expression of Wnt5a in normal, paraneoplastic, primary and metastatic prostate cancer tissues in the GSE6919 dataset from the GEO Database. (**B**) Expression of Wnt5a in patients with different Gleason score levels or (**C**) tissue type were analyzed using GSE70768 dataset from the GEO database. (**D**) Expression levels of Wnt5a in tumor tissues formed by LNCaP prostate cancer cells in mice with or without surgical castration in GSE33316 dataset from the GEO database. (**E**) Wnt5a expression of LNCaP cells cultured in regular medium and hormone-deprived medium were compared using GSE8702 dataset from the GEO database. (**F**) Protein and mRNA expression levels of Wnt5a in LNCaP cells with or without Wnt5a overexpression were tested by Western blot and RT-qPCR respectively. (**G**) Protein and mRNA expression of Wnt5a in 22RV1 cells with Wnt5a knockdown (Wnt5a-KD) or not (Control) were tested by Western blot and RT-qPCR respectively. (**H**) The proliferation capacity of LNCaP cells with Wnt5a overexpression or 22RV1 cells with Wnt5a knockdown were assessed by colony formation assay and (**I**) MTS assay. (**J**) Androgen-independent growth of LNCaP cells with Wnt5a overexpression or 22RV1 cells with Wnt5a knockdown were detected by colony formation assay and (**K**) MTS assay. *$P < 0.05$; **$P < 0.01$; *ns*, not significant

Wnt5a promotes bicalutamide resistance of prostate cancer cells

Androgen receptor pathway is a crucial stimulator for the development of prostate cancer, which is the basis for the clinical endocrine therapy of the disease. Although endocrine therapy effectively enhances the survival of prostate cancer patients, the inevitable resistance of the therapy is a difficulty in clinical. Since Wnt5a could promote androgen-independent growth of prostate cancer cells, we further investigated whether Wnt5a is involved in endocrine therapy resistance. To this end, we established a bicalutamide-resistant LNCaP line (named as LNCaP-BicR). LNCaP-BicR cells showed reduced bicalutamide sensitivity compared with LNCaP-Parental cells (Fig. 2A); the IC50 of bicalutamide for LNCaP-BicR was 94.15 μM, which is substantially higher than that for LNCaP-Parental cells (48.51 μM) (Fig. 2B). Of note, Wnt5a expression was obviously upregulated in the bicalutamide-resistant LNCaP cells compared with the parental cells (Fig. 2C). More importantly, overexpression of Wnt5a reduced the inhibitory effect of bicalutamide on LNCaP cells (Fig. 2D, E); the IC50 of bicalutamide for Wnt5a-overexpressing LNCaP cells and control cells were 102.0 μM and 76.66 μM respectively (Fig. 2F). Whereas, knockdown of Wnt5a enhanced the sensitivity of 22RV1 cells to bicalutamide (Fig. 2G); the IC50 of bicalutamide for 22RV1 cells with or without Wnt5a knockdown was 171.3 μM and 457.2 μM respectively (Fig. 2H).

Wnt5a affects steroid hormone anabolic pathway in PCa cells

In order to unravel the mechanism through which Wnt5a facilitates androgen-independent growth and bicalutamide resistance in prostate cancer, we conducted RNA-seq analysis to identify changes in the transcriptome of Wnt5a-overexpressing LNCaP cells under androgen deprivation conditions in comparison to control cells. The results indicated that overexpression of Wnt5a caused a significant upregulation of 967 genes and downregulation of 307 genes in the cells (Fig. 3A), of which the top 100 genes with the most significant differential changes are shown as heat maps in Fig. 3B. The top 20 significantly enriched pathways of the differentially expressed genes were shown in Fig. 3C. Among them, the steroid hormone biosynthesis pathway drew our focus, as intratumoral androgen biosynthesis has been reported to contribute to endocrine therapy resistance in prostate cancer, and enhanced androgen synthesis in prostate cancer cells stands as a key mechanism in the progression of CRPC [20]. The enzymes responsible for androgen synthesis and transformation were depicted in Fig. 4A, and the expression fold changes of differential genes involved in the steroid hormone anabolic pathway were shown in Fig. 4B. We next applied RT-qPCR to validate the results of RNA-seq. As shown in Fig. 4C, except HSD17B1, alterations in the expression levels of UGT2B10, AKR1C1, AKR1C2, AKR1C3, HSD3B1, and CYP1A1 in LNCaP cells after Wnt5a overexpression were consistent with RNA-seq analysis results. Meanwhile, knockdown of Wnt5a in 22RV1 cells had the opposite effect (Fig. 4D).

To further investigate whether Wnt5a could affect the synthesis of androgen, we measured the levels of steroid hormones in the LNCaP cells with different Wnt5a expression levels. The results showed that only Dehydroepiandrosterone, Dihydrotestosterone (DHT), Progesterone, and Pregnenolone levels were identified out of the 14 hormones examined (Fig. 5A-D, supplementary Fig. 1). Unexpectedly, while the levels of Dehydroepiandrosterone, Progesterone, and Pregnenolone decreased in Wnt5a-overexpressing LNCaP cells, there was no notable distinction in DHT levels between Wnt5a-overexpressing LNCaP cells and control cells (Fig. 5D-F). These results indicate intratumoral androgen synthesis might not be the key factor responsible for the stimulatory impact of Wnt5a on androgen-independent growth and bicalutamide resistance.

Wnt5a upregulates free cholesterol levels and activates AR signaling in PCa cells

Cholesterol is the material for the synthesis of steroid hormones, which implicates in the process of tumorigenesis through regulating hormone levels or signaling pathways. In the analysis of steroid hormones, we found prognenolone level was remarkably decreased in Wnt5a-overexpressing LNCaP cells (Fig. 5F). Prognenolone can be produced from cholesterol by the catalysis of CYP11A1. We then wondered whether the cholesterol levels in the cells is disturbed by Wnt5a. Through

the assessment of intracellular total and free cholesterol levels using cholesterol enzymatic assays and Filipin cholesterol fluorescence staining, we found a significant upregulation of free cholesterol content in LNCaP cells under hormone-deprived medium (HDM) conditions; whereas, 22RV1 cells with Wnt5a knockdown exhibited reduced total and free cholesterol levels under hormone-deprived conditions (Fig. 6A-C). Consistently, the free cholesterol content in LNCaP-BicR cells were elevated compared to the parental cells (Fig. 6C). These findings indicate the heightened free cholesterol content represents one of the potential mechanisms through which Wnt5a fosters castration-resistant prostate cancer. Previously, studies reported cholesterol could activate AKT signaling [21, 22], which further phosphorylates AR to promote its translocation into nucleus and transcriptional activation capacity. Consistently, we confirmed Wnt5a overexpression in LNCaP cells under HDM culturing condition upregulated the expression levels of AR target genes, PSA and TMPRSS2 (Fig. 6D), while knockdown of Wnt5a in 22RV1 cells resulted in the reduced expression of PSA and TMPRSS2 (Fig. 6E).

