INTRODUCTION

Prostate cancer (PCa) is the most prevalent male-specific malignancy and the second leading cause of cancer deaths among men worldwide. Androgen ablation is an effective therapy for the treatment of advanced PCa, and many biomarkers of PCa are androgen-regulated genes, including prostate-specific antigen (PSA). However, androgen deprivation therapy invariably induces androgen independence, leading to PCa recurrence, which is referred to as castration-resistant prostate cancer (CRPCa). CRPCa often exhibits enhanced androgen receptor (AR) signaling due to hypersensitivity to androgen, intratumoral steroidogenesis, AR gene amplifications, and AR mutations. For the purpose of exploring the treatment of CRPCa, it is important to identify novel AR-related pathways that drive or contribute to the growth and progression of PCa.

The human testis-specific protein Y-encoded 1 (TSPY1) gene is the largest tandem-repeat multicopy gene in the genome. Normally, TSPY1 is specifically expressed in testicular germ cells and
contributes to spermatogonial cell renewal and propagation.\textsuperscript{11-13} Under pathological conditions, TSPY1 is not only highly expressed in various types of germ cell tumors\textsuperscript{8,14,15} but is also often ectopically expressed in different somatic cell carcinomas.\textsuperscript{16-22} Many studies have focused on the role of TSPY1 during tumor progression. The results have shown that TSPY1 can interact with the cyclin B/CDK1 complex to accelerate cell G2/M phase transition\textsuperscript{23} and interplay with the translation elongation factor eEF1A1/2 to stimulate protein synthesis during promoting cell proliferation.\textsuperscript{24-27} Recently, our research elucidated that TSPY1 increases the formation of the TSPY1-TSPYL5-USP7 complex and decreases the p53 level so as to promote cell proliferation\textsuperscript{28} and that TSPY1 activates the PI3K/AKT and RAS signaling through suppressing the IGFBP3 transcription.\textsuperscript{29} Taken together, the findings suggest an oncogene function of TSPY1.

In PCa cells, TSPY1 is frequently highly-abundantly expressed and positively correlated with the grade of prostate cancer.\textsuperscript{16,17} Li et al (2017) demonstrated that TSPY1, constructing a complex with AR, exacerbates the AR-targeted gene activation and promotes PCa cell proliferation.\textsuperscript{30} Therefore, the causes of the ectopic TSPY1 expression in PCa deserve more attention considering the potential of TSPY1 as a diagnostic marker and therapeutic target.

To address this issue, we investigated the epigenetic regulation and androgen stimulation on TSPY1 expression. Our observations identified that DNA demethylation of the CpG islands (CGI) and enrichment of histones with specific post–translational modifications (PTM), creating a relaxed chromatin in the TSPY1 promoter, are two necessary conditions for the TSPY1 activation. Interestingly, we identified an AR-binding site in the 5′ upstream region of the TSPY1 promoter. The findings reveal that TSPY1 is a novel AR-targeted gene, suggesting a persuasive mechanism underlying the stimulation of androgen on TSPY1 expression.

2 | MATERIALS AND METHODS

2.1 | Human samples

Human testicular biopsies were obtained from three obstructive azoospermic patients with normal spermatogenesis. Peripheral blood samples were collected from 10 male volunteers. Six PCa tissues were selected from 56 localized PCa patients who obtained a histopathologic diagnosis from 2016-2018 at the Department of Urology, West China Hospital, Sichuan University. Written informed consent was obtained from each participant. This study was authorized by the Ethical Committee of West China Hospital, Sichuan University.

2.2 | Cell lines

Five cell lines, including a non–tumorigenic prostate epithelial cell (RWPE1), three PCa cells (LNCaP, 22RV1, and DU145), and one teratoma cell (NT2/D1), were originally purchased from the American Type Culture Collection and maintained in our laboratory. VCaP cell line was kindly gifted by Professor Qiao Zhou, Department of Pathology, West China Hospital, Sichuan University. RWPE1 cells were grown in Defined Keratinocyte-SFM medium (ThermoFisher). LNCaP and 22RV1 cells were grown in RPMI-1640 supplemented with 10% FBS. DU145, NT2/D1, and VCaP cells were maintained in DMEM supplemented with 10% FBS. All cells were incubated at 37°C with 5% CO₂.

2.3 | Antibodies

As shown in Table S1, rabbit polyclonal antibodies for acetyl-histone H3 (ab47915), mouse monoclonal antibodies for H3K9me2 (ab1220), H3K27me3 (ab6002), and H3K4me3 (ab6000) were obtained from Abcam. The rabbit polyclonal antibodies for acetyl-histone H4 (06-598) and AR (39781) were purchased from Millipore and Active Motif, respectively. Mouse and rabbit IgG were purchased from Cell Signaling Technology.

2.4 | Chemical treatments

DNA methyltransferase inhibitor 5´-aza-2´-deoxycytidine (AZA) and HDAC inhibitor trichostatin A (TSA) were purchased from Sigma-Aldrich. Cells were treated separately or simultaneously with different doses of AZA and TSA. Androgen boldenone undecylenate (B) and AR inhibitor enzalutamide (E) were purchased from MedChemExpress. Before treatment, cells were cultured in phenol red-free medium containing 5% charcoal-stripped FBS (SeraPure) for 72 hours. Then cells were cultured for an additional 6 days in the same medium supplemented with different doses of B and E. The culture media were changed every 2 days.

