CYP19A1 Is Regulated by BRD4 and Suppresses Castration-resistant Prostate Cancer Cell Invasion and Proliferation by Decreasing AR Expression

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Research Article

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Abstract

Background

Castration-resistant prostate cancer (CRPC) is the final stage of prostate cancer (PCa). Thus far, there is no effective method for CRPC treatment. Androgen and androgen receptor (AR) play a vital role in CRPC occurrence and drug tolerance. Testosterone is the main androgen in males. CYP19A1 can encode aromatase, a key enzyme that can catalyze the conversion of testosterone to estrogen, and may affect both androgen and AR. CYP19A1 may play a role in the occurrence of CRPC; however, the function of CYP19A1 in CRPC remain unclear.

Methods

Using data from public databases and clinical samples, we analyzed the expression of CYP19A1 in PCa and CRPC specimens. In addition, the influence of CYP19A1 on cell invasion and proliferation was detected in vivo and vitro. The affect of BRD4 on CYP19A1 and AR was detected by qRT-PCR and western blot experiments.

Results

We found that CYP19A1 was downregulated in CRPC samples and cells. CYP19A1 overexpression decreased CRPC cell invasion and proliferation. In addition, CYP19A1 expression was negatively correlated with AR expression. CYP19A1 affected CRPC cell invasion and proliferation ability by suppressing the expression of AR, and this may be attributed to the metabolism of testosterone by CYP19A1. Moreover, the BRD4 inhibitor-JQ1 induced the expression of CYP19A1 and suppressed the expression of AR. Following BRD4 knockdown, CYP19A1 showed a higher expression level, and, AR expression was decreased.

Conclusions

In conclusion, our findings demonstrated that CYP19A1 could reduce CRPC cell invasion and proliferation by targeting AR, and this process could be regulated by BRD4. The BRD4-CYP19A1-AR pathway may be a potential cause of CRPC occurrence. Therefore, CYP19A1 may be a potential therapeutic target for treating CRPC.

Background

Prostate cancer (PCa) remains one of the most common carcinomas in elderly men. It is the second leading cause of cancer-related deaths in Western countries[1]. There are various effective methods for treating PCa, including androgen deprivation therapy (ADT)[2]. As the first line therapy for treating PCa, ADT is effective at the primary stage[3]. However, patients with PCa will inevitably develop castration-resistant prostate cancer (CRPC) after ADT treatment for less than 2 years[4]. When patients relapse into
the castration-resistant stage, the median survival rate is less than 20 months[5]. Moreover, there is still a lack of effective methods for treating CRPC.

Cytochromes P450 (CYPs) are one of the largest and most diverse superfamily of enzymes[6]. CYPs are involved in the metabolism of endogenous and exogenous substances, including drugs, and environmental compounds. As a terminal oxygenase, they are involved in sterol hormone in living organisms[7, 8]. The CYP19A1 gene is a member of the CYP family, which can encode aromatase, a key enzyme that can catalyzes the conversion of androgen to estrogen[9, 10]. Therefore, the expression level of CYP19A1 can affect the testosterone level and may be correlated with the occurrence of CRPC. However, the role of CYP19A1 in CRPC is still unclear.

Bromo-domain-containing protein 4 (BRD4) belongs to the bromo-domain and extraterminal (BET) family. It has been reported to exert carcinogenic functions in various cancers, including prostate cancer[11–13]. As a nuclear protein, BRD4 can bind to histone- acetylated lysine residues via its bromo-domains, and further recruit transcription factors to activate downstream gene expression[11, 14]. JQ1 is a BRD4 inhibitor, which has been developed to target BRD4 and play an anti-tumor role[12, 14, 15]. The function of JQ1 in treating PCa has been demonstrated. However, the use of JQ1 in treating CRPC requires further investigation.

Androgen receptor (AR) is one of the key factors that promote CRPC[16, 17]. In addition, as the main component of androgen, testosterone can impact AR expression and cause CRPC[18]. In the prostate, when AR binds to androgen, the AR-androgen complex translocates to the nucleus. Once in the nucleus, AR binds to androgen response elements upstream of target genes, leading to DNA transcription, and inducing prostate epithelial cell proliferation [17, 19]. Although the mechanisms of CRPC are still unclear, the abnormal amplification of AR by testosterone and structure changes are the main underlying causes of CRPC [20].