Finally, we used drug target Mendelian randomization (MR) analysis to investigate the causal relationship between inhibitor of PCSK9 (PCSK9i), a lipid-lowering drug target, and the risk of prostate cancer. In line with expectations, the positive control analysis confirmed that PCSK9i significantly reduced the risk of CHD through MR Egger, weighted median, inverse variance weighted (IVW), simple mode, and weighted mode analyses. Genetically predicted inhibition of PCSK9 had an obvious protective effect on PCa in both the IVW method (OR [95%] = 0.81 [0.71 to 0.92], $p < 0.001$), weighted median method (OR [95%] = 0.86 [0.74 to 0.97], $p = 0.0083$), and weighted mode method (OR [95%] = 0.87 [0.74 to 0.99], $p = 0.0445$) (Fig. 7A). The results of sensitivity analysis showed that there were no heterogeneity and horizontal pleiotropy in all other outcomes ($p > 0.05$), and the leave-one-out method showed that there would be no significant difference in the results after removing any SNP for CHD and PCa (Supplementary Table 4, Fig. 7B, C).

Collectively, the aforementioned findings suggest that Wnt5a may increase free cholesterol levels in prostate cancer cells, potentially contributing to the activation of AR signaling. Lowering cholesterol levels could be a potential approach for treating prostate cancer as well as CRPC.

Discussion

Most patients with prostate cancer are sensitive to initial androgen deprivation therapy, but after a median remission of 18 to 24 months, the vast majority will develop castration-resistant prostate cancer (CRPC). At this stage, many patients develop bone or distant organ metastases, leading to a grim prognosis with an estimated survival of less than 19 months. The challenge of managing prostate cancer clinically primarily stems from this progression to metastatic CRPC and its associated poor outcomes.

It is reported that Wnt5a regulates epithelial-mesenchymal transition in colorectal, prostate, non-small cell lung, and breast cancers [23–26]. High expression of Wnt5a and CAMK2N1 leads to a worse prognosis in prostate cancer [23]. The specific mechanism of Wnt5a in CRPC is not clear. In the present study, we found Wnt5a plays a cancer-promoting role in prostate cancer and is associated with castration therapy resistance in prostate cancer. Wnt5a-overexpressing PCa cells showed significantly enhanced cell proliferation under both regular culture conditions and androgen deprivation conditions; in contrast, knockdown of Wnt5a in PCa cells significantly inhibited cell proliferation.

Androgen receptor antagonists are frequently employed for prostate cancer treatment in clinic due to their favorable effect. Bicalutamide is the first-generation non-steroidal androgen receptor antagonist. Unlike agents that suppress androgen production, bicalutamide operates by competitively binding to the androgen receptor, thereby exerting its therapeutic effects [27]. As AR levels increase, prostate cancer shifts from hormone-dependent to hormone-independent, resulting in resistance to anti-prostate cancer drugs such as bicalutamide [28]. Our study revealed that Wnt5a has the ability to reduce the sensitivity of prostate cancer cells to bicalutamide. Furthermore, we observed that prostate cancer cells resistant to bicalutamide showed increased expression level of Wnt5a. These findings provide evidence supporting the promoting role of Wnt5a in the development of resistance to castration therapy in prostate cancer.

In CRPC, increased expression of testosterone and dihydrotestosterone (DHT) synthetase that catalyzes the synthesis of adrenal androgens, including DHEA and androstenedione, has been observed [29]. In our study, we found significant differences in the expression of enzymes involved in steroid anabolic pathways between PCa cells with different Wnt5a expression levels. CYP1A1 and HSD3B1 were up-regulated in Wnt5a-overexpressing cells, but down-regulated in Wnt5a knockdown cells. In contrast, UGT2B10, AKR1C1 and AKR1C3 were down-regulated in Wnt5a-overexpressing cells, but upregulated in Wnt5a knockdown cells. CYP1A1 is a member of the cytochrome P450 family, which hydroxylates testosterone at the C-6 site, producing hydrophilic metabolites and promotes the excretion of testosterone [30]. HSD3B1 catalyzes the conversion of dehydroepiandrosterone to 17α-hydroxypregnenolone, and also the conversion of pregnenolone and 17α-hydroxypregnenolone to progesterone [31]. HSD17B1 is an enzyme preferably converting estrone to highly potent estradiol, but it is reported in Sertoli cells,