2.5 | RT-PCR

Total RNA was isolated from cell lines and tissues using a SuperPurity RNA Exaction Kit (BioTeke). First-strand cDNA was reverse-transcribed using a RevertAid First-Strand cDNA Synthesis Kit (Vazyme). Quantitative RT-PCR (qRT-PCR) was performed using the AceQ qPCR SYBR Green Master Mix (Vazyme). Reactions were run using a Bio-Rad CFX96 RT-PCR Detection System. The ΔΔCT method was used for data analysis. Each assay was performed in triplicate. The GAPDH gene was used as an internal control. The primers that were used for RT-PCR are listed in Table S2.

2.6 | Bisulfite genomic DNA sequencing

Genomic DNA was bisulfite-treated with the DNA Methylation-Gold Kit (Zymo research) according to the manufacturer’s instructions. The PCR primers used for amplifying the TSPY-CGI are shown.
in Table S2. All PCR products were separated on agarose gels, recovered, and inserted into a pMD19-T vector (TaKaRa Bio). More than 20 clones for each DNA sample were sequenced.

2.7 | siRNA

Transient AR knockdown was performed using the identified siRNA oligos (#390824) obtained from Thermo Fisher. Negative control (#siN00000001-1-5) was purchased from RiboBio.

2.8 | Cell proliferation assay

The overexpressed or knocked down TSPY1 in LNCaP cells was obtained by the infection of lentiviral vectors according to our previous report. 29 Cells were seeded into a 96-well plate (3000 cells/well) and incubated overnight in the previously described conditions. Cell numbers were measured using the Cell Counting Kit-8 (Vazyme) at different times (0, 24, 48, and 72 hours).

2.9 | ChIP and quantitative PCR

ChIP assay was performed using the ChIP-IT Express Enzymatic Kit (Active Motif) according to the manufacturer’s protocol. Briefly, the cells were fixed by 1% formaldehyde. After sonication, the chromatin was immunoprecipitated with antibodies against different histone-PTM or mouse/rabbit IgG for 16 hours. The DNA purification was performed using the ChIP-IT Express Enzymatic Kit (Active Motif). A total of 5 μL of ChIP-enriched DNA was used as a template for quantitative PCR (qPCR) amplification with designated primers (Table S2). The cycle threshold (Ct) values from each run were averaged per tissue or cell, and the ΔΔCT method was then used for the data analysis, in which the value of target DNA fragments that were enriched in each tissue or cell was normalized to the value of the 5% input DNA of each sample. The qPCR data were expressed as the mean ± SD. The ChIP-qPCR assay was repeated twice to confirm the reproducibility of the results. The results were represented as fold changes compared with the control group.

2.10 | Plasmid construction

Three lentiviral vectors for overexpressing or knocking down TSPY1 and a partial TSPY promoter-diverting luciferase gene were constructed by GeneCopoeia. Among them, the full-length cDNA encoding FLAG-tagged TSPY1 was synthesized and cloned into pLVX-ZsGreen1-N1 vector (Clontech). TSPY1-specific shRNA (shTSPY1) were synthesized and inserted into the pLKO.1 vector (Addgene). The partial TSPY1 promoter fragment, from −1530 bp to +34 bp, was synthesized and inserted into the pEZ-LvPG04 vector (Figure S1).

In addition, the TSPY1 promoter-deleted mutants were amplified and subcloned into the pGL3 Basic Luciferase Reporter Vector (Promega). pEZ-M13-AR encoding full-length AR were constructed by GeneCopoeia. The PCR primers for amplifying the TSPY1 promoter are listed in Table S2. The cDNA encoding the AR-DBD domain were amplified with primers listed in Table S2 and inserted into a prokaryotic expression vector pGEX-5x-3 (GE Healthcare [Amersham Biosciences]).

2.11 | Site-directed mutagenesis

To investigate the putative androgen response element (ARE) sites in the TSPY1 promoter, we separately mutated the ARE sequences using the specific primers (Table S2) and a site-directed mutagenesis kit (Mut Express II Fast Mutagenesis Kit V2, Vazyme).

2.12 | In vitro methylation of CpG sites in the TSPY1 promoter

The plasmids containing the partial TSPY1 promoter were treated with a CpG methyltransferase M.Sss I and S-adenosylmethionine (New England Biolabs). The enzymes of HpaII (methylation-sensitive) and MspI (methylation-non-sensitive) were used to verify the methylation efficiency (Figure S2).

2.13 | Luciferase activity assay

After transfection with each construct of TSPY promoter-diverting luciferase for 48 hours, the cells were collected and lysed. The luciferase activity in the cell lysate was measured using a Dual-Luciferase Reporter Assay System (Promega). Renilla luciferase activity was used as an internal control. In addition, the Gaussia luciferase activity of the LNCaP cells infected with the lentiviral TSPY1 promoter-luciferase vector was detected using a Secrete-Pair Dual Luminescence Assay Kit (GeneCopoeia). The activity of secreted alkaline phosphatase was used as an internal control.