In this study, we found that the expression of CYP19A1 was lower in CRPC specimens than in normal tissues. CYP19A1 overexpression decreased CRPC cell invasion and proliferation. In addition, the expression of CYP19A1 directly modulated AR expression, affecting cell invasion and proliferation. CYP19A1 could suppress AR expression because it could metabolize testosterone. Moreover, the expression of CYP19A1 was increased when CRPC cells were subjected to JQ1 treatment or BRD4 knockdown. Therefore, CYP19A1 may be a potential therapeutic target for treating CRPC. BRD4-CYP19A1-AR may be an oncogenic pathway in CRPC development.

Methods

Bioinformatic analysis

starBase (http://starbase.sysu.edu.cn/starbase2/index) is a public database that provides gene expression data for different tumors based on The Cancer Genome Atlas (TCGA). UALCAN (http://ualcan.path.uab.edu/home) is another online web tool that contains sequence data from the
TCGA database. The Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/) database is supported by the NCBI, which is used to collect gene expression chip data. We obtained GSE21034 from GEO database, which had gene chip data from both prostate cancer specimens and para-cancerous samples. The Chinese Prostate Cancer Genome and Epigenome Atlas (CPGEA) (http://www.cpgea.com/), is a database that collects the sequence data of prostate cancer patients in China. We downloaded the data and analyzed the gene expression using R software (R version 4.0.3).

Tissue samples

CRPC samples and para-cancerous samples were collected at Tongji Hospital, School of Medicine, Tongji University. The methods used for collecting the samples were approved by the Ethics Committee of Tongji Hospital, School of Medicine, Tongji University (SBKT-2021-220). Patients who provided the samples were familiar with the process of the experiment and gave informed consent.

Cell culture and drug treating

Prostate cell lines were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). The human normal prostate epithelial cell line RWPE-1 and human prostate cancer cells LNCaP, 22Rv1, and C4-2 were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, Darmstadt, Germany) with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). The human prostate cancer cell VCaP was cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma) with 10% FBS. All cell lines were cultured in a humid environment with 5% CO$_2$ and 95% air at 37°C. JQ1 was purchased from SelleckChem (S7110). The prostate cancer cell lines were treated with different drug concentrations following the manufacturers’ instructions. In addition, prostate cancer cells was cultured in culture medium without androgen.

Cell transfection and lentivirus production

Cell transfection assays were performed with Lipofectamine 2000 (Thermo Fisher Scientific) and polyethylenimine (PEI) (Sigma-Aldrich, St. Louis, MO USA) following the manufacturer's instructions. The shRNA lentivirus was constructed for the knock-down of specific gene. The shRNAs were purchased from Youze Biotechnology Company (Guangzhou, China). The lentivirus packaging plasmids PSPAX2 and PMD2.G, together with shRNAs for specific genes, were transfected into 293 T cells with PEI. The culture medium was replaced after 24 h of transfection, and the medium was collected after another 48 h of culture. Then the medium was added to 22Rv1 and C4-2 cells. The shRNA sequence is shown in Table S1.

Cell invasion assay

After 48 h of transfection, the transfected 22Rv1 and C4-2 cells were digested and seeded into the upper chambers ($N = 1 \times 10^5$) with a non-coated membrane and 200 µl 1640+ 2% FBS; 500 µl of 1640 medium +10% FBS was added to the lower chambers. After 48 h, cells on the upper filters were gently removed with a cotton swab. Cells that migrated to the lower chambers were fixed with carbinol for 30 min. Then, the cells were washed three times to remove the carbinol. The chambers were stained by crystal violet for
20 min and washed three times to remove the crystal violet. Finally, the membranes were dried with a blower and observed using an Olympus microscope (Olympus Corp., Tokyo, Japan).

**Cell proliferation assay**

Cell proliferation was detected using the CCK-8 kit (Dojindo, Kumamoto, Japan). In brief, cells were seeded in 96-well plates (3000 cells/well), and cultured in 200µL 1640 medium+ 10% FBS for 0, 24, 48, 72, and 106 h. After incubation, the cells were treated with CCK-8 reagents according to the manufacturer's instructions, and the absorbance was measured at 450 nm using a multi-mode reader (LD942, Beijing, China).