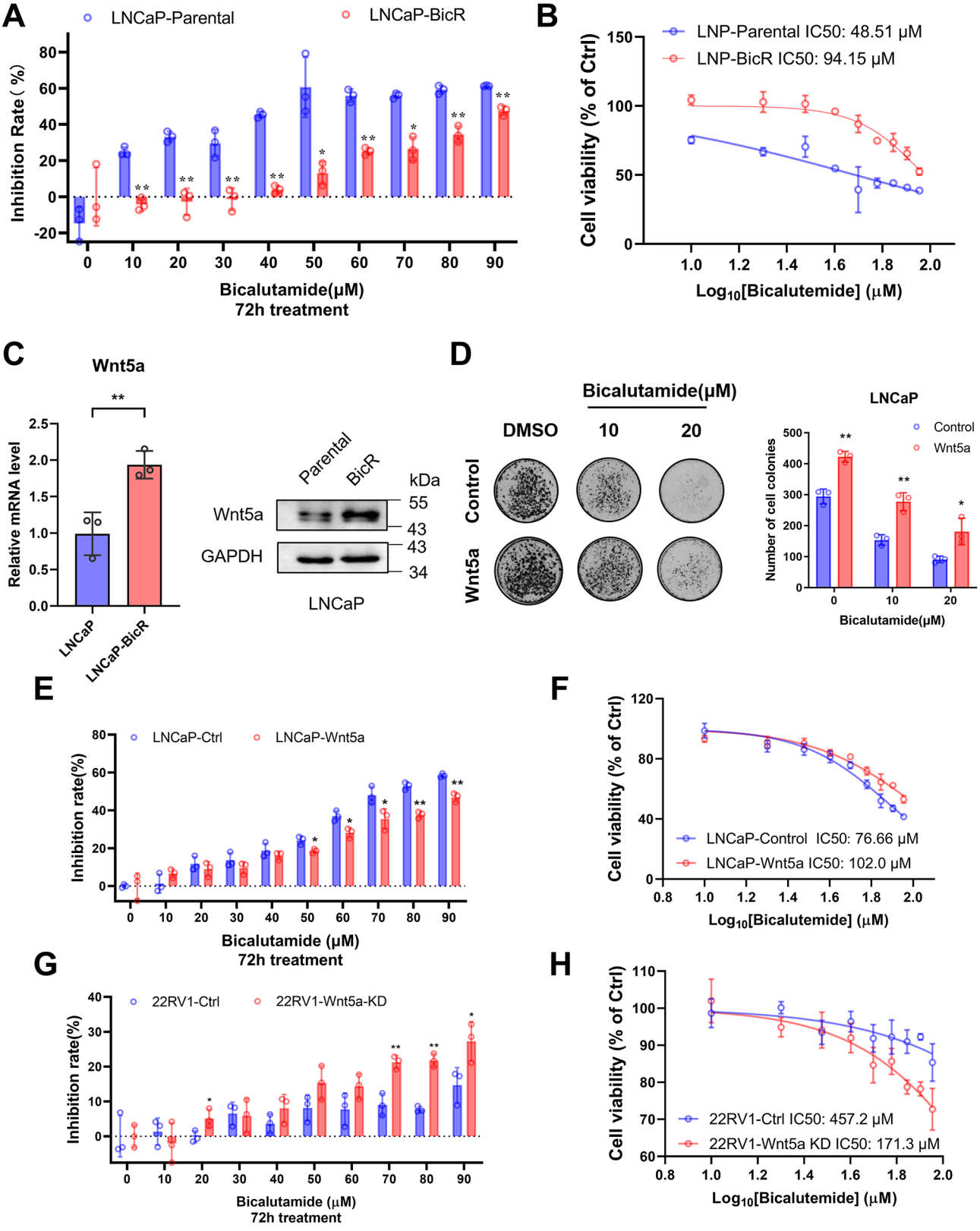

Fig. 2 Wnt5a promotes bicalutamide resistance of prostate cancer cells. (**A**, **B**) Comparison of the sensitivity of bicalutamide-resistant LNCaP cell line (LNCaP-BicR) and LNCaP parental cell line (LNCaP-Parental) to bicalutamide by MTS assay. (**C**) Protein and mRNA levels of Wnt5a in LNCaP-BicR cells and LNCaP-Parental cells were detected by Western blot analysis and RT-qPCR. (**D**) The inhibitory effect of bicalutamide on LNCaP cells with or without Wnt5a overexpression was tested by colony formation assay and (**E**, **F**) MTS assay. (**G**, **H**) The inhibitory effect of bicalutamide on 22RV1 cells with or without Wnt5a knockdown was tested by MTS assay. *$P < 0.05$; **$P < 0.01$

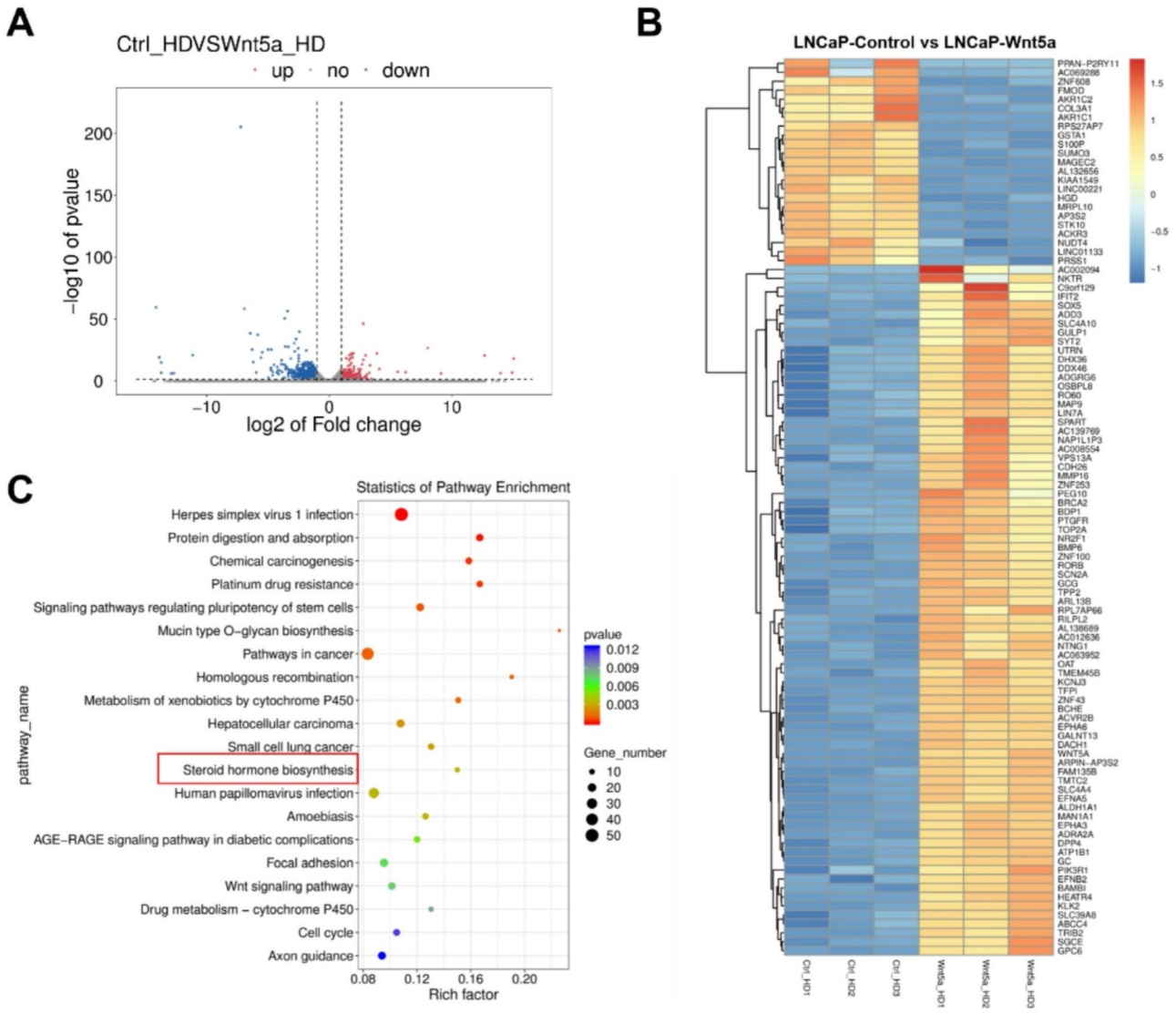

Fig. 3 Wnt5a affects steroid hormone anabolic pathway in PCa cells. (**A**) Volcano plot showing differentially expressed genes between LNCaP-Wnt5a and LNCaP-Control cells under hormone-deprived medium (HDM) culturing conditions. (**B**) Heat map displays the expression levels of the top 100 genes with the most significant differences. (**C**) Differential genes were enriched for the pathways with significant differences after KEGG analysis