2.14 | Expression and purification of GST-fused AR-DBD protein

After the construct of pGEX-5x-AR-DBD was transformed into the *Escherichia coli* host BL21 (DE3), the GST-fused AR-DBD protein was expressed under the induction with 0.1 mmol/L isopropy-β-D-thiogalactoside (Sigma-Aldrich). Then, the harvested bacterial pellet was homogenized by sonication. After centrifugation, the supernatant was purified using Glutathione Sepharose 4B (GE Healthcare). Finally, the purified proteins were detected using the Coomassie brilliant blue method after SDS-PAGE electrophoresis.
Biotin-labeled, biotin-unlabeled (cold competitor), and ARE-mutated (mut competitor) oligonucleotides corresponding to the TSPY1 promoter (Table S2) were synthesized and annealed into double-stranded probes. EMSA was carried out using a Chemiluminescent EMSA Kit (Beyotime) according to the manufacturer’s instructions. Briefly, AR-DBD fusion protein (2 μg) was incubated with 20 fmol of probes at room temperature for 20 minutes. The entire reaction mixtures were separated on a 6.5% nondenaturing, native polyacrylamide gel, and then transferred on a positively-charged nylon membrane (Beyotime). After the membrane was cross-linked using UV light, the biotin signals were successively developed with Streptavidin-HRP and BeyoECL Moon (Beyotime) and visualized after exposure to X-ray films.

**2.15 | EMSA**

The homogenous gene copies of TSPY1 provided a chance to analyze its promoter region. To investigate whether the expression of the TPSY1 gene is regulated by DNA methylation in the promoter region, we first predicted two CpG islands in the 5’ upstream region, spanning ~981 bp to ~676 bp and ~135 bp to +463 bp relative to the putative transcription initiation site of the TSPY1 gene, using the bioinformatics method (http://genome.ucsc.edu/) (Figure 1A). Then, we observed that the CpG sites of the two CGI were hypermethylated, with average methylation (AM) level >0.86 in the peripheral blood cells with TSPY1 silence (Figure 1B, Figure S3). However, the CpG sites were not frequently methylated in the TSPY1-expressed testicular tissues derived from three patients with obstructive azoospermia (Figure 1B, Figure S3). Further investigation of the DNA methylation status of TSPY1 CpG sites in TSPY1-expressed and TSPY1-silent prostate cancer tissues indicated a negative correlation between the TSPY1 expression and the CpG methylation status of the TSPY1 promoter (Figure 1C, Figure S3), revealing that the demethylation of TSPY1 CpG sites is necessary for its transcription activation in prostate tumors.

**2.16 | Statistical analyses**

All data were expressed as the mean ± SD of at least three independent experiments. Statistical significance was tested using Student’s t-test between any two groups. P < 0.05 was considered significant. GraphPad Prism 7 software was used for the statistical analysis.

**3 | RESULT**

**3.1 | Identification of the negative correlation between the TSPY1 expression level and DNA methylation in the TSPY1 promoter**

DNA methylation in the CpG-rich promoter region is the primary silencing mechanism of most genes related to prostate cancer. DNA methylation in the CpG-rich promoter region is the primary silencing mechanism of most genes related to prostate cancer.

![Figure 1](https://example.com/fi1.png)

**FIGURE 1** The CpG methylation status in the 5’ upstream region of the TSPY1 gene in human samples. A, Schematic diagram of two CpG islands in the 5’ upstream region of the TSPY1 gene. Arrows show the primers for BSP-PCR amplification. TSS, transcription start site. B, The two TSPY1 CGI are hypermethylated in the peripheral blood cells while frequently unmethylated in human testicular tissues. C, The two TSPY1 CGI are hypermethylated in TSPY1-silent while hypomethylated in TSPY1-expressed prostate cancer tissues. AM, average methylation level of CpG sites in the two TSPY1 CGI.
and NT2 (Figure 2A), and the CpG sites of the TSPY1 CGI were heavily methylated (AM >81.7%) (Figure 2B). Then, when we treated 22RV1 and DU145 cells with 5′-aza-2′-deoxycytidine (AZA), a DNA methyltransferase inhibitor, the TSPY1 CGI methylation decreased (Figure 2C) and the TSPY1 expression increased (Figure 2D, E). These results suggested that DNA demethylation activated the TSPY1 expression in vitro.

In addition, we constructed the partial TSPY1 promoter into a luciferase reporter vector. When the TSPY1 promoter was artificially methylated using M.SssI, a CpG methyltransferase, the promoter activity was significantly decreased in vitro (Figure 2F). The observation further supports that the methylation of TSPY1 CGI is the primary mechanism of TSPY1 silence.