**In vivo mouse xenografts**

NOD-SCID mice (4 weeks old, 20-22g) were used in the study. These mice were raised in our experiment center, at 22 ± 0.5°C with a relative humidity of 60 ± 2%. The mice were fed with sufficient food and water. Stable CYP19A1 knock down and shControl 22Rv1 cell lines were constructed. Then, 5 × 10^6 22Rv1 cells were injected subcutaneously into the mice. Tumor size was measured using a scale plate externally every 3 days. The weight of the tumor was also measured. The animal experiment was approved by the Ethics Committee of Tongji Hospital of Tongji University.

**RNA extraction and qRT-PCR**

RNA was isolated from tissue samples and cell samples with TRIzol reagent (Sigma-Aldrich) according to the manufacturer's instructions. The RNA was reversed-transcribed to cDNA using a reverse transcription kit (Advantage® RT-for-PCR Kit, Takara Bio Inc., Kusatsu, Japan). qRT-PCR was performed using Applied Biosystems 7500 Sequence Detection System with qRT-PCR reagents and a kit (TB Green® Premix Ex Taq™ II, Takara Bio Inc.) according to the manufacturer's instructions. GAPDH was used as the normal control (NC). RNA expression was quantified according to the 2^−ΔΔCt method. The forward and reverse primer sequences are shown in Table S2.

**Western blot**

Total proteins from samples and cell lines were extracted with RIPA lysis buffer. Protein samples were treated with Dual Color Protein Loading Buffer (Thermo Fisher Scientific). The proteins were separated on SDS–PAGE gels (7.5%, 10%), followed by transfer to nitrocellulose membranes (Merck KGaA, Darmstadt, Germany). Protein-Free Rapid Blocking Buffer (Thermo Fisher Scientific) was used to block the membranes. Then, the membranes were incubated overnight at 4°C with primary antibodies against CYP19A1 (1:1000), AR (1:1000), BRD4 (1:1000), and GAPDH (1:1000) (Abcam UK, Cambridge, UK). On the next day, 1×TBST was used to wash the membranes three times (10 min, each). Then, the membranes were incubated at room temperature for 1 h with a matched secondary antibody (HRP-labeled Goat Anti-Human IgG (H+L), Beyotime Biotechnology, Shanghai, China). Lastly, the membranes were exposed to X-ray irradiation.

**Exogenous testosterone culture and test**
Testosterone was purchased from Beyotime (Beyotime Biotechnology, China) and added (1 nmol/ml) to the culture medium. At 0, 2, 48, and 72 h of culture, the testosterone in the cells was analyzed by using the Testosterone ELISA Kit ((Beyotime Biotechnology, Shanghai, China) S according to the manufacturer's instructions. The absorbance was measured at 450 nm using a multi-mode reader (LD942, Beijing, China).

**Statistical analysis**

The data represent the results of at least three independent experiments. The results are shown as the mean ± standard deviation (SD). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons or Student's t-test for comparison between two groups. A $P$ value less than 0.05 was considered statistically significant.

**Results**

**CYP19A1 downregulation in PCa and CRPC specimens**

To examine the expression of CYP19A1 in PCa, we analyzed the CPGEA, GEO, and TCGA databases. We found that the mRNA level of CYP19A1 was significantly downregulated in Chinese patients (Figure 1A). The result was similar for GSE21034, which included 130 tumor and 18 normal sample tissues (Figure 1B). Similar results were also obtained using the TCGA database. We found that CYP19A1 was downregulated in PCa samples compared with normal samples in the starBase and UALCAN databases (Figure 1C-D). Next, to investigate the expression of CYP19A1 in CRPC specimens, we collected both CRPC tissue samples and para-cancerous normal tissues at our hospital. Similar to the results using the GEO, CPGEA, and TCGA, the expression of CYP19A1 was downregulated at both the mRNA and protein levels (Figure 1E-F). In addition, we determined the expression of CYP19A1 in the normal prostate epithelial cell line RWPE-1 and PCa cell lines. We found that CYP19A1 was downregulated in PCa cell compared with RWPE-1. In addition, CYP19A1 was lower in non-androgen-dependent PCa cells (Figure 1G-H). The results demonstrated that CYP19A1 was downregulated in PCa and CRPC samples.