HSD17B1 contribututes to steroid synthesis and is required for male fertility [32]. AKR1C1 and AKR1C3 catalyze the dehydrogenation of DHT to 3β-androstenediol and 3α-androstenediol, respectively [33]. UGT2B10 is a member of the UDP-glucuronosyltransferase family, responsible for transferring the glucuronyl moiety from 5'-diphosphoglucuronic acid (UDPGA) to substrates such as steroid hormones and xenobiotics, facilitating their metabolism [34]. The alterations of these enzymes led us to speculate whether Wnt5a promotes CRPC by promoting intratumoral hormone levels. Unexpectedly, although the levels of dehydroepiandrosterone, progesterone, and pregnenolone were reduced in LNCaP cells overexpressing Wnt5a, there was no significant difference in DHT levels between the cells with or without Wnt5a overexpression.

Studies have shown that serum cholesterol and HDL are associated with prostate cancer [35–38]. Elevated levels of LDL cholesterol are linked to a higher risk of developing high-grade prostate cancer [38, 39]. Cholesterol is usually found in the form of free cholesterol and cholesteryl esters. Cholesteryl esters are produced by combining free cholesterol with fatty acids and are the storage and transport form of cholesterol. Free cholesterol is a common precursor for the synthesis of steroid hormones such as androgens and has been implicated in the development of castration-resistance in prostate cancer [40]. Studies that have found out LDL cholesterol promotes proliferation of prostate cancer cells by activating the STAT3 pathway [41]. Cholesterol levels in lipid raft microdomains regulate apoptotic cell death in prostate cancer cells through EGFR-mediated Akt and

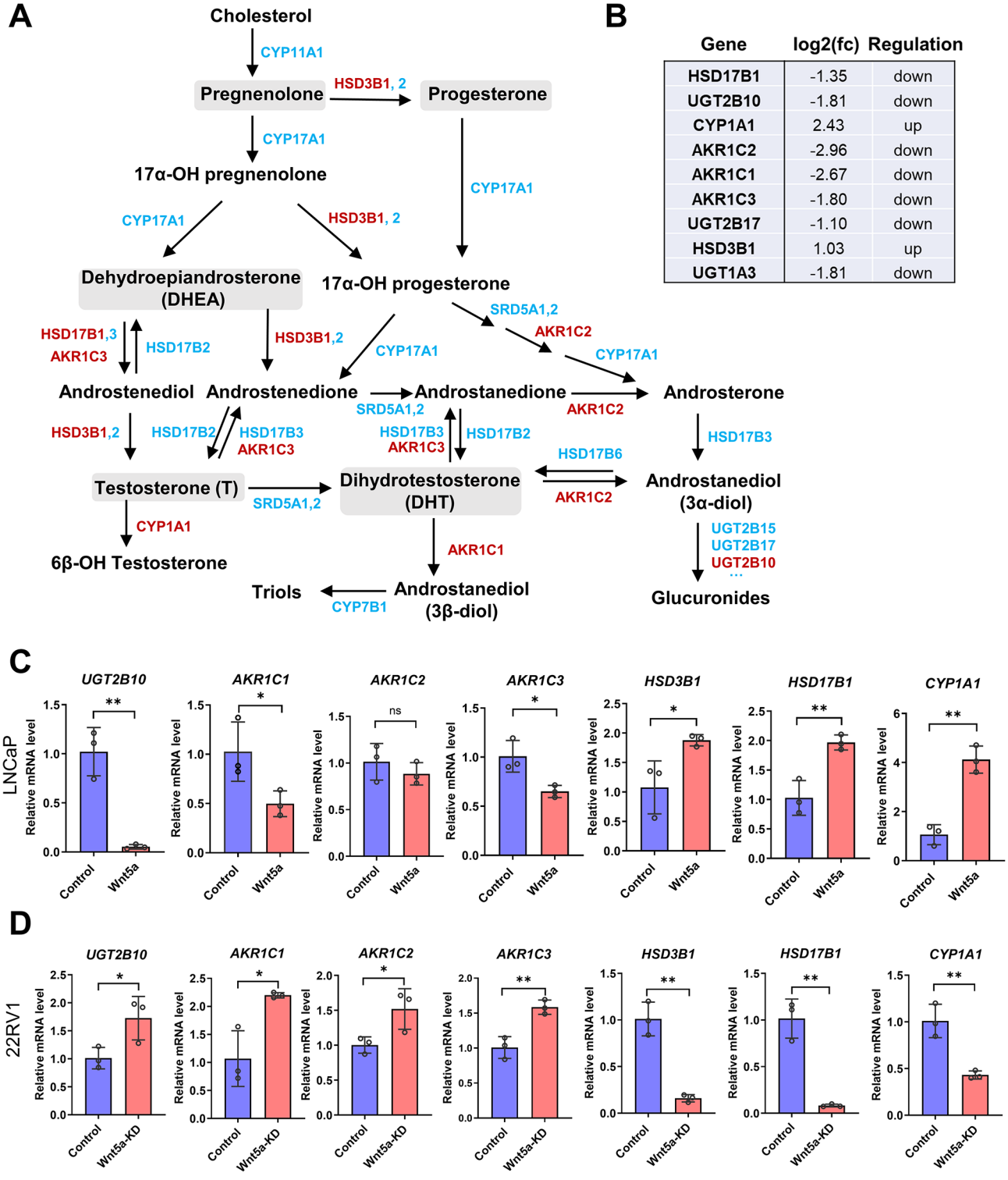

Fig. 4 Wnt5a regulates expression of enzymes involved in steroid hormone biosynthesis. **(A)** Diagram of metabolites and enzymes involved in androgen biosynthesis, with enzymes differentially expressed in Wnt5a-overexpressed LNCaP cells marked in red. **(B)** Differential genes enriched in steroid hormone anabolic pathways and their expression ploidy changes in data of RNA-seq analysis (LNCaP-Wnt5a vs. LNCaP-Control). **(C)** RT-qPCR was employed to investigate the impact of Wnt5a on the expression levels of enzymes related to steroid hormone anabolic pathways in LNCaP cells and **(D)** 22RV1 cells. $^{*}P < 0.05$; $^{**}P < 0.01$; *ns*, not significant