### 3.3 Regulation of histone post-translational modifications on the TSPY1 expression

Histone-PTM affect chromatin decondensation and DNA accessibility to induce the transcriptional activation of
surrounding genes.\textsuperscript{33-37} To investigate the influence of histone-PTM on the TSPY1 expression, we analyzed the occupancies of acetylated histones H3 (acH3) and H4 (acH4) and methylated histones H3K4me3, H3K9me2, and H3K27me3 on the TSPY1 promoter in different human tissues and cells. The ChIP-qPCR results showed that the amount of acH3 and acH4 binding to the TSPY1 promoter in human testis was 2.2 and 8.9 times that in human blood (Figure 3A). The amount of trimethyl-histone H3K4me3 on the TSPY1 promoter in testicular tissues was 4.8-fold higher than that in human blood (Figure 3A). In contrast, the occupancy of the dimethyl-histone H3K9me2 on the TSPY1 promoter in testicular tissues were lower than that in human blood (Figure 3A). Consistently, in prostate cancer tissues ectopically expressing TSPY1, the amount of acH3, acH4, and H3K4me3 binding to the TSPY1 promoter was 2.4, 4.2, and 2.9 times that in prostate cancer tissue with TSPY1 silence, respectively (Figure 3B). Moreover, the amount of acH4 and H3K4me3 binding to the TSPY1 promoter in the LNCaP cells was 23-fold and 8-fold higher than that in DU145 cells (Figure 3C). These findings suggested that the enrichment of acH3, acH4, and H3K4me3 created a relaxed chromatin environment and facilitated the subsequent TSPY1 expression.

We further investigated whether TSA, a histone deacetylases inhibitor, can activate the expression of the TSPY1 gene in

![Figure 3](https://example.com/figure3.png)

**FIGURE 3** The impact of histone-PTMs on the TSPY1 promoter activity. A-C, ChIP-qPCR analysis comparing the enrichment level of the TSPY1 promoter region occupied by the histone-PTM in different tissues and cells, including testis and blood (A), TSPY1 expression and non-expression PCa (B), and LNCaP and DU145 (C). D and E, Expression of TSPY1 mRNA was activated in 22RV1 (D) and DU145 (E) cells that were treated with the HDAC inhibitor trichostatin A (TSA). F, TSA treatment alone activated the TSPY1 expression in 22RV1 cells. TSA further increased the TSPY1 expression in 22RV1 cells to a higher level than treatment with AZA alone. G, The DNA methylation level of the TSPY1 CGI in 22RV1 cells that were treated with TSA, AZA alone, or a combination of AZA and TSA.
human cells with a hypermethylated TSPY1 CGI. Previous reports demonstrated that AZA and TSA could act synergistically\(^\text{28,39}\) and TSA could induce DNA demethylation.\(^\text{40,41}\) We treated 22RV1 and DU145 cells with TSA and observed that the TSPY1 transcription could be activated in both cell lines (Figure 3D,E). However, the expression level of TSPY1 in 22RV1 treated by TSA was 6.0-fold lower than that treated by AZA (Figure 3F). In addition, the TSPY1 expression level was enhanced approximately 1.5-fold when treated with a combination of AZA and TSA relative to the treatment with AZA alone (Figure 3F). Subsequent BSP sequencing showed a slight demethylation of the TSPY1 CGI in the TSA-treated cells (Figure 3G), while the difference in the demethylation level of the TSPY1 CGI was not observed in the cells exposed to both AZA and TSA and the cells treated with AZA alone (Figure 3G). These results further supported that the presence of acetyl-histones facilitated the TSPY1 expression, and the upregulation was synergistically enhanced by DNA demethylation.

### 3.4 Involvement of TSPY1 in androgen-induced proliferation of prostate cancer cells

Previous studies have shown that both androgen and TSPY1 can stimulate the proliferation of prostate cancer cells\(^\text{30,42}\) and the TSPY1 expression is upregulated by androgen.\(^\text{43,44}\) With the constructed LNCaP cell models stably overexpressing or knocking down TSPY1, we confirmed that TSPY1 overexpression promoted and TSPY1 knockdown decreased the proliferation of LNCaP cells (Figure 4A,B). The treatment of the synthetic steroidal androgen B also increased the proliferation of LNCaP cells with TSPY1 overexpression or decreased TSPY1 expression (Figure 4A,B). However, the treatment of androgen B in the cells with decreased TSPY1 expression cannot significantly promote the cell proliferation (Figure 4B). Furthermore, we investigated whether TSPY1 overexpression can promote the proliferation of PCa cells treated with AR antagonist enzalutamide (E). We observed that the treatment of E inhibited the cell proliferation of LNCaP (Figure 4C). Then, TSPY1 overexpression

---

**FIGURE 4** Androgen B promotes the TSPY1 expression and the LNCaP cell proliferation. A and B, CCK-8 assays showing the increased LNCaP cell proliferation when the TSPY1 expression was enhanced and cells were treated by androgen B. C, TSPY1 rescued the decreased cell proliferation of LNCaP treated with the androgen receptor (AR) antagonist enzalutamide (E, 1 μmol/L). D and E, The TSPY1 and PSA expression level was raised or dropped in LNCaP cells following treatment with different doses of androgen B or androgen receptor inhibitor E. F, AR-targeted siRNA decreased the AR mRNA level in LNCaP cells. G, Decreased AR expression reducing TSPY1 mRNA level and the downregulation of AR not affecting the exogenous TSPY1 expression. H, The TSPY1 expression was not activated by androgen B in DU145 cells. I, After treatment by AZA, the TSPY1 expression was increased by androgen B in DU145 cells.
rescued the decreased proliferation trend in LNCaP cells treated with E (Figure 4C). Taken together, these observations implied that androgen-induced TSPY1 overexpression may be involved in androgen-induced proliferation of PCa cells.