**Effect of CYP19A1 on CRPC cell invasion and proliferation in vivo and vitro**

As CYP19A1 was downregulated in PCa and CRPC specimens, we hypothesized that CYP19A1 may prevent CRPC occurrence. To verify our hypothesis, we constructed a CYP19A1 overexpression (OE) plasmid and CYP19A1 knockdown (shCYP19A1) lentivirus. We transfected the OE plasmid and shCYP19A1 lentivirus into 22Rv1 and C4-2 prostate cancer cells, which are CRPC cell lines that can grow without androgen[21, 22]. After transfection, the effect of transfection and the function of CYP19A1 in cell invasion and proliferation ability were examined. We found that after transfection with the OE plasmid, the expression of CYP19A1 was upregulated at both the mRNA and protein levels in 22Rv1 and C4-2 cells (Figure 2A-B). On the other hands, after transfection with the shCYP19A1 lentivirus, the expression of CYP19A1 was markedly downregulated in 22Rv1 and C4-2 cell at both mRNA and protein
level (Figure 2C-D). As expected, CYP19A1 could affect cell invasion and proliferation. When transfected with CYP19A1 OE plasmid, invasion ability was decreased for both 22Rv1 and C4-2 cells. However, after transfection with the CYP19A1 knockdown lentivirus, the invasion ability of 22Rv1 and C4-2 cells was increased (Figure 2E-F). In addition, cell growth was slower after 22Rv1 and C4-2 cells were transfected with the CYP19A1 OE plasmid. On the other hand, when transfected with the shCYP19A1 lentivirus, the cells showed faster growth (Figure 2G-H). We injected stable shCYP19A1 and shControl 22Rv1 cells subcutaneously into NOD-SCID mice. Based on the in vivo results, shCYP19A1 increased tumor size and tumor growth in vivo (Figure 2I-K). Collectively the results indicated that CYP19A1 could suppress CRPC cell invasion and proliferation.

**Tumor suppressive function of CYP19A1 via decreased AR expression**

AR is well known to play an oncogenic role in the occurrence of PCa and even CRPC. Moreover, AR plays an essential role in the resistance to anti-androgen therapy. As an important member in the androgen metabolic pathway, AR is essential for the development of PCa and CRPC[23–25]. As mentioned above, CYP19A1 can encode aromatase, which can catalyze the conversion of androgen to estrogen. Therefore, CYP19A1 may regulate the expression of AR by metabolizing testosterone. We hypothesized that there may be a correlation between CYP19A1 and AR.

To investigate our hypothesis, we observed the expression of AR after 22Rv1 and C4-2 prostate cancer cells were transfected with the CYP19A1 OE (oeCYP19A1) plasmid or CYP19A1 knock-down (shCYP19A1) lentivirus. After transfection with the oeCYP19A1 plasmids, the expression of AR in 22Rv1 and C4-2 cells was decreased at both the mRNA and protein levels (Figure 3A-B). We found that the expression of AR was increased following CYP19A1 knockdown (Figure 3C-D). The results indicated that CYP19A1 could affect the expression of AR. Subsequently, we investigated whether CYP19A1 suppresses cell invasion and proliferation by regulating AR. We constructed a shAR lentivirus and found that the shRNA lentivirus can inhibit the expression of AR at both the mRNA and protein levels truly (Figure 3E-F). Following CYP19A1 knockdown, 22Rv1 and C4-2 cell invasion was increased as shown in the results above. Likewise, cell invasion ability was markedly reduced after the shAR lentivirus transfected into 22Rv1 and C4-2 cells. However, there was no difference between shCYP19A1+shAR and shAR for both 22Rv1 and C4-2 cells (Figure 3G-H). Similar results were also obtained for cell proliferation. In comparison with NC cells, shCYP19A1-transfected 22Rv1 and C4-2 cells grew faster. With shAR transfection, the proliferative ability of 22Rv1 and C4-2 cells was markedly decreased compared with that of NC cells. However, the proliferative ability of CRPC cells was not significantly different between shCYP19A1+shAR and shAR (Figure 3I-J).

Taken together, the results demonstrated that CYP19A1 could affect cell invasion and proliferation by targeting AR.