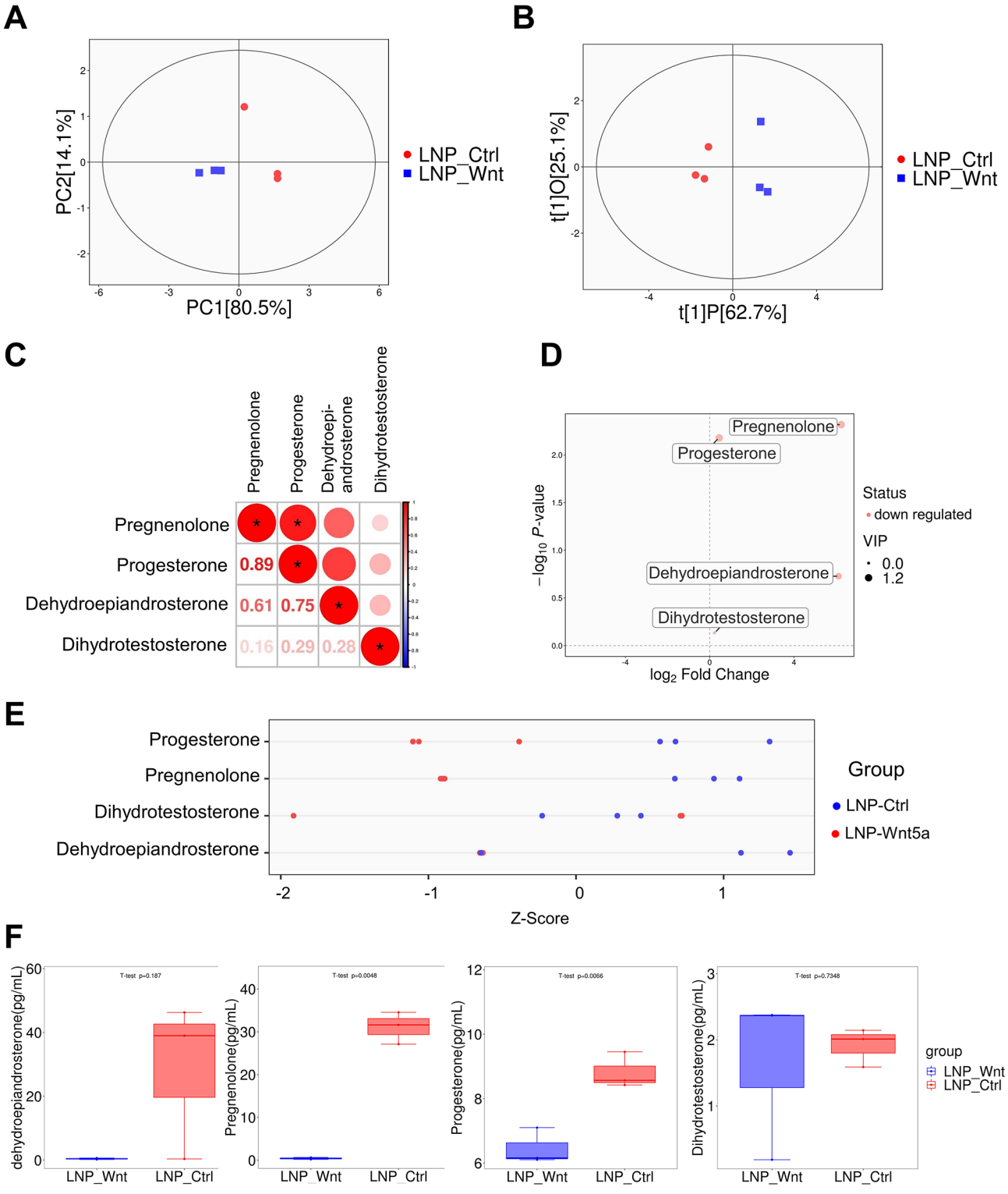

Fig. 5 Detection of steroid hormones in LNCaP-Wnt5a and LNCaP-Control cells under HDM culturing condition. (**A**) Score scatter plot of PCA model for group LNP_Wnt (LNCaP cells with Wnt5a overexpression) vs. LNP_Ctrl (LNCaP control cells). (**B**) Score scatter plot of OPLS-DA model for group LNP_Wnt vs. LNP_Ctrl. (**C**) Correlation analysis of the four detected hormones in the indicated LNCaP cells. (**D**) Volcano plot, (**E**) Z-score plot, and (**F**) Boxplot analysis for group LNP_Wnt vs. LNP_Ctrl

