Nuclear receptor LRH-1 functions to promote castration-resistant growth of prostate cancer via its promotion of intratumoral androgen biosynthesis

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Abstract

Targeting of steroidogenic enzymes (e.g. abiraterone acetate targeting CYP17A1) has been developed as a novel therapeutic strategy against metastatic castration-resistant prostate cancer (CRPC). However, resistance to steroidal inhibitors inevitably develops in patients, the mechanisms of which remain largely unknown. Liver receptor homolog-1 (LRH-1, NR5A2) is a nuclear receptor, originally characterized as an important regulator of some liver-specific metabolic genes. Here we report that LRH-1, which exhibited an increased expression pattern in high-grade prostate cancer and CRPC xenograft models, functions to promote de novo androgen biosynthesis via its direct transactivation of several key steroidogenic enzyme genes, elevating intratumoral androgen levels and reactivating AR signaling in CRPC xenografts as well as abiraterone-treated CRPC tumors. Pharmacological inhibition of LRH-1 activity attenuated LRH-1-mediated androgen deprivation and antiandrogen resistance of prostate cancer cells. Our findings not only demonstrate the significant role of LRH-1 in the promotion of intratumoral androgen biosynthesis in CRPC via its direct transcriptional control of steroidogenesis but also suggest targeting LRH-1 could be a potential therapeutic strategy for CRPC management.

Precis

Our present findings not only demonstrate the significant role of the nuclear receptor LRH-1 in the promotion of intratumoral androgen biosynthesis in castration-resistant prostate cancer (CRPC) via its direct transcriptional control of steroidogenesis but also suggest targeting LRH-1 could be a potential therapeutic strategy for CRPC management.
Introduction

Hormone therapy is still the mainstay medical treatment option for most prostate cancer patients relapsed from prostatectomy or with metastasis, largely based on its androgen-dependent characteristic. However, this treatment is only effective temporarily, as many patients will inevitably fail in this therapy and progress to a fatal hormone-refractory (or androgen-insensitive) castration-resistant (CRPC) stage within 2-3 years. Accumulated evidences suggest that this advanced disease progression involves multiple but interconnected molecular mechanisms related to dysregulated persistent androgen receptor (AR) signaling and altered androgen biosynthesis and metabolism (1). Among these mechanisms, intraprostatic conversion of adrenal androgens to testosterone and intratumoral androgen biosynthesis via the *de novo* pathway resulting in AR reactivation in CRPC has received particular attention and persistent AR-axis signaling is regarded as a critical therapeutic target, as the involved steroidogenic enzymes are potential therapeutic targets leading to AR inactivation in prostate cancer cells.

The normal prostate gland and prostate tumors express the full set of steroidogenic enzymes necessary for the conversion of adrenal androgens to testosterone and DHT. Previous studies on CRPC tissues and experimental xenografts clearly show that intratumoral androgen levels still remain moderately high and also expressions of androgen-responsive genes (*AR, PSA*) are not suppressed or AR is still highly expressed and transcriptionally reactivated following androgen deprivation therapy (2,3), indicating the AR signaling still remains activated in CRPC tumors or tumors remain androgen-dependent. Moreover, many enzyme genes involved in androgen biosynthesis (e.g. *HSD3B2, AKR1C3, CYP17A1*) show up-regulation in metastatic CRPC tissues and CRPC xenograft tumor models (4,5). Studies performed in experimental CRPC xenograft models provide evidence that tumor explants show increased expressions of steroidogenic enzymes (*CYP11A1* and *CYP17A1*) and are accompanied with enhanced capacity of *de novo* synthesis of androgens from cholesterol.
during castration-resistant progression (2,6). These studies support the mechanism on that intratumoral androgen biosynthesis by conversion from adrenal androgens or de novo androgen biosynthesis causing the reactivation of AR signaling in prostate cancer cells makes a key contribution to drive the CRPC progression. Based on this, inhibition of systemic or intraprostatic biosynthesis of androgens by targeting CYP17A1, a key enzyme in androgen biosynthesis, has thus been applied as a novel therapeutic approach in the treatment of CRPC (7). The recently reported phase-2/3 clinical trials indicate that combined treatment with abiraterone acetate (a selective irreversible CYP17A1 inhibitor) and prednisone (a synthetic glucocorticoid) can improve the overall survival of chemotherapy-treated or -naïve metastatic CRPC patients (8,9). However, progression to acquired resistance to abiraterone treatment is still invariably developed in CRPC patients shortly, and further increased up-regulation of steroidogenic enzymes (including CYP17A1 and other steroidogenic enzymes) and restored AR reactivation are also detected in abiraterone-treated CRPC xenograft models (6,10). However, how the steroidogenic enzymes are regulated in prostate cancer cells and also the exact mechanisms involved in abiraterone-resistance in CRPC patients, particularly the induction of increased CYP17A1 expression and reactivation of AR, still remain unclear.