**CYP19A1 mediated androgen-dependent regulation of AR expression**
The main function of CYP19A1 in the human body is to metabolize androstenedione and testosterone. The level of testosterone can affect the expression level of AR[26]. Therefore, we hypothesized that the regulation of AR by CYP19A1 attributed to the metabolism of testosterone. To investigate the hypothesis, we added exogenous testosterone to the culture medium without androgen.

First, we measured the testosterone level in 22Rv1 and C4-2 cells after transfection with the CYP19A1 OE (oeCYP19A1) plasmids and shCYP19A1 lentivirus. We added exogenous testosterone to the culture medium of the oeCYP19A1 group and oeControl group for both CRPC cell lines. After 48 h of culture, the level of testosterone was markedly lower in the cytoplasm of the oeCYP19A1 group compared with the oeControl group (Figure 4A-B). Following CYP19A1 knock-down by shCYP19A1 lentivirus, the level of testosterone was markedly increased (Figure 4C-D). Subsequently, we determined whether the testosterone level would change with the addition of exogenous testosterone to the culture medium of the oeCYP19A1 group. We found although CYP19A1 could decrease the level of testosterone, the level of testosterone in the cytoplasm was not significantly different when exogenous testosterone was added in the culture medium (Figure 4E-F). To determine whether the change in AR expression in CRPC cells following CYP19A1 level alteration is associated with a change in testosterone level, we measured CYP19A1, and AR expression at both the mRNA and protein levels after exogenous testosterone was added in the oeCYP19A1 group. We found that testosterone increased the expression of CYP19A1, and AR at both the mRNA and protein levels. An increase in the level of testosterone reduced the effect of CYP19A1 on AR (Figure 4G-J). However, when we added dihydrotestosterone (DHT), a compound that cannot be metabolized by CYP19A1, to the culture medium, CYP19A1 did not affect AR expression (Figure 4K-L).

Taken together, the results showed that CYP19A1 could regulate the expression of AR by metabolizing testosterone in vitro.

**Upregulation of CYP19A1 by suppressing BRD4 and modulating the sensitivity of the CRPC cell response to JQ1 treatment**

The BRD4 protein has been found to play an important role in the occurrence of PCa. JQ1 as a BRD4 inhibitor may be used to treat PCa. However, the mechanism of JQ1 in the treatment of PCa requires further investigation. In a previous study, researchers found that some anti-cancer compounds can collectively affect the function of BRD4 and aromatase [27]. BRD4 is involved in the regulation of downstream target gene expression[15]. Therefore, we hypothesized that CYP19A1 may be a downstream target regulated by BRD4.

First, we examined the expression of BRD4 using the TCGA database. We found that BRD4 was upregulated in tumor tissues compared with normal tissues (Figure 5A). Then, we examined the correlation between BRD4 and CYP19A1 for GSE21034. We found that there was a negative correlation
between BRD4 and CYP19A1 ($R = -0.32, P = 6.0 \times 10^{-5}$) (Figure 5B). To further confirmed the correlation between BRD4 and CYP19A1, we purchased a BRD4 inhibitor (JQ1) and constructed a shBRD4 lentivirus. We found that after 22Rv1 and C4-2 cells were treated with JQ1, the expression of CYP19A1 was increased at both the mRNA and protein levels; simultaneously, the expression of AR was decreased. In addition, the expression level of CYP19A1 was increased with a higher concentration of JQ1 (Figure 5C-F). BRD4 knockdown in 22Rv1 and C4-2 cells transfected with the shBRD4 lentivirus showed that the expression of CYP19A1 was increased at the both mRNA and protein levels (Figure 5G-I). Furthermore, when CYP19A1 overexpression or downregulated, the expression of BRD4 did not change (Figure S1A-D). The results indicated that BRD4 may be an oncogenic protein that contributes to PCa, and it may play a potential role in suppressing the expression of CYP19A1. Finally, we investigated whether the expression of CYP19A1 modulates the effect of JQ1. We treated shCYP19A1-transfected CRPC cells and NC cells with different concentrations of JQ1. We found that when CYP19A1 was downregulated, the cells were more resistant to JQ1. The IC50 value of JQ1 was increased from 8.92 µM to 16.74 µM for 22Rv1 cells and from 7.62µM to 14.86µM for C4-2 cells (Figure 5L-M). These results demonstrated that CYP19A1 could be regulated by BRD4, which could affect the sensitivity of CRPC cells to treatment with JQ1.