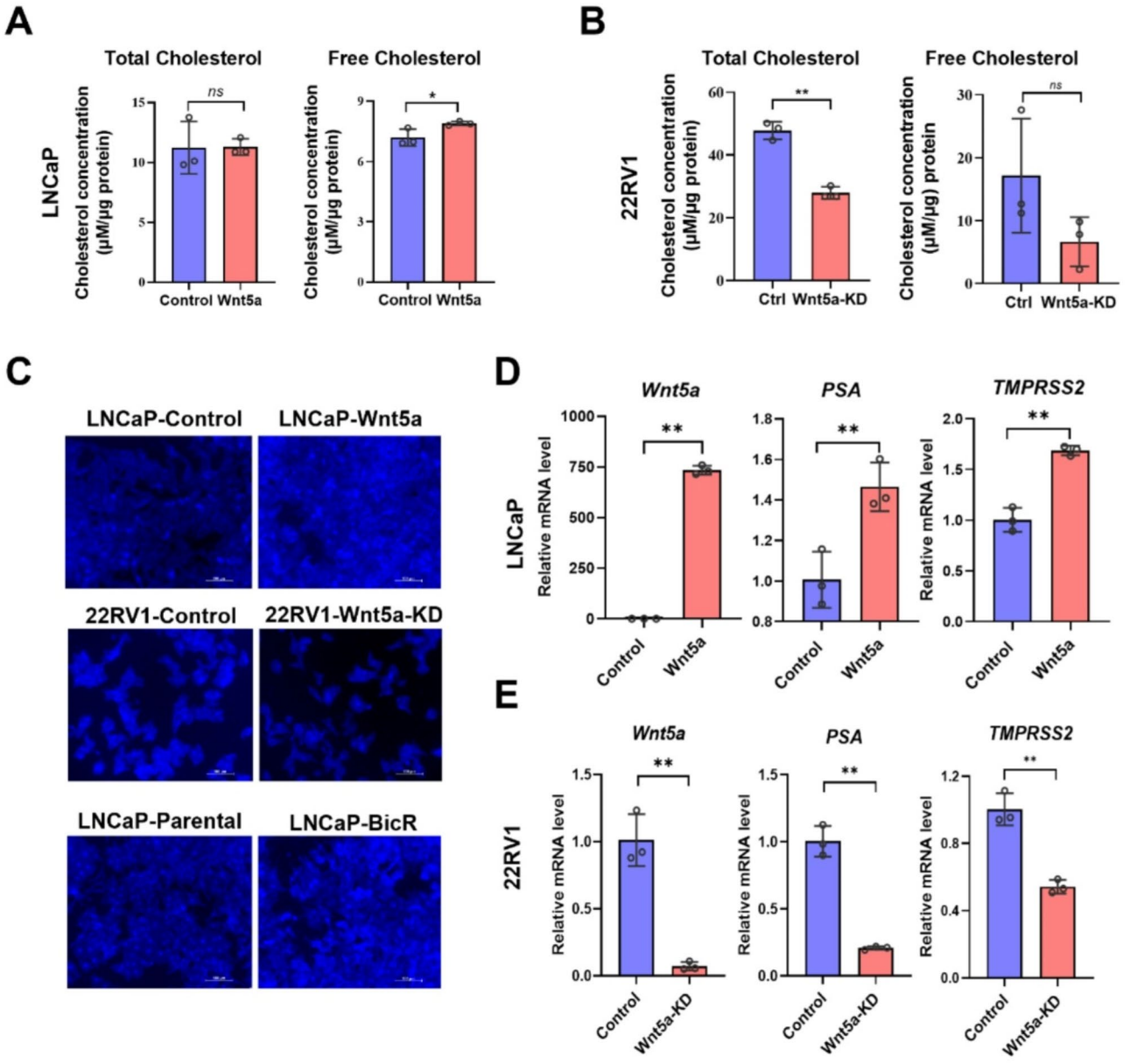

Fig. 6 Wnt5a upregulates free cholesterol levels and activates AR signaling. (**A**) Enzymatic assays of total and free cholesterol in LNCaP and (**B**) 22RV1 cells with different Wnt5a expression levels. (**C**) Filipin staining was used to detect the content of free cholesterol in the indicated cells. (**D**) RT-qPCR was performed to detect the effect of Wnt5a on the expression levels of AR target genes in LNCaP and (**E**) 22RV1 cells. Data are presented as mean ± SD. *$P < 0.05$; **$P < 0.01$; *ns*, not significant

ERK signaling [42]. We conducted further research to determine the influence of Wnt5a on intracellular cholesterol levels. Our results demonstrate that overexpression of Wnt5a in LNCaP cells resulted in elevated free cholesterol content under hormone-deprived culture conditions compared to control cells. Conversely, knocking down Wnt5a in 22RV1 cells led to a decrease in free cholesterol content. Additionally, we observed that Wnt5a overexpression activates the androgen receptor (AR) signaling pathway while Wnt5a depletion resulted in dampened AR signaling, as evidenced by altered expression of AR target genes including PSA and TMPRSS2. These findings suggest that Wnt5a may elevate free cholesterol levels to activate AR signaling pathway, thereby promoting hormone-dependent and -independent growth in prostate cancer cells.

Conclusion

In conclusion, our study demonstrates that Wnt5a plays a pivotal role in enhancing the growth of prostate cancer cells and boosting their resistance to bicalutamide. Importantly, we discovered that Wnt5a's influence on promoting androgen-independent growth and resistance to bicalutamide

A

Outcome	Target	method	nsnp	pval	OR (95% CI)
CHD	PCSK9	MR Egger	13	<0.001	0.5467 (0.3502 − 0.7431)
		Weighted median	13	<0.001	0.5380 (0.3777 − 0.6982)
		Inverse variance weighted	13	<0.001	0.5404 (0.4267 − 0.6542)
		Simple mode	13	<0.001	0.4777 (0.1953 − 0.7601)
		Weighted mode	13	<0.001	0.5297 (0.3622 − 0.6972)
PC	PCSK9	MR Egger	12	0.1754	0.8903 (0.7341 − 1.0465)
		Weighted median	12	0.0083	0.8583 (0.7448 − 0.9718)
		Inverse variance weighted	12	<0.001	0.8130 (0.7088 − 0.9171)
		Simple mode	12	0.0725	0.8189 (0.6216 − 1.0161)
		Weighted mode	12	0.0445	0.8664 (0.7425 − 0.9903)

Fig. 7 PCSK9 inhibitor significantly reduced the risk of PCa. (**A**) The effect of PCSK9 inhibitor on the risk of coronary heart disease and PCa. OR, odds ratio; CI, confidence interval; PCSK9, proprotein convertase subtilisin/kexin 9; CHD, coronary heart disease; PC, prostate cancer. (**B**) Sensitivity analysis of PCSK9 on coronary heart disease (CHD) and (**C**) PCa

is not strongly linked to the local production of androgens within the tumor. Notably, Wnt5a can elevate intracellular free cholesterol levels, potentially activating the androgen receptor (AR) pathway, which may contribute to the progression of castration-resistant prostate cancer. Further systemic investigations are warranted to elucidate the specific impact of alterations in free cholesterol content on the activation of the AR pathway by Wnt5a.

Abbreviations

PCa	prostate cancer
CRPC	castration-resistant prostate cancer
AR	androgen receptor
ADT	androgen deprivation therapy
PSA	prostate specific antigen

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Acknowledgements
Not applicable.