Nuclear receptor (NR) liver receptor homolog-1 (LRH-1, NR5A2) belongs to NR5A subfamily of NR superfamily. Although LRH-1 is constitutively active and regarded as an orphan NR, some crystallographic studies suggest that some phospholipids can bind and act as endogenous ligands to LRH-1 and modulate its transactivation via selectivity of coregulators (11,12). LRH-1 is expressed at moderate to high levels in fetal and adult organs of endodermal origin, including liver, pancreas and intestine, steroidogenic organs (adrenal gland) and gonads (testis and ovary), placenta and adipose tissue.

LRH-1 has been implicated in tumorigenesis of some cancers, though its exact oncogenic roles are still unclear and controversial. Increased LRH-1 expression is shown in breast cancer (13), colon cancer (14), pancreatic cancer (15) and ovarian granulosa cell tumor.
(16). A few functional analyses show that LRH-1 can promote breast cancer growth via its transcriptional regulation of aromatase expression (13), auto-regulatory loop with ERα (17), and enhance breast cancer cell motility and invasiveness by reorganization of actin cytoskeleton and E-cadherin cleavage (18). Its overexpression can facilitate tumor immune escape in colon cancer cells by local promotion of glucocorticoid synthesis (14) and estradiol biosynthesis in endometrial cancer cells by regulation of steroidogenic genes (19), suggesting that LRH-1 may act as a prosteroidogenic factor. A recent genome-wide association study (GWAS) also links mutation in *LRH-1* gene to the development of pancreatic cancer (20). Transgenic knockout studies show that heterozygous deletion of LRH-1 can suppress the intestinal tumorigenesis in mouse intestinal tumor models (21), whereas its loss can promote the oncogenic Kras-driven pancreatic tumorigenesis (22).

The main goal of this study was to determine the role of LRH-1 in the growth regulation of CRPC. Here, we showed that LRH-1 displayed an increased expression pattern in high Gleason-score prostate cancer tissues, CRPC xenograft models and also abiraterone-treated CRPC tumors, and its overexpression could promote both *in vitro* androgen deprivation-resistant and *in vivo* castration-resistant growth capacity in AR-positive prostate cancer cells via its direct transactivation of some key steroidogenic enzyme genes including *CYP17A1* and increased intratumoral production of androgens in a CYP17A1-dependent manner. Importantly, the resistance of prostate cancer cells to androgen deprivation could be attenuated by suppression of LRH-1 activity by a LRH-1-specific inverse agonist ML-180. Thus, our results demonstrate for the first time a novel role for LRH-1 in the regulation of intratumoral androgen biosynthesis in prostate cancer and also suggest a potential therapeutic strategy for CRPC by targeting LRH-1.
Materials and Methods

The detailed methods (plasmid construction, retroviral transduction, in vitro cell growth assays and drug treatments, RNA interference, RNA and protein analyses, luciferase reporter assay, chromatin immunoprecipitation) are described in Supplementary Methods, and sequence information on oligonucleotides used for various applications was listed in Supplementary Tables S1-S4.

Human prostatic tissues and immunohistochemistry

A set of prostatic tissue microarray (TMA) slide containing a total of 15 validated cases of hormone-resistant prostate cancer, 26 cases of hormone-naïve or neo-adjuvant-treated prostate cancer and 9 cases of normal prostatic tissues (Prostate Cancer Biorepository Network) and also a self-constructed TMA set containing a total of 108 validated cases of prostate cancer with various Gleason-scores (GS), 20 cases of benign prostatic hyperplasia and 10 cases of normal or adjacent normal prostatic tissues for LRH-1 immunohistochemistry using a rabbit polyclonal NR5A2/LRH-1-antibody (LS-A2447, LifeSpan BioSciences) and a streptavidin-biotin-peroxidase amplification method (23). The LRH-1 immunosignals in stained sections were evaluated by a semi-quantitative immunoreactivity scoring (IRS) method as described previously (24). All human tissues were obtained from patients with informed consent and approvals from the institutional clinical research ethics committees, and studies were conducted in accordance with the Declaration of Helsinki.

Cell lines and cell culture

A panel of immortalized human prostatic epithelial cell (RWPE-1, RWPE-2, HPr-1, HPr-1AR, PWR-1E, PNT1A, PNT2, BPH-1, BPH-1-AR, RC-165N, PZ-HPV-7) and prostate cancer cell lines (LNCaP, VCaP, DuCaP, LAPC-4, MDAPCa2b, CWR22Rv1, CA-HPV-10, DU145, RC-58T) were used in this study. RWPE-1, RWPE-2, PWR-1E, PZ-HPV-7, LNCaP, LAPC-4, MDAPCa2b, CWR22Rv1, CA-HPV-10, PC-3 and DU145 were obtained from ATCC; PNT1A and PNT2 from ECACC; metastatic LNCaP sublines (C4, C4-2, C4-2B)
from ViroMed Laboratories. These cell lines were provided by the original investigators: BPH-1 from Dr. S. Hayward; BHP-1-AR and LNCaP-BC from Dr. S. Yu; RC-165N and RC-58T from Dr. J.S. Rhim; HPr-1 and HPr-1AR from Dr. Y.C. Wong; VCaP and DuCaP from Dr. K. Pienta; androgen-insensitive LNCaP sublines (104S, 104R1, 014R2) from Prof. S. Liao. Various cells were cultured in different media as described previously (23,25,26). All cell lines were used within 10-20 passages of thawing original stocks every 1-2 months, and were routinely tested for mycoplasma contamination and authenticated by short tandem-repeat (STR) DNA profiling as described previously before the study (27).