Taken together, CYP19A1 could be downregulated by BRD4, and its function could be reversed by JQ1. In addition, CYP19A1 could suppress CRPC cell invasion and proliferation by regulating AR (Figure 5N).

Discussion

PCa is one of the most common malignant tumors in elderly men. In the United States, the incidence rate of PCa is nearly 20%. It is also the second leading causing of death from cancer in men[1]. In China, PCa is a serious health problem among men. According to the data from the National Cancer Center of China, the incidence and mortality rates of PCa have greatly increased[28]. Androgen plays an important role in the occurrence of PCa and thus is a target in ADT. ADT is a first line treatment for PCa[3]. However, after a treatment period of 18-24 months, PCa patients will inevitably relapse into the castration-resistant stage[4]. Patients with CRPC typically have a median survival time of no more than 2 years[5]. Thus far, there is no effective method for treating CRPC, and the mechanism of CRPC is still unclear.

The androgen metabolic pathway is important in androgen synthesis and metabolism. It has been demonstrated that the abnormal function of the androgen metabolic pathway plays key role in the occurrence of CRPC[29]. The androgen metabolic pathway consists of several components, which include cytochromes P450 (CYPs) [30].CYPs are one of the largest and most diverse superfamily of enzymes[6]. CYPs are involved in the process of metabolizing various substances. As a terminal oxygenase, they are involved in sterol hormone synthesis in living organisms[7, 8]. The CYP19A1 gene is a member of CYPs, which can encode aromatase, a key enzyme that catalyzes the conversion of androgen to estrogen[9, 10]. Therefore, the higher the expression of CYP19A1, the lower the androgen level in serum. In addition, CYP19A1 has been demonstrated to play an important role in the occurrence of breast cancer and even affect patients’ survival[31]. Various drugs can inhibit the function of CYP19A1, such as Letrozole, have been used in clinical settings to treat breast cancer[32]. PCa and breast
cancer share many similarities; for example, they are both hormone-dependent neoplasms. In addition, CYP19A1 can metabolize testosterone to estrogen. Therefore, CYP19A1 may be correlated with the occurrence of CRPC and may be a potential therapeutic target for treating CRPC.

AR binds to androgen and has been found to play an important role in both hormone-dependent PCa and CRPC occurrence and development[33]. AR is a member of the nuclear steroid receptor superfamily of transcription factors. It is located at Xq11-12 and contains eight exons that encode a protein of ~919 amino acids[34–37]. Since 1995, there is a consensus that AR can regulate multiple cellular events such as proliferation, apoptosis, migration, invasion, and differentiation. AR mutation and amplification occur in PCa and CRPC. Moreover, AR expression changes in PCa with lymph node, visceral, and bone metastases[38, 39]. Apart from primary PCa, AR is also highly expressed and transcriptionally active in CRPC[25]. Given its involvement, AR is a target in enzalutamide treatment[40]. A series of studies with vitro models similarly showed increased AR expression and restoration of AR activity in tumors that relapsed after castration, and RNA interference and related approaches established that AR was required for growth in these CRPC models[41–45].

BRD4 has been reported to be overexpressed in various tumors including CRPC[11–13]. As a carcinogenic factor, BRD4 can promote breast cancer cell invasion and migration by targeting the Jagged1/Notch1 pathway[13]. Increased BRD4 expression is related to lung cancer lymph node metastasis, and the mechanism may involve the binding of BRD4 to RelA, an important component of the NF-κB complex, then leading to inflammatory responses[46]. In addition, BRD4 can regulate the transcription of the oncoprotein c-Myc and influence the downstream gene expression[15]. JQ1, a BET inhibitor, has been demonstrated to function by targeting BRD4, which is a transcription factor that belongs to the BET family and has been reported to be a novel and important oncogenic protein in human prostate cancer[47, 48]. Moreover, JQ1 disrupts the BRD4-acetylated lysine interaction, further suppressing the transcriptional activity of BRD4[15]. Here, we found that both BRD4 and JQ1 could affect the expression of CYP19A1, which indicating that CYP19A1 may be a potential downstream gene of BRD4.