Author contributions
Y.G., Y.F., and M.L. performed the study concept and design; Y.G., X.S., Z.Z., and L.D., and X.L. provided technical and material support; Y.G., Z.Z., L.D., X.S., and X.L. provided acquisition, analysis, and interpretation of data, and statistical analysis; Y.G., Z.Z., and X.S. wrote the original draft; Y.G., Y.F., and M.L. performed review and revision of the paper. All authors read and approved the final paper.

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Data availability
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethical approval and consent to participate
The study was reviewed and approved by the Ethics Committee of Ningxia Medical University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023.[J]. Cancer J Clin. 2023;73(1):17–48. https://doi.org/10.3322/caac.21763.
2. Ji Z, Zhao W, Lin HK, Zhou X. Systematically Understanding the immunity leading to CRPC progression.[J]. PLoS Comput Biol. 2019;15(9):e1007344. https://doi.org/10.1371/journal.pcbi.1007344.
3. Onozawa M, Akaza H, Hinotsu S, Oya M, Ogawa O, Kitamura T, et al. Combined androgen Blockade achieved better oncological outcome in androgen deprivation therapy for prostate cancer: analysis of community-based multi-institutional database across Japan using propensity score matching.[J]. Cancer Med. 2018;7(10):4893–902. https://doi.org/10.1002/cam4.1735.
4. Hotte SJ, Saad F. Current management of castrate-resistant prostate cancer.[J]. Current oncology (Toronto, Ont.), 2010:S72–9;https://doi.org/10.3747/co.v17i0.718
5. Karantanos T, Corn PG, Thompson TC. Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches.[J]. Oncogene. 2013;32(49):5501–11. https://doi.org/10.1038/onc.2013.206.
6. Ozyigit G, Hurmuz P, Yuce D, Akyol F. Prognostic significance of castrate testosterone levels for patients with intermediate and high risk prostate cancer.[J]. World J Clin Oncol. 2019;10(8):283–92. https://doi.org/10.5306/wjco.v10.i8.283.
7. Yamamoto S, Sakamoto S, Minhui X, Tamura T, Otsuka K, Sato K, et al. Testosterone reduction of ≥ 480 Ng/dL predicts favorable prognosis of Japanese men with advanced prostate Cancer treated with Androgen-Deprivation Therapy.[J]. Clin Genitourin Cancer. 2017;15(6):e1107–15. https://doi.org/10.1016/j.clgc.2017.07.023.
8. Kirby M, Hirst C, Crawford ED. Characterising the castration-resistant prostate cancer population: a systematic review.[J]. Int J Clin Pract. 2011;65(11):1180–92. https://doi.org/10.1111/j.1742-1241.2011.02799.x.
9. Moreira DM, Howard LE, Sourbeer KN, Amarasekara HS, Chow LC, Cockrell DC, et al. Predicting time from metastasis to overall survival in Castration-Resistant prostate cancer: results from SEARCH.[J]. Clin Genitourin Cancer. 2017;15(1):60–6. https://doi.org/10.1016/j.clgc.2016.08.018.
10. Carneiro I, Quintela-Vieira F, Lobo J, Moreira-Barbosa C, Menezes FD, Martins AT, et al. Expression of EMT-Related genes CAMK2N1 and WNT5A is increased in locally invasive and metastatic prostate Cancer.[J]. J Cancer. 2019;10(24):5915–25. https://doi.org/10.7150/jca.34564.
11. Ren D, Dai Y, Yang Q, Zhang X, Guo W, Ye L, et al. Wnt5a induces and maintains prostate cancer cells dormancy in bone[. J] J Exp Med. 2018;216:0doi. https://doi.org/10.1084/jem.20180661.
12. Sandsmark E, Hansen AF, Selnæs KM, Bertilsson H, Bofin AM, Wright AJ, Viset T, Richardsen E, Drabløs F, Bathen TF, Tessem, et al. A novel non-canonical Wnt signature for prostate cancer aggressiveness.[J]. Oncotarget. 2017;8(6):9572–86. https://doi.org/10.18632/oncotarget.14161.
13. Murillo-Garzón V, Gorroño-Etxebarria I, Åkerfelt M, Puustinen MC, Sistonen L, Nees M, et al. Frizzled-8 integrates Wnt-11 and transforming growth factor-β signaling in prostate cancer.[J]. Nat Commun. 2018;9(1):1747. https://doi.org/10.1038/s41467-018-04042-w.
14. Jędroszka D, Orzechowska M, Hamouz R, Górniak K, Bednarek AK. Markers of epithelial-to-mesenchymal transition reflect tumor biology according to patient age and Gleason score in prostate cancer.[J]. PLoS ONE. 2017;12(12):e0188842. https://doi.org/10.1371/journal.pone.0188842.
15. Arisan ED, Rencuzogullari O, Freitas IL, Radzali S, Keskin B, Kothari A, et al. Upregulated Wnt-11 and miR-21 expression trigger epithelial mesenchymal transition in aggressive. Prostate Cancer Cells [J] Biology. 2020;9(3). https://doi.org/10.3390/biology9030052.
16. Luo J, Wang D, Wan X, Xu Y, Lu Y, Kong Z, et al. Crosstalk between AR and Wnt signaling promotes Castration-Resistant. Prostate Cancer Growth [J] Onco Targets Ther. 2020;13:9257–67. https://doi.org/10.2147/OTT.S245861.
17. Wang XF, Xu WJ, Wang FF, Leng R, Yang XK, Ling HZ, et al. Telomere length and development of systemic lupus erythematosus: A Mendelian randomization Study[J]. Arthritis Rheumatol. 2022;74(12):1984–90. https://doi.org/10.1002/art.42304.
18. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome[J]. Elife. 2018;7. https://doi.org/10.7554/eLife.34408.
19. Verbanck M, Chen CY, Neale B, et al. Detection of widespread horizontal Pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases[J]. Nat Genet. 2018;50(5):693–8. https://doi.org/10.1038/s41588-018-0099-7.
20. Armstrong CM, Gao AC. Dysregulated androgen synthesis and anti-androgen resistance in advanced prostate cancer. Am J Clin Exp Urol. 2021;9(4):292–300. PMID:34541028.
21. Dufour J, Viennois E, De Boussac H, Baron S, Lobaccaro JM. Oxysterol receptors, AKT and prostate cancer.[J]. Curr Opin Pharmacol. 2012;12. https://doi.org/10.1016/j.coph.2012.06.012.
22. Le NT, Abe JI. Regulation of Kir2.1 function under shear stress and cholesterol Loading.[J]. J Am Heart Assoc. 2018;7. https://doi.org/10.1161/JAHA.118.008749.
23. Carneiro I, Quintela-Vieira F, Lobo J, Moreira-Barbosa C, Menezes FD, Martins AT, Oliveira J, Silva R, Jerónimo C, Henrique R. Expression of EMT-Related Genes CAMK2N1 and WNT5A is increased in Locally Invasive and Metastatic Prostate Cancer. J Cancer. 2019;10(24):5915-25. https://doi.org/10.7150/jca.34564
24. Prasad CP, Chaurasiya SK, Guilmain W, Andersson T. WNT5A signaling impairs breast cancer cell migration and invasion via mechanisms independent of the epithelial-mesenchymal transition[J]. J Exp Clin Cancer Res. 2016;35(1):144. https://doi.org/10.1186/s13046-016-0421-0.
25. Wang B, Tang Z, Gong H, Zhu L, Liu X. Wnt5a promotes epithelial-to-mesenchymal transition and metastasis in non-small-cell lung cancer[J]. Biosci Rep. 2017;37(6). https://doi.org/10.1042/BSR20171092.
26. Liu Q, Yang C, Wang S, Shi D, Wei C, Song J, et al. Wnt5a-induced M2 polarization of tumor-associated macrophages via IL-10 promotes colorectal cancer progression[J]. Cell Commun Signal. 2020;18(1):51. https://doi.org/10.1186/s12964-020-00557-2.
27. Hodgson MC, Astapova I, Hollenberg AN, Balk SP. Activity of androgen receptor antagonist bicalutamide in prostate cancer cells is independent of NCoR and SMRT corepressors.[J]. Cancer Res. 2007;67(17):8388–95. https://doi.org/10.1158/0008-5472.CAN-07-0617.
28. Feng Q, He B. Androgen receptor signaling in the development of Castration-Resistant prostate Cancer.[J]. Front Oncol. 2019;9:858. https://doi.org/10.3389/fonc.2019.00858.
29. Penning TM. Dehydroepiandrosterone (DHEA)-SO(4) depot and Castration-Resistant prostate Cancer.[J]. Vitam Horm. 2018;108. https://doi.org/10.1016/bs.vh.2018.01.007.