Castration-resistant prostate cancer xenograft tumors

Xenograft model of primary prostate cancer tissue CWR22 and its castration-relapse subline CWR22-AIM were established previously (23). To establish the VCaP-CRPC xenografts, VCaP cells (2×10^6 cells/100 μl mixed 1:1 in Matrigel) were injected subcutaneously into the flanks of 6-8 week old intact male SCID mice and allowed to grow for 14 weeks totally. Host mice bearing xenograft tumors were then orchiectomized when tumor sizes reached about 0.8 cm³. The relapsed xenograft tumors were allowed to grow to sizes of about 1.2 cm³. Biopsies of tumors were acquired at same days when castration was performed (pre-castration), 4-day post-castration and 8-week post-castration (castration-relapse) for mRNA analysis and steroid hormone measurements. For in vivo abiraterone treatment study, once the castration-relapsed VCaP tumors regrew to the pre-castration sizes, tumor bearing mice were randomly assigned to daily intraperitoneal injection of vehicle (5% benzyl alcohol plus 95% olive oil mixture) or abiraterone (0.5 mmol/kg/day in vehicle) for 3 weeks. Tumor needle biopsies were acquired at pre-treatment and 3-week post-treatment for mRNA analysis.

In vivo tumorigenicity assay

In vivo tumorigenicity and castration-resistance of LRH-1- and vector-transduced clones (3×10^6 cells suspended in 100 μl 1:1 growth medium-Matrigel mixture) were evaluated by s.c.
injection of cells into the flanks of intact male SCID mice as described previously (28) and allowed to grow for 7 weeks. At about 7th-week, mice were orchiectomized bilaterally and tumors were allowed to grow in castrated mice until at 10th-week. Tumor volumes (mm$^3$) were measured by electronic calipers using the formula (width$^2$×length×0.52). At the end of experiments, tumors were dissected and snap-frozen in liquid nitrogen for steroid extraction. All animal experiments were performed in accordance with the university laboratory animal guidelines and with approval from the institutional animal experimentation ethics committee.

**Steroid measurements**

Steroids were extracted from cultured cells and snap-frozen tumor tissues by diethyl ether following previous procedures with modifications (4,29). For steroid extraction from cultured cells, pelleted cells were washed twice with PBS and lysed with cell lysis buffer. For frozen tumor tissues, samples were weighed and homogenized in PBS. To serve as an internal control, 50 pg of 3-deuteride-testosterone (T-d3) and dihydrotestosterone (DHT)-d3 was added to the homogenates. The samples were extracted with 8 mL of diethyl ether and the aqueous phase was frozen in a dry-ice/ethanol bath with the organic phase decanted. The residue was then resuspended in 0.5 mL of water before extraction with methylene chloride. Standards for T and DHT (Fluka Chemika) were prepared in parallel. The resulting oximes were analyzed by ultra-high performance liquid chromatography (UPLC) coupled with electrospray ionization tandem mass spectrometry (LC-MS-MS) using an Agilent 1290 LC and Agilent 6460 Triple Quadrupole LC/MS system. Ions monitored were 289.4 > 109.2 and 292.4 > 109.2 for T and T-d3, respectively; and 291.2 > 255.4 and for 294.2 > 258.4 DHT and DHT-d3, respectively. The lower limits of quantification (LLOQ defined as a signal/noise ≥ 5) for T and DHT were 5 pg/ml and 10 pg/ml, respectively.

**Statistical analysis**
All results were expressed as mean ± SD from at least three independent experiments. Statistical analyses of data were performed using two-tail Student’s $t$-test and differences were considered significant where $P < 0.05$. 


Results

LRH-1 shows an up-regulation expression in prostate cancer tissues and AR-positive prostate cancer cells

Immunohistochemistry of LRH-1 showed that the cancer cells in lesions of hormone-resistant prostate cancer expressed higher nuclear immunoreactivity, as compared to lesions of hormone-naïve or neo-adjuvant-treated prostate cancers and normal adjacent tissues, the latter expressed relatively weak or negative LRH-1 immunoreactivity (Fig. 1A). Similar increased nuclear LRH-1 immunoreactivity was also seen in cancer cells in high GS hormone-naïve prostate cancers, as compared to low GS hormone-naïve prostate cancers and non-cancerous (normal or benign hyperplastic) glandular epithelial cells (Supplementary Figs. S1A and S1B). Immunoreactivity score (IRS) analysis further demonstrated that hormone-resistant lesions showed significantly higher LRH-1 expression than the lesions of hormone-naïve or neo-adjuvant-treated prostate cancers and adjacent normal tissues (Fig. 1B). To further validate the increased expression pattern of LRH-1 in CRPC, we analyzed the LRH-1 expression profile in clinical prostatic tumors using two public available Gene Expression Omnibus (GEO) datasets (GSE35988 and GSE32269), both revealed that LRH-1 exhibited a significant increased expression pattern in hormone-refractory prostate cancer or CRPC as compared to primary prostate cancer tissues or benign prostate tissues (Fig. 1C, Supplementary Figs. S1C and S1D). Quantitative qRT-PCR analysis on prostatic cell lines showed that LRH-1 exhibited higher mRNA levels in several AR-positive prostate cancer cell lines, as compared to immortalized prostatic epithelial cell and AR-negative prostate cancer cell lines (Supplementary Fig. S2A). Similar increased LRH-1 expression was also observed in several androgen-insensitive and metastatic LNCaP-derived sublines, as compared to their parental androgen-sensitive LNCaP cells (Supplementary Fig. S2B). These results suggest that increased LRH-1 expression may be associated with the advanced progression of prostate cancer.
Castration-relapse prostate cancer xenograft tumors show elevated intratumoral androgen levels and up-regulation of steroidogenic enzyme genes and LRH-1