In this study, we found that the expression of CYP19A1 was lower in CRPC samples. In addition, CYP19A1 affected CRPC cell invasion and proliferation, which may be attributed to the regulation of AR by CYP19A1. Moreover, as CYP19A1 could metabolize testosterone, it could affect AR expression. We found that CYP19A1 expression could be influenced by BRD4, and it could modulate the effect of JQ1 in CRPC. The results suggest that CYP19A1 may be a potential target for treating CRPC, especially when using BET inhibitors.

**Conclusion**

In conclusion, CYP19A1 may be one of the causes for the occurrence of CRPC. CYP19A1 may affect CRPC cell invasion and proliferation. In CRPC cells, CYP19A1 may target AR and may be regulated by BRD4. The expression of CYP19A1 may influence cell sensitivity to BET inhibitors. Therefore, the BRD4-
CYP19A1-AR pathway may be a novel oncogenic pathway in CRPC and CYP19A1 may be a potential target for treating CRPC.

Abbreviations

PCa Prostate cancer
CRPC Castration-resistant prostate cancer
AR Androgen receptor
BRD4 Bromo-domain-containing-protein 4
ADT Androgen deprivation therapy
CYPs Cytochromes P450
BET Bromo-domain and extraterminal
TCGA The Cancer Genome Atlas
GEO Gene Expression Omnibus
CPGEA Chinese Prostate Cancer Genome and Epigenome Atlas
DHT Dihydrotestosterone

Declarations

Acknowledgments

Not applicable.

Authors’ contributions

Xi Chen designed the study, analyzed the data, and wrote the manuscript, Chengdang Xu, Xinan Wang, and Yicong Yao finished the experiments and collected the data, Gang Wu collected the clinical specimens, Denglong Wu revised the manuscript. All authors read and approved the final manuscript.

Founding

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Availability of data and materials

The datasets analyzed in the study can be acquired from the corresponding author. The data from public databases can be acquired from TCGA (https://www.cancer.gov/about-ncc/organization/ccg/research/structural-genomics/tcga), GEO (https://www.ncbi.nlm.nih.gov/gds), and CPGEA (http://www.cpgea.com/) databases.

Ethic approval and consent to participate

The study was approved by the ethic committee of Tongji Hospital, School of Medicine, Tongji University (SBKT-2021-220). Each participate volunteered to join and signed the informed consent form. The study conformed to the provisions of the Declaration of Helsinki.

Consent for publication

Not applicable.

Conflict of Interests Statement

The authors declare that they have no competing interests.

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**Figures**
Figure 1

**CYP19A1 downregulated expression in PCa and CRPC specimens.** (A) CYP19A1 downregulated in Chinese PCa patients (data from CPGEA database). (B) The expression of CYP19A1 was lower in tumor samples than normal samples (data from GSE21034). (C-D) The transcript expression of CYP19A1 using the TCGA data depend on starBase and UALCAN databases (E) Comparison of mRNA level of CYP19A1 between CRPC and para-cancerous normal prostate tissues from Tongji Hospital. (F) Protein level of CYP19A1 in CRPC patients. Protein was extracted from tumor tissues and normal tissues respectively. (G) The mRNA expression of CYP19A1 among normal prostate epithelial cell: RWPE-1, and human prostate cancer cell lines: VCaP, LNCaP, 22Rv1, and C4-2. (H) Protein level of CYP19A1 at both normal prostate epithelial cell line and prostate cancer cell lines. The experiment of qRT-PCR and western blot used GAPDH as an inner control. * represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$. 
Figure 2

**CYP19A1** affect CRPC cell lines invasion and proliferation in vivo and vitro. (A) The mRNA expression of CYP19A1 after 22Rv1, and C4-2 cells transfected by CYP19A1 overexpression (OE) plasmids and normal control (NC) plasmids. (B) Protein level of CYP19A1 after OE plasmids and NC plasmids transfected into CRPC cells. (C) The mRNA level of CYP19A1 after shCYP19A1#1, shCYP19A1#2, and shControl lentivirus transfected into 22Rv1 and C4-2 cells, respectively. (D) Protein level of CYP19A1 after 22Rv1 and C4-2
cells was transfected with different lentivirus, respectively. (E) 22Rv1 and C4-2 cells which overexpressed CYP19A1 had lower invasion ability than untreated cells. (F) CYP19A1 knockdown 22Rv1 and C4-2 cells have higher invasion ability than NC cells. (G) Cell proliferative ability was decreased after OE plasmids transfected into 22Rv1 and C4-2 cells. (H) Cell proliferative ability was increased after shCYP19A1 lentivirus transfected into 22Rv1 and C4-2 cells. (I-K) The volume, size and weight of tumors in NOD-SCID mice after injected shCYP19A1 and shControl 22Rv1 cells. Every 3 days, the tumors size would be measured. At 27 days, the tumors were excised and the weight was measured. * represents $p<0.05$, ** represents $p<0.01$, *** represents $p<0.001$. GAPDH as inner control at both qRT-PCR and western blot experiments.
**Figure 3**