Expectedly, the expression level of the TSPY1 gene was gradually increased when treated with androgen B in a dose-dependent manner while decreased when treated with E (Figure 4D). As a control, the expression of the AR-targeted gene prostate-specific antigen (PSA) was also significantly promoted or inhibited in LNCaP cells when treated with androgen B or AR antagonist E (Figure 4E). In addition, when we knocked down AR expression in LNCaP cells through AR-targeted siRNA (Figure 4F), we observed that the TSPY1 transcription was reduced by approximately 30% (Figure 4G). However, the exogenous TSPY1 expression was not inhibited (Figure 4G). Moreover, when we added different doses of androgen B to the DU145 cells, whose proliferation is known to not respond to androgen, the TSPY1 expression was not activated (Figure 4H).

Considering that DU145 contains hypermethylated TSPY1 CGI, we then treated the cells with AZA and androgen B together, and observed that the TSPY1 transcriptional level was increased 1.3-fold in such cells compared to cells treated with AZA alone (Figure 4I). These results suggested that androgen-mediated AR activation can induce TSPY1 expression, and the DNA demethylation of TSPY1 CGI is an indispensable condition for the stimulation.

3.5 Identification of the androgen receptor binding sites in the TSPY1 promoter region

First, we found and located six potential AR-binding elements (ARE) through an in silico analysis with jaspar (http://jaspar.genereg.net) and animal TFDB (V3.0) (http://bioinfo.life.hust.edu.cn/AnimalTFDB/#/) of the TSPY1 promoter region from −1530 bp to +34 bp (Figure 5A). After constructing a lentiviral vector containing the partial TSPY1 promoter region (from −1530 bp to +34 bp) driving a luciferase reporter gene (Figure S1) and infecting the LNCaP cells, we observed increased expression of luciferase with androgen B treatment, while decreased expression when AR antagonist E was added to the cultured cells (Figure 5B). These observations supported our speculation that the AR may bind to the TSPY1 promoter.
promoter. Furthermore, by conducting ChIP-qPCR and amplifying the TSPY1 promoter sequence enriched by the AR antibodies in the cells of LNCaP and DU145, an abundant amplification of the TSPY1 promoter region from −1288 bp to −1127 bp was observed in LNCaP cells (Figure 5C). The region contains one of six potential ARE, ARE2. After constructing the mutants according to the ARE2 motif sequence and co-transfecting each mutant with AR expression vector, we observed that the activity of the TSPY1-promoter containing mutant ARE2 was not accelerated significantly under the stimulation of androgen B (Figure 5D), suggesting that the disruption of the consensus motif in the putative ARE2 locus results in the disappearance of the regulation of androgen B on the activity of the TSPY1 promoter.

Moreover, we performed a gel EMSA to further assess the probability of AR binding on the TSPY1 promoter. First, we obtained the purified GST-fused AR-partial protein, including DNA binding domain (AR-DBD) (Figure S4). Using a partial DNA sequence, each of which contained single ARE, acting as a template, we synthesized biotin-labeled and unlabeled competitor probes (Figure 5E) and then co-incubated the probes with AR-DBD proteins. The subsequent EMSA results showed that the protein-DNA complexes were formed for the ARE2 probes (Figure 5E), while not for other ARE probes, and the DNA-protein complexes disappeared completely when excess amounts of the unlabeled competitor probes were added (Figure 5F, lane 3). The levels of the DNA-protein complexes were not obviously changed when the mutated probes were added (Figure 5F, lane 4 and 5). All of this suggests that AR binds on the ARE2 site of the TSPY1 promoter.

4 | DISCUSSION

In this study, we first identified that the methylation status of the TSPY1 CGI was negatively correlated with its expression level, and the histone-PTM, especially acH4 and H3K4me3, bound to the TSPY1 promoter and stimulated the TSPY1 expression. Our findings suggest that epigenetic modifications, acting as a primary mechanism, regulate the ectopic activation of TSPY1 in PCa. In addition, AR, acting as an important regulator in PCa progression activated by androgen, was found to accelerate the TSPY1 transcription through occupying the TSPY1 promoter. All of this suggested that TSPY1 was a novel targeted gene of the transcription factor deeply involved in the occurrence and development of PCa (Figure 6).

DNA methylation, as a stable epigenetic modification mark, can silence or downregulate the following gene transcription. DNA methylation changes have been associated with a long list of pathologies of disorders, including cancers. DNA hypomethylation often occurs in repeat DNA sequences and large regions of DNA in tumors. Coincidentally, TSPY1 is the largest tandem-repeat gene in the human genome. We proposed that the demethylation of TSPY1-CGI was programmed to transcribe the gene for normal spermatogenesis, while the expression of the gene contributed to the malignant behavior of PCa, although it is unknown whether the activation of the TSPY1 gene is a result or a cause of the development of PCa.

Histone-PTM are also important epigenetic factors that determine the gene transcriptional activation or inactivation. Much evidence has revealed that the alteration of histone-PTM is involved in tumorigenesis. Recently, a histone demethylase Jumonji domain containing 1A (JMJD1A) was revealed to play a tumor-promoting role in several cancers, including PCa. JMJD1A upregulates the activities of AR and c-Myc and promotes PCa progression. JMJD1A also enhances alternative splicing of AR variant 7 (AR-V7), a hormone-independent truncated form of AR in CRPCa. The acetylation of JMJD1A is critically important for maintaining JMJD1A stability and AR activity in CRPCa. H3K9 demethylated by JMJD1A promotes
the chromatin recruitment of AR. In this study, we found that H3K9me2 occupancies on the TSPY1 promoter may be involved in keeping its silence and that the demethylated H3K9 accompanied by AR recruitment enhances the TSPY1 transcription. Taken together, JMJD1A may be critical in the regulation of the TSPY1 activity during PCa progression.