To further elucidate whether LRH-1 would play a role in the castration-relapse growth and intratumoral steroidogenesis of prostate cancer, we examined the expression patterns of LRH-1 and several major steroidogenic enzyme genes involved in androgen biosynthesis in two tumor xenograft models of CRPC. Besides previously established CWR22-AIM xenograft model (23), we also established another CRPC tumor xenograft model VCaP-CRPC, based on the post-castration relapse growth of AR-positive VCaP cells upon orchiectomy (Fig. 2A). qRT-PCR analysis showed that the AR-axis signaling was reactivated in castration-relapse VCaP-CRPC xenograft tumors, and maintained at significantly higher levels as compared to that in pre-castration (Fig. 2B). Measurements of androgens in tumors by LC-MS/MS confirmed that high amounts of androgens (testosterone and DHT), at levels comparable to that in pre-castration, were detected in castration-relapse VCaP-CRPC tumors (Figs. 2C and 2D). Results also revealed that mRNA levels of LRH-1 and most key steroidogenic enzymes including CYP17A1 in VCaP-CRPC tumors increased significantly at 4-day post-castration and dropped dramatically in the relapsed tumors, but still at higher levels than that at pre-castration (Fig. 2E). Similar increased expressions of LRH-1 and major steroidogenic enzymes were also detected in another castration-refractory CWR22-AIM xenograft model as compared to its parental androgen-sensitive CWR22 tumors (Supplementary Fig. S3A). However, the protein levels of LRH-1 and two key steroidogenic enzymes (CYP17A1 and AKR1C3) in VCaP-CRPC xenograft tumors remained unchanged or slightly increased at 4-day post-castration but significantly increased in relapsed tumors as compared to pre-castration (Fig. 2F). These observations indicate that the transcript levels of LRH-1 and most of the major steroidogenic enzymes are simultaneously and acutely up-regulated in VCaP-CRPC tumors in response to host castration and that further up-regulation of LRH-1 and key steroidogenic enzymes (e.g., CYP17A1 and AKR1C3) may eventually endow the tumors...
with adequate androgen for AR reactivation. To support this, we also showed that LRH-1 and CYP17A1 manifested a positive expression correlation in clinical prostate cancer tissues as shown in a GEO dataset (GSE32269; Supplementary Fig. S1C). These expression data strongly demonstrated that LRH-1 exhibited significant up-regulation in castration-relapse growth of prostate cancer and its up-regulation was in close association with the increased expression of steroidogenic enzyme genes and elevated intratumoral androgen levels.

In another independent experiment, castrated mice bearing castration-relapsed VCaP tumors were further treated with abiraterone or vehicle for additional 3 weeks. Abiraterone treatment could significantly retard the CRPC xenograft growth as compared to vehicle control (Supplementary Fig. S3B). qRT-PCR analysis of tumors acquired at pre- and post-treatment showed that abiraterone, but not vehicle control, could significantly decrease the expressions of two AR target genes (KLK3 and NKX3-1), consistent with abiraterone-induced suppression of AR transcriptional activity, but increase the expressions of two major steroidogenic enzymes (CYP17A1 and AKR1C3) and LRH-1 (Supplementary Fig. S3C and S3D). Together, these results indicate that LRH-1 plays a role in the intratumoral androgen biosynthesis in the castration-resistant growth of prostate cancer and may further act to maintain the high expression levels of some key steroidogenic enzymes expressed in castration-relapsed CRPC tumors upon abiraterone treatment.

**LRH-1 overexpression confers resistance to androgen-deprivation and antiandrogen in AR-positive but not AR-negative prostate cancer cells**

Since LRH-1 exhibited an up-regulation expression pattern in high-grade prostate cancer tissues and CRPC xenograft tumors, we hypothesize that LRH-1 might play a positive role in CRPC growth. We next evaluated the functional significant of its overexpression in prostate cancer cell growth. We generated stable LRH-1-transduced clones in two prostate cancer cell lines (AR-positive LNCaP and AR-negative DU145) for *in vitro* and *in vivo* growth phenotype studies (Supplementary Figs. S4 and S5A). When cultured in conditions with...
normal FBS, both immunoblot-validated LNCaP-LRH-1 and DU145-LRH-1 clones proliferated at rates similar to their empty vector clones (Fig. 3A and Supplementary Fig. S5B). *In vitro* phenotype analyses showed that the AR-positive LNCaP-LRH-1 clones exhibited significant resistance to antiandrogen bicalutamide and androgen-deprivation condition (CS-FBS) and enhanced anchorage-independent growth capacity, as compared to empty vector clones (Figs. 3A and 3B). Intriguingly, the AR-negative DU145-LRH-1 clones showed no significant changes in their responsiveness to androgen-deprivation condition and anchorage-independent growth (Supplementary Figs. S5C and S5D). Together, these results suggest that overexpression of LRH-1 can confer insensitivity to androgen-deprivation and antiandrogen, and promote anchorage-independent growth capacity in AR-positive but not AR-negative prostate cancer cells.