**CYP19A1 affects CRPC cell invasion and proliferation though targeting AR.** (A) The mRNA level of AR after 22Rv1 and C4-2 cells transfected with oeCYP19A1 and oeControl plasmids. (B) Protein expression of AR after 22Rv1 and C4-2 cell were transfected by different plasmids. (C) The mRNA expression of AR after shControl and shCYP19A1 lentivirus transfected into 22Rv1 and C4-2 cells. (D) The protein level of AR after CYP19A1 knockdown in both 22Rv1 and C4-2 cells. (E-F) The efficiency of shAR lentivirus was...
verified at both mRNA and protein levels in different cell lines. (G-H) 22Rv1 and C4-2 cells invasion ability was detected after transfected with different lentivirus. (I-J) After 22Rv1 and C4-2 cells transfected with different lentivirus, the cell proliferative ability was detected. ns represents no significant, * represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$. GAPDH as an inner control at both qRT-PCR and western blot experiments.

**Figure 4**

**CYP19A1 regulates the expression of AR by metabolizing testosterone.** (A) The level of testosterone in cytoplasm after oeControl and oeCYP19A1 plasmids transfected into 22Rv1 cells. (B) Testosterone level in cytoplasm after ocControl and oeCYP19A1 plasmids transfected into C4-2 cells. (C) The level of testosterone in cytoplasm after shControl and shCYP19A1 lentivirus transfected into 22Rv1 cells. (D) Testosterone level in cytoplasm after shControl and shCYP19A1 lentivirus transfected into C4-2 cells. (E) Testosterone level in cytoplasm after exogenous testosterone added into culture medium between different groups. (F) Testosterone level in cytoplasm after exogenous testosterone added into culture medium between different C4-2 cells. (G-H) The mRNA level of CYP19A1 and AR after exogenous
testosterone added into culture medium between oeControl and oeCYP19A1 groups. (I-J) The protein level of CYP19A1 and AR after exogenous testosterone added into culture medium between different CRPC cell groups. (K-L) CYP19A1 and AR protein expression after exogenous DHT added into culture medium between different CRPC cell groups. ns represents no significant, * represents $P<0.05$, ** represents $P<0.01$.

Figure 5
CYP19A1 regulated by BRD4 and affect the sensitivity of CRPC cell response to JQ1 treatment. (A) The transcript expression level of BRD4 in PRAD using TCGA data. (B) The mRNA expression correlation of CYP19A1 and AR in human PCa samples was detected by GSE21034. (C) The expression of CYP19A1 at mRNA level after 22Rv1 cells treated by different dose of JQ1. (D) Protein expression of CYP19A1 after 22Rv1 cells treated by different dose JQ1. (E) The mRNA level of CYP19A1 in C4-2 cells after treated with different dose of JQ1. (F) The expression of CYP19A1 and AR protein in C4-2 cells after treated with different dose of JQ1. (G) The mRNA expression level of CYP19A1 after 22Rv1 and C4-2 cells transfected with shBRD4 lentivirus. (H-I) Protein level of CYP19A1 and AR after 22Rv1 and C4-2 cells transfected after shBRD4 lentivirus. (L-M) The dose-effect relation curves of JQ1 in 22Rv1 and C4-2 cell after transfected by different lentivirus. (N) The hypothetical model depicting that CYP19A1 regulated by BRD4 then affecting CRPC cell invasion and proliferation by targeting AR. GAPDH as inner control at both qRT-PCR and western blot experiments. * represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$, **** represents $P<0.0001$.

Supplementary Files

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