Androgen and its receptor are critical regulators involved in PCa development and progression. AR, activated by androgen, binds to cis DNA elements in the promoter or enhancer regions and promotes or suppresses the expression of target genes. In this study, we found that AR could occupy the 5’ upstream region of the TSPY1 gene and upregulate TSPY1 transcription. Therefore, the importance of TSPY1 in androgen/AR signal axis is highlighted by our finding. Previous reports on the interaction between TSPY1 and AR demonstrate that TSPY1 complexes with AR and its variant AR-V7 to exacerbate their transactivation, that TSPY1 increases the AR expression in the cells derived from male hepatocellular carcinoma, and that TSPY1 can amplify its self-transcription through acting on its first exon region. Taken together, our finding is crucial because it completes and constructs a positive feedback loop presenting the interaction of androgen, AR, and TSPY1 (Figure 6). It is highly likely that the positive feedback mechanism drives or contributes to the tumorigenic processes in PCa with the ectopic TSPY1 expression.

With the help of ChIP techniques combined with DNA microarray (ChIP on chip) and deep sequencing (ChIP-Seq), the AR-targeted gene network has been revealed to be deeply involved in the PCa progression. In particular, quite a few AR-targeted genes act as critical regulators in CRPCa, including neuroendocrine PCa. In this study, our findings suggested that TSPY1 was a new AR-targeted gene. To the best of our knowledge, TSPY1 is the first AR-related gene to construct a positive feedback loop with AR. Besides interacting with AR, TSPY1, harboring a highly conserved SET/NAP domain (SET, suppressor of variegation, enhancer of zeste, and trithorax; NAP, nucleosome assembling protein), can function as other protumorigenic regulators involved in nucleosome assembly, DNA replication, and transcriptional regulation. All of this supports that TSPY1 may be a new target to use in PCA therapy.

However, due to the limitation of PCa samples, our current study is deficient for the correlation analysis of clinical outcomes of PCa patients with different TSPY1 transcription levels. Considering the heterogeneity of PCa cells, we will further investigate whether the TSPY1-expressing PCa cells survive in radiotherapy and chemotherapy, or the therapy stress activates the TSPY1 expression in PCa cells through the epigenetic alteration.

In conclusion, we found that the epigenetic modifications, including the hypomethylation status of the TSPY1-CGI and the histone the nearby gene expression, are necessary for the TSPY1 transcriptional activation in PCa. More importantly, we identified that TSPY1 was a novel target gene of AR and suggested a positive feedback loop in the androgen-AR-TSPY1 signal axis. Our work provided evidence for the potential of TSPY1 as a new therapy target for PCa.

ACKNOWLEDGMENTS

We are grateful to the study participants for their cooperation. We thank the staff of the Department of Pathology and the Department of Urological Surgery, West China Hospital, Sichuan University, China for collecting PCa samples. We also acknowledge financial support from the National Natural Science Foundation of China (research grants: 81871203 and 81773159).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

Yunqiang Liu https://orcid.org/0000-0001-7691-7630
Yuan Yang https://orcid.org/0000-0002-9206-0312

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7-30.
2. Scher HI, Buchanan G, Gerald W, Butler LM, Tilley WD. Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer. Endocr Relat Cancer. 2004;11:459-476.
3. van der Kwast TH, Schalken J, Ruizeveld de Winter JA, et al. Androgen receptors in endocrine-therapy-resistant human prostate cancer. Int J Cancer. 1991;48:189-193.
4. Chen CD, Welsbie DS, Tran C, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004;10:33-39.
5. Waltering KK, Helenius MA, Sahu B, et al. Increased expression of androgen receptor sensitizes prostate cancer cells to low levels of androgens. Cancer Res. 2009;69:8141-8149.
6. Locke JA, Guns ES, Lubik AA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res. 2008;68:6407-6415.
7. Zhang JS, Yang-Feng TL, Muller U, Mohandas TK, de Jong PJ, Lau YF. Molecular isolation and characterization of an expressed gene from the human Y chromosome. Hum Mol Genet. 1992;1:717-726.
8. Lau YF, Li Y, Kido T. Gonadoblastoma locus and the TSPY gene on the human Y chromosome. Birth Defects Res C Embryo Today. 2009;87:114-122.
9. Salo P, Kääriäinen H, Petrovic V, Lutomski P, Page DC, de la Chapelle A. Molecular mapping of the putative gonadoblastoma locus on the Y chromosome. Genes Chromosomes Cancer. 1995;14:210-214.
10. Tsuchiya K, Reijo R, Page DC, Distèche CM. Gonadoblastoma: molecular definition of the susceptibility region on the Y chromosome. Am J Hum Genet. 1995;57:1400-1407.
11. Honecker F, Stoop H, Reijo R, Lau YF, Bokemeyer C, Looijenga LH. Pathobiological implications of the expression of markers of testicular carcinoma in situ by fetal germ cells. J Pathol. 2004;203:849-857.
12. Lau YF, Li Y, Kido T. Role of the Y-located putative gonadoblastoma gene in human spermatogenesis. Syst Biol Reprod Med. 2011;57:27-34.
13. Schnieders F, Dörk T, Arnemann J, Vogel T, Werner M, Schmidtke J. Testis-specific protein, Y-encoded (TSPY) expression in testicular tissues. Hum Mol Genet. 1996;5:1801-1807.
14. Kersemaekers AM, Honecker F, Stoop H, et al. Identification of germ cells at risk for neoplastic transformation in gonadoblastoma: an immunohistochemical study for OCT3/4 and TSPY. Hum Pathol. 2005;36:512-521.
15. Li Y, Tabatabai ZL, Lee TL, et al. The Y-encoded TSPY protein: a significant marker potentially plays a role in the pathogenesis of testicular germ cell tumors. Hum Pathol. 2007;38:1470-1481.