**LRH-1 overexpression promotes *in vivo* castration-resistant growth capacity and induces elevated intratumoral androgen levels in prostate cancer cells**

*In vivo* tumorigenicity study showed that the AR-positive LNCaP-LRH-1 clones formed larger xenograft tumors at faster rates as compared to vector clones in intact SCID mice (Figs. 3C and 3D). More significantly, tumors formed by LNCaP-LRH-1 cells showed no response to castration of host mice and continued to grow aggressively, in sharp contrast to tumors formed by vector clones that ceased to grow or became shrunk in castrated hosts (Fig. 3C). To further investigate the possible driving mechanism that is responsible for the *in vitro* androgen deprivation-resistant and *in vivo* castration-resistant growth phenotypes acquired in LNCaP-LRH-1 cells, we measured the intracellular and intratumoral androgen levels (testosterone and DHT) in both cultured LNCaP-LRH-1 cells and also tumors formed by LNCaP-LRH-1 cells in castrated mice by LC-MS-MS. Results revealed that significantly higher levels of androgens were detected in both cultured LNCaP-LRH-1 cells and LNCaP-LRH-1-derived tumors, as compared to their vector counterparts (Figs. 3E and 3F). These results strongly suggest that the castration-resistant LNCaP-LRH-1 xenograft tumors could be
capable of \textit{de novo} androgen synthesis. It has been demonstrated that the castration-resistant LNCaP xenografts can synthesize DHT directly from acetic acid (2) and the rodent adrenal glands do not synthesize androgens due to lack of enzyme 17α-hydroxylase (CYP17A1) expression (30). Together, these results suggest that LRH-1 overexpression could augment the intracellular or intratumoral \textit{de novo} production of androgens in LNCaP-LRH-1 cells (at least with no DHEA-s contributed by the rodent adrenal glands) and contribute to their castration-resistant growth \textit{in vivo}.

**LRH-1 can directly transactivate key steroidogenic enzyme genes in prostate cancer cells**

Based on results as described, we hypothesize that LRH-1 overexpression could promote the \textit{in situ} steroidogenesis or androgen biosynthesis in prostate cancer cells, resulting in increased androgen production in cultured LNCaP-LRH-1 cells and also their derived xenograft tumors under androgen-deprivation condition. qRT-PCR analysis revealed that endogenous expressions of LRH-1 and \textit{CYP17A1} were significantly up-regulated in bicalutamide-resistant LNCaP-BC cells as compared to their parental LNCaP cells (Fig. 4A). Similar elevation of \textit{CYP17A1} gene expression was also verified in AR-positive LRH-1-transfected VCaP and LAPC-4 prostate cancer cells (Supplementary Figs. S6A and S6B). Besides, six key enzyme genes (\textit{CYP17A1, CYP11A1, STAR, HSD3B1, HSD3B2} and \textit{SRD5A2}) involved in steroidogenesis were significantly up-regulated in both LNCaP-LRH-1 and DU145-LRH-1 transduced clones (Figs. 4B and 4C). Furthermore, treatment of LNCaP-LRH-1 cells with a LRH-1 agonist RJW100 could further enhance \textit{CYP17A1} mRNA expression in a dose-dependent manner (Fig. 4D). These expression results suggested that LRH-1 expression was positively correlated with the up-regulation of steroidogenic enzyme genes in prostatic cells. Since \textit{CYP17A1} is the key enzyme catalyzing the major steps in androgen biosynthesis, we next investigated if LRH-1 could directly transactivate the \textit{CYP17A} gene promoter. Reporter gene assay showed that \textit{CYP17A1} gene promoter-driven reporter could be dose-dependently
transactivated by the transfected wild-type LRH-1, with further potentiation by the LRH-1 agonist RJW100 (Fig. 4E). Truncation analysis of LRH-1 functional domains showed that CYP17A1-Luc reporter could only be transactivated by intact LRH-1 but not its truncated mutants (-ΔZF1, -ΔCTE, -ΔFtz-f1, and -ΔLBD), suggesting that an intact LRH-1 would be required for the transactivation of CYP17A1 gene (Fig. 4E). ChIP assay further confirmed that a 183-bp DNA fragment of CYP17A1 gene promoter (located between +119 and -390 bp) could be PCR-amplified in the immunoprecipitated DNA extracted from HEK293 cells transfected with intact LRH-1 but not LRH-1-ΔZF1 mutant or empty vector, indicating that LRH-1 could directly bind to the CYP17A1 gene promoter in vivo and with such binding requiring an intact DBD (Fig. 4F). Furthermore, intact LRH-1 but not its DBD-truncated mutant could also transactivate the reporters driven by promoters of other steroidogenic enzyme genes, including STAR, CYP11A1 and HSD3B2 (Supplementary Figs. S6C-S6E). To further confirm whether the induction of CYP17A1 expression by LRH-1 in LNCaP-LRH-1 cells could be cell-type specific or not, we repeated the experiments in two other AR-positive prostate cancer cell lines, VCaP and LAPC-4. In vitro analyses confirmed that transient LRH-1 transfection could induce CYP17A1 expression and dose-dependently transactivate the CYP17A1-Luc reporter in both VCaP and LAPC-4 cells, with such induction or transactivation further potentiated by co-transfection with the NR coactivator PGC-1α but repressed by a NR corepressor DAX-1 (Supplementary Figs. S6F and S6G). Taken together, these results demonstrate that LRH-1 can enhance the expressions of several key steroidogenic enzyme genes, including CYP17A1, in prostate cancer cells via its direct transactivation of their genes.