30. Lu J, Shang X, Zhong W, Xu Y, Shi R, Wang X. New insights of CYP1A in endogenous metabolism: a focus on single nucleotide polymorphisms and diseases. Acta Pharm Sin B. 2020;10(1):91–104. https://doi.org/10.1016/j.apsb.2019.11.016.
31. Hettel D, Sharifi N. HSD3B1 status as a biomarker of androgen deprivation resistance and implications for prostate cancer[J]. Nat Rev Urol. 2018;15(3):191–6. https://doi.org/10.1038/nrurol.2017.201.
32. Hakkarainen J, Zhang FP, Jokela H, Mayerhofer A, Behr R, Cisneros-Montalvo S et al. Hydroxysteroid (17β) dehydrogenase 1 expressed by Sertoli cells contributes to steroid synthesis and is required for male fertility.[J]. FASEB journal: official publication of the federation of American societies for experimental biology, 2018,32(6):3229–41; https://doi.org/10.1096/fj.201700921R
33. Jin Y, Mesaros AC, Blair IA, Penning TM. Stereospecific reduction of 5beta-reduced steroids by human ketosteroid reductases of the AKR (aldo-keto reductase) superfamily: role of AKR1C1-AKR1C4 in the metabolism of testosterone and progesterone via the 5beta-reductase pathway[J]. Biochem J. 2011;437(1):53–61. https://doi.org/10.1042/BJ20101804.
34. Yu L, Li RW, Huang H, Pham Q, Yu L, Wang TTY. Transcriptomic analysis of LNCaP tumor xenograft to elucidate the components and mechanisms contributed by tumor environment as targets for dietary prostate Cancer prevention Studies[J]. Nutrients. 2021;13(3). https://doi.org/10.3390/nu13031000.
35. Morin EE, Li XA, Schwendeman A. HDL in endocrine carcinomas: biomarker, drug carrier, and potential Therapeutic.[J]. Front Endocrinol. 2018;9:715. https://doi.org/10.3389/fendo.2018.00715.
36. Ruscica M, Botta M, Ferri N, Giorgio E, Macchi C, Franceschini G, et al. High density lipoproteins inhibit oxidative Stress-Induced prostate Cancer cell Proliferation.[J]. Sci Rep. 2018;8(1):2236. https://doi.org/10.1038/s41598-018-19568-8.
37. Jamnagerwalla J, Howard LE, Allott EH, Vidal AC, Moreira DM, Castro-Santamaria R, et al. Serum cholesterol and risk of high-grade prostate cancer: results from the REDUCE study.[J]. Prostate Cancer Prostatic Dis. 2018;21(2):252–9. https://doi.org/10.1038/s41391-017-0030-9.
38. Murtola TJ, Kasurinen TVJ, Talala K, Taari K, Tammela TLJ, Auvinen A. Serum cholesterol and prostate cancer risk in the Finnish randomized study of screening for prostate cancer. Prostate Cancer Prostatic Dis. 2019;22(1):66–76. https://doi.org/10.1038/s41391-018-0087-0
39. Hu P, Cheng R, Chen G, Hu X, Shen Y. Low-Density Lipoprotein Cholesterol and Prostate Cancer: A Retrospective Study. Arch Esp Urol. 2024;77(3):229–34. https://doi.org/10.56434/j.arch.esp.urol.20247703.30
40. Leon CG, Locke JA, Adomat HH, Etinger SL, Twiddy AL, Neumann RD, et al. Alterations in cholesterol regulation contribute to the production of intratumoral androgens during progression to castration-resistant prostate cancer in a mouse xenograft model. Prostate. 2010;70(4):390–400. https://doi.org/10.1002/pros.21072.
41. Jung YY, Ko JH, Um JY, Chinnathambi A, Alharbi SA, Sethi G, et al. LDL cholesterol promotes the proliferation of prostate and pancreatic cancer cells by activating the STAT3 pathway[J]. J Cell Physiol. 2021;236(7):5253–64. https://doi.org/10.1002/jcp.30229.
42. Oh HY, Lee EJ, Yoon S, Chung BH, Cho KS, Hong SJ. Cholesterol level of lipid raft microdomains regulates apoptotic cell death in prostate cancer cells through EGFR-mediated Akt and ERK signal transduction[J]. Prostate. 2007;67(10):1061–9. https://doi.org/10.1002/pros.20593.

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