16. Lau YF, Lau HW, Kömüves LG. Expression pattern of a gonadoblastoma candidate gene suggests a role of the Y chromosome in prostate cancer. Cytogetenet Genome Res. 2003;101:250-260.

17. Kido T, Hatakayama S, Ohyama C, Lau YF. Expression of the Y-Encoded TSPY is associated with progression of prostate cancer. Genes(Basel). 2010;1:283-293.

18. Gallagher WM, Bergin OE, Rafferty M, et al. Multiple markers for melanoma progression regulated by DNA methylation: insights from transcriptomic studies. Carcino genesis. 2005;26:1856-1867.

19. Kido T, Lau YC. Identification of a TSPY co-expression network associated With DNA hypomethylation and tumor gene expression in somatic cancers. J Genet Genomics. 2016;43:577-585.

20. Kido T, Lau YC. The Y-linked proto-oncogene TSPY contributes to poor prognosis of the male hepatocellular carcinoma patients by promoting the pro-oncogenic and suppressing the anti-oncogenic gene expression. Cell Biosci. 2019;9:22.

21. Yin YH, Li YY, Qiao H, et al. TSPY is a cancer testis antigen expressed in human hepatocellular carcinoma. Br J Cancer. 2005;93:458-463.

22. Kido T, Lo RC, Li Y, et al. The potential contributions of a Y-located protooncogene and its X homologue in sexual dimorphisms in hepatocellular carcinoma. Hum Pathol. 2014;45:1847-1858.

23. Li Y, Lau YF. TSPY and its X-encoded homologue interact with cyclin B but exert contrasting functions on cyclin-dependent kinase 1 activities. Oncogene. 2008;27:6141-6150.

24. Kido T, Lau YF. The human Y-encoded testis-specific protein interacts functionally with eucharyotic translation elongation factor eEF1A, a putative oncprotein. Int J Cancer. 2008;123:1573-1585.

25. Spilka R, Ernst C, Mehta AK, Haybaec et. J. Eukaryotic translation initiation factors in cancer development and progression. Cancer Lett. 2013;340:9-21.

26. Kido T, Lau YF. Roles of the Y chromosome genes in human cancers. Asian J Androl. 2015;17:373-380.

27. Oram SW, Liu XX, Lee TL, Chan WY, Lau YF. TSPY potentiates cell proliferation and tumorigenesis by promoting cell cycle progression in HeLa and NIH3T3 cells. BMC Cancer. 2006;6:154.

28. Shen Y, Tu W, Liu Y, et al. TSPY1 suppresses USP7-mediated p53 function and promotes spermatogonial proliferation. Cell Death Dis. 2018;9:542.

29. Tu W, Yang B, Leng X, et al. Testis-specific protein, Y-linked 1 activates PI3K/AKT and RAS signaling pathways through suppressing IGFBP3 expression during tumor progression. Cancer Sci. 2019;110:159-167.

30. Li Y, Zhang DJ, Qiu Y, Kido T, Lau YC. The Y-located proto-oncogene TSPY exacerbates and its X-homologue TSPX inhibits transactivation functions of androgen receptor and its constitutively active variants. Hum Mol Genet. 2017;26:901-912.

31. Nowacka-Zawisza M, Wiśniewski E. DNA methylation and histone modifications as epigenetic regulation in prostate cancer (Review). Oncol Rep. 2017;38:2587-2596.

32. Skawran B, Schubert S, Dechend F, et al. Characterization of a human TSPY promoter. Mol Cell Biochem. 2005;276:159-167.

33. Esteller M. Epigenetics in cancer. N Engl J Med. 2008;358:1148-1159.

34. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128:683-692.

35. Turner BM. Defining an epigenetic code. Nat Cell Biol. 2007;9:2-6.

36. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006;5:769-784.

37. Zhang Z, Yamashita H, Toyama T, et al. Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast. Breast Cancer Res Treat. 2005;94:11-16.

38. Momparler RL. Cancer epigenetics. Oncogene. 2003;22:6479-6483.

39. Walton TJ, Li G, Seth R, McArdle SE, Bishop MC, Rees RC. DNA demethylation and histone deacetylation inhibition co-operate to re-express estrogen receptor beta and induce apoptosis in prostate cancer cell-lines. Prostate. 2008;68:210-222.

40. Ou JN, Torrisani J, Unterberger A, et al. Histone deacetylase inhibitor Trichostatin A induces global and gene-specific DNA demethylation in human cancer cell lines. Biochem Pharmacol. 2007;73:1297-1307.