**LRH-1-induced androgen-deprivation or antiandrogen-resistant growth in prostate cancer cells can be attenuated by the CYP17A1 inhibitor abiraterone**
The increased expression of some key steroidogenic enzyme genes in LRH-1-transduced prostate cancer cells and also the determination of these enzyme genes, including the rate limiting enzyme gene \textit{CYP17A1}, as the direct targets of LRH-1 as demonstrated above suggested that the steroidogenesis or \textit{de novo} biosynthesis of androgens could be potentiated in LRH-1-overexpressed prostate cancer cells. We hypothesize that increased intracellular or intratumoral androgen biosynthesis is responsible for the reactivation of AR signaling in therapy-resistant prostate cancer cells. We next determined the status of AR signaling in prostate cancer cells under androgen-deprivation condition (CS-FBS). Immunoblot and RT-PCR analyses showed that the protein levels of nuclear AR (not total cellular) and the transcript levels of its targets (\textit{KLK3, KLK2} and \textit{NKX3-1}) were maintained at higher levels in LNCaP-LRH-1 cells than their control LNCaP-vector cells under CS-FBS or serum-free culture condition, suggesting that the AR signaling still remained activated in prostate cancer cells under androgen-deprivation condition (Figs. 5A-D). On the other hand, treatment with the CYP17A1 inhibitor abiraterone could dose-dependently suppress the expression levels of these AR target genes (Figs. 5B-D), further suggesting that the increased intracellular androgen biosynthesis could lead to activation of AR signaling in LRH-1-overexpressed prostate cancer cells. To further elucidate the significance of increased expression of \textit{CYP17A1} in the resistant growth to androgen-deprivation and antiandrogen conferred in LRH-1-transduced prostate cancer cells, we next examined the \textit{in vitro} growth effect of abiraterone on the LNCaP-LRH-1 transduced cells and also bicalutamide-resistant LNCaP-BC cells. Results showed that treatment with abiraterone could dose-dependently restore the sensitivity of LNCaP-LRH-1 cells to androgen-deprivation (CS-FBS) and bicalutamide, but not the vector clones (Figs. 5E and 5F), the latter cells expressed low endogenous levels of \textit{CYP17A1}. Furthermore, abiraterone treatment could also attenuate the resistant growth of LNCaP-BC cells to androgen-deprivation condition (Fig. 5G). Together, these results demonstrate that the increased expression of \textit{CYP17A1} induced by LRH-1 overexpression...
can significantly contribute to the reactivation of AR signaling and resistance of prostate cancer cells to androgen-deprivation and antiandrogen.

**Sensitivity of prostate cancer cells to androgen-deprivation can be potentiated by suppression of LRH-1**

Since LRH-1-overexpression could promote the androgen deprivation-resistant growth of AR-positive but not AR-negative prostate cancer cells in a CYP17A1-dependent manner, we next examined whether suppression of LRH-1 in VCaP prostate cancer cells, which express high endogenous levels of LRH-1 and are also active in *de novo* androgen synthesis (6), could restore their sensitivity to androgen-deprivation condition. We first used siRNA-mediated approach to knockdown the endogenous LRH-1 in VCaP cells (Fig. 6A). qRT-PCR analysis showed that decreased expressions of three steroidogenic enzyme genes (*CYP17A1, HSD3B1, HSD3B2* and *AKR1C3*) were induced in immunoblot-validated VCaP-siLRH-1 cells (Fig. 6B). *In vitro* studies showed that siLRH-1 knockdown in VCaP-siLRH-1 cells could enhance their sensitivity to androgen-deprivation condition (CS-FBS) and antiandrogen (bicalutamide), with further potentiation by combined treatment with abiraterone (Figs. 6C and 6D). In the second approach, we used an LRH-1 inverse agonist ML-180 to inhibit the LRH-1 activity in VCaP cells. qRT-PCR analysis showed that ML-180 treatment could dose-dependently suppress the expression of *CYP17A1* and also three other steroidogenic genes (*STAR, HSD3B1* and *AKR1C3*) in VCaP cells (Fig. 6E). Luciferase reporter assay showed that ML-180 could dose-dependently block the LRH-1-driven transactivation of CYP17A1-Luc reporter in transfected HEK293 cells (Fig. 6F). Finally, *in vitro* studies showed that ML-180 treatment could dose-dependently sensitize the sensitivity of both VCaP and LNCaP-BC cells to androgen-deprivation condition (Figs. 6G and 6H). Together, these results suggest that LRH-1 is a potential therapeutic target of prostate cancer and suppression of LRH-1 could potentiate or enhance the sensitivity of prostate cancer cells to hormone or AR-axis targeting therapy.
Discussion

Persistent intratumoral androgen biosynthesis is regarded as one key factor responsible for the hormone-refractory growth of prostate cancer and the reactivation of AR signaling in CRPC. Based on this fact, inhibition of steroidogenic enzymes has been developed as a novel therapeutic strategy in the hormone therapy of metastatic CRPC and also clinically proved that the CYP17A1-targeting abiraterone acetate can markedly lower serum and intratumoral androgens and improve the overall survival of CRPC patients (31). However, resistance to steroidal inhibitors still invariably develops in patients shortly, and the involved mechanisms and also the up-stream regulation of steroidogenic enzymes in abiraterone-resistant prostate cancer still remain largely unknown. Here we show that the NR LRH-1 could be an important factor for the up-regulation of key steroidogenic enzyme genes in prostate cancer cells and responsible for the advanced therapy-resistant growth of prostate cancer.

In the present study, we show that LRH-1 displayed a high expression pattern in high-grade prostate cancer and CRPC tissues, xenograft CRPC models, abiraterone-treated CRPC tumors and many hormone-insensitive prostate cancer cell line models, suggesting that elevated LRH-1 expression may be associated with the development and progression of CRPC. Interestingly, an increased LRH-1 expression is also observed in primary breast cancer tissues and its immunoreactivity is positively associated with the expression of sex hormone receptors and steroidogenic enzymes (13). However, how LRH-1 expression is up-regulated in prostate cancer or CRPC is still yet unclear. LRH-1 gene (NR5A2) is located at the chromosome arm 1q32.1. Susceptibility of hereditary prostate cancer has been linked to genes or loci located broadly at chromosome 1, spanning the regions from 1p13 to 1q32 (32). A recent GWAS study conducted in cohorts of Korean population identifies that single-nucleotide polymorphism (SNP) variants located at 1q32.1 are associated with log-transformed serum PSA levels (33).
analyses show that gain of chromosome arm 1q is detected in some clinical hormone-refractory and metastatic prostate cancers (34-36) and also some prostate cancer cell lines, including the androgen-insensitive PC-3 and CWR22R (37). These studies provide indirect evidences suggesting that genetic factors, including gene amplification, could be one of reasons for the elevated expression of LRH-1 in advanced prostate cancer. However, further study is needed to confirm if genetic change on LRH-1 gene is also involved in advanced progression or increased risk of prostate cancer. Besides gene amplification, a few studies show that gene expression of LRH-1 can be regulated by some activated signal pathways, including inflammatory TNF-α-dependent pathway in injured liver (38), p38/MAPK pathway in FSH-stimulated steroidogenesis in ovarian granulosa cells (39), ERK1/2 pathway in heat-treated testicular Sertoli cells (40); and also LRH-1 is characterized as a direct target of pancreatic-duodenal homeobox 1 (PDX-1) in pancreas development (41), β-catenin for the pluripotency maintenance in mouse embryonic stem cells (ESC) (42) and NR SF-1 for steroidogenesis in ovarian granulosa cells (43). Among these up-stream regulatory pathways and targets, dysregulation of pathways of TNF-α (44), Wnt/β-catenin (45), PI3K/AKT (46) and MAPK (47) have been characterized and implicated in the advanced progression of prostate cancer. Recently, it is shown that testosterone can induce the expression of LRH-1 in granulosa cells mediated through the binding of AR-aryl hydrocarbon receptor (AHR) complex to LRH-1 gene promoter (48). However, it remains to be further determined if dysregulation of AR or signal transduction pathways could contribute to the up-regulation of LRH-1 in advanced prostate cancer.

One important finding in this study is that besides its promotion of malignant growth of prostate cancer cells, LRH-1 overexpression could also promote the in vitro resistance to androgen-deprivation and in vivo castration-resistant growth capacity of AR-positive prostate cancer cells via a mechanism on its direct transactivation of multiple key enzymes involved
in androgen biosynthesis, leading to enhanced intracellular or intratumoral de novo androgen production in prostate cancer cells. We also showed that the LRH-1-induced androgen-deprivation or castration-resistant growth in prostate cancer cells was mediated through its direct transactivation of CYP17A1 gene, as such induced effects could be attenuated by the CYP17A1 inhibitor abiraterone. LRH-1 and also another NR5A subfamily member steroidogenic factor-1 (SF-1) are characterized as important regulators of steroidogenesis via their direct transcriptional regulation of multiple steroidogenic enzymes and related protein genes (including STAR, CYP11A1, HSD3B2, CYP17A1, CYP11B1, CYP11B2, CYP19) in different steroidogenic and non-steroidogenic tissues (49,50). However, so far there is still no evidence in support of a direct regulatory role of LRH-1 in androgen biosynthesis. Previously, Sirianni et al. show that LRH-1 can directly transactivate the CYP17A1 gene (49). Its ectopic expression can induce both CYP17A1 gene expression and the differentiation of bone marrow mesenchymal stem cells into Leydig-like cells (51).