41. Arzenani MK, Zade AE, Ming Y, et al. Genomic DNA hypomethylation by histone deacetylase inhibition implicates DNMT1 nuclear dynamics. Mol Cell Biol. 2011;31:4119-4128.

42. van Steenbrugge GJ, van Uffelen CJ, Bolt J, Schröder FH. The human prostatic cancer cell line LNCaP and its derived sublines: an in vitro model for the study of androgen sensitivity. J Steroid Biochem Mol Biol. 1991;40:207-214.

43. Lau YC, Li Y, Kido T. Battle of the sexes: contrasting roles of testis-specific protein Y-encoded (TSPY) and TSPX in human oncogenesis. Asian J Androl. 2019;21:260-269.

44. Lau YF, Zhang J. Expression analysis of thirty one Y chromosome genes in human prostate cancer. Mol Carcinog. 2000;27:308-321.

45. Ehrlich M. DNA hypermethylation in disease: mechanisms and clinical relevance. Epigenetics. 2019;14:1141-1163.

46. Kirby MK, Ramaker RC, Roberts BS, et al. Genome-wide DNA methylation measurements in prostate tissues uncovers novel prostate cancer diagnostic biomarkers and transcription factor binding patterns. BMC Cancer. 2017;17:273.

47. Zelic R, Fiano V, Grasso C, et al. Global DNA hypomethylation in prostate cancer development and progression: a systematic review. Prostate Cancer Prostatic Dis. 2015;18:1-12.

48. Hon GC, Hawkins RD, Caballero OL, et al. Global DNA hypomethylation coupled to repressive chromatin domain formation and gene silencing in breast cancer. Genome Res. 2012;22:246-258.

49. Hansen KD, Timp W, Bravo HC, et al. Increased methylation variation in epigenetic domains across cancer types. Nat Genet. 2011;43:678-677.

50. Fan L, Peng G, Sahgal N, et al. Regulation of c-Myc expression by the histone demethylase JMJD1A is essential for prostate cancer cell growth and survival. Oncogene. 2016;35:2441-2452.

51. Wilson S, Fan L, Sahgal N, et al. The histone demethylase KDM3A regulates the transcriptional program of the androgen receptor in prostate cancer cells. Oncotarget. 2017;8:30328-30343.

52. Fan L, Zhang F, Xu S, et al. Histone demethylase JMJD1A promotes alternative splicing of AR variant 7 (AR-V7) in prostate cancer cells. Proc Natl Acad Sci USA. 2018;115:E4584-E4593.

53. Xu S, Fan L, Jeon HY, et al. p300-mediated acetylation of histone demethylase JMJD1A prevents its degradation by ubiquitin ligase STUB1 and enhances its activity in prostate cancer. Cancer Res. 2020;80:3074-3087.

54. Labbé DP, Brown M. Transcriptional regulation in prostate cancer. Cold Spring Harb Perspect Med. 2018;8:a030437.

55. Zhu S, Zhao D, Li C, et al. BMI1 is directly regulated by androgen receptor to promote castration-resistance in prostate cancer. Oncogene. 2020;39:17-29.

56. Gritsina G, Gao WQ, Yu J. Transcriptional repression by androgen receptor: roles in castration-resistant prostate cancer. Asian J Androl. 2019;21:215-223.

57. Li S, Mo C, Huang S, et al. Over-expressed Testis-specific Protein Y-encoded 1 as a novel biomarker for male hepatocellular carcinoma. PLoS ONE. 2014;9:e89219.

58. Kido T, Lau YF. The Y-located gonadoblastoma gene TSPY amplifies its own expression through a positive feedback loop in prostate cancer cells. Biochem Biophys Res Commun. 2014;446:206-211.

59. Takayama K, Kaneshiro K, Tsutsui S, et al. Identification of novel androgen response genes in prostate cancer cells by coupling chromatin immunoprecipitation and genomic microarray analysis. Oncogene. 2007;26:4453-4463.
60. Takayama K, Tsutsumi S, Katayama S, et al. Integration of cap analysis of gene expression and chromatin immunoprecipitation analysis on array reveals genome-wide androgen receptor signaling in prostate cancer cells. Oncogene. 2011;30:619-630.
61. Massie CE, Lynch A, Ramos-Montoya A, et al. The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. EMBO J. 2011;30:2719-2733.
62. Hoang DT, Iczkowski KA, Kilari D, See W, Nevalainen MT. Androgen receptor-dependent and-independent mechanisms driving prostate cancer progression: opportunities for therapeutic targeting from multiple angles. Oncotarget. 2017;8:3724-3745.
63. Centenera MM, Selth LA, Ebrahimie E, Butler LM, Tilley WD. New opportunities for targeting the androgen receptor in prostate cancer. Cold Spring Harb Perspect Med. 2018;8:a030478.
64. Fujita K, Nonomura N. Role of androgen receptor in prostate cancer: a review. World J Mens Health. 2019;37:288-295.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Leng X, Liu M, Tao D, et al. Epigenetic modification-dependent androgen receptor occupancy facilitates the ectopic TSPY1 expression in prostate cancer cells. Cancer Sci. 2021;112:691–702. https://doi.org/10.1111/cas.14731