Another noteworthy finding in this study is that LRH-1, which is regarded as an orphan NR, is druggable as its transactivation activity could be modulated by LRH-1 specific ligands (agonist RJW100 and inverse agonist ML-180) in prostate cancer cells. Importantly, our current study also implicates that targeting the steroidogenesis-regulatory LRH-1 could be a potential therapeutic approach for the inhibition of persistent AR signaling in CRPC, as evidenced by that the androgen deprivation-resistant growth capacity of advanced prostate cancer cells could be attenuated by the LRH-1 inverse agonist via its suppression on the genes encoding key steroidogenic enzymes.

Although recent clinical trials indicate that AR-targeting treatment with abiraterone alone or combined with the next-generation antiandrogen enzalutamide can significantly prolong the overall survival of patients with metastatic CRPC (8,9), acquired cross-resistance to these agents still develops gradually in patients. The involved mechanisms are still not yet fully understood. It is recently reported that expression of an AR splice variant AR-V7, which
encodes a constitutively active AR with truncated LBD, in circulating tumor cells from metastatic CRPC patients is associated with the resistance to abiraterone and enzalutamide (52). Previously, Tamae et al. demonstrate that despite drastic reductions (> 90%) in the adrenal androgen precursors following abiraterone treatment, a significant amount of circulating DHEA-S (~20 µg/dL) may serve as a depot for intratumoral conversion to T and DHT bypassing CYP17A1 (53). Recently, Powell et al. report that AKR1C3 functions downstream of CYP17A1 and catalyzes the biochemical reduction of 5a-Adione to DHT in prostate cancer, and that ERG expressed by T:E fusion could enhance this step through up-regulation of AKR1C3 expression, suggesting that AKR1C3 may be a potentially therapeutic target in treatment of T:E fusion-positive CRPC. Our present findings also showed that further increased expressions of steroidogenic enzymes (including CYP17A1 and AKR1C3) and AR reactivation were detected in abiraterone-treated CRPC xenograft tumors, as consistent with previous studies (6,10), and that was also accompanied with simultaneous increased expression of LRH-1. These results suggest that persistent intratumoral de novo androgen biosynthesis or steroidogenesis activation induced by LRH-1 overexpression could be at least partially responsible for the resistance to abiraterone and enzalutamide in abiraterone-treated CRPC patients. In addition, our results showed that CYP17A1 and some upstream or downstream steroidogenic enzymes (including AKR1C3) could be suppressed upon ML-180 treatment or LRH-1 silencing, suggesting that targeting LRH-1 may potentiate the efficacy of abiraterone alone for the treatment of CRPC by further inhibiting the key steroidogenic enzymes involved in androgen biosynthesis.

In summary, our present study shows for the first time that the druggable NR LRH-1 performs an oncogenic role in the advanced growth of prostate cancer by enhancing the intratumoral androgen biosynthesis and promoting the castration-resistant growth of prostate cancer via its direct transactivation of several key steroidogenic enzyme genes (Fig. 6I).
study also provides a novel insight that targeting LRH-1 signaling could be a valuable therapeutic strategy approach for CRPC treatment.
Disclosure of Potential Conflicts of Interest

All authors declare no conflict of interest of any kind in this work.

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References


Figure Legends

Figure 1. Increased expression of LRH-1 in castration-resistant prostate cancer (CRPC) tissues. **A,** LRH-1 immunohistochemistry. Representative micrographs of LRH-1-immunostained tissue microarray spots of adjacent normal prostatic tissues, hormone-naïve prostate cancer, neo-adjuvant-pretreated prostate cancer and CRPC. Magnification, × 40; bars, 250 μm. Inserts show the enclosed areas at higher magnification. Magnification, × 400; bars, 30 μm. The glandular epithelial cells in normal tissues showed barely detectable or negative nuclear immunoreactivity. Intense nuclear immunosignals were detected in cancer cells in hormone-resistant lesions as compared to lesions in hormone-naïve or neo-adjuvant pretreated prostate cancers. **B,** LRH-1-IRS analysis performed on TMA slide containing microarray spots of prostate cancer lesions as mentioned above. Results showed that hormone-resistant lesions showed significantly higher LRH-1 expression than lesions from hormone-naïve or neo-adjuvant-pretreated prostate cancers and normal prostatic tissues. **C,** Expression profile of LRH-1 as revealed from a GEO dataset (GSE35988). Results showed that LRH-1 mRNA levels exhibited a significant increase in CRPC tissues as compared to primary prostate cancer and benign prostate tissues. ***, P < 0.01.

Figure 2. Castration-relapse prostate cancer xenograft tumors exhibit increased expression levels of steroidogenic enzymes and LRH-1 and contain higher intratumoral levels of androgens. **A-E,** castration-resistant VCaP-CRPC xenograft model. **A,** growth curve of VCaP xenograft tumors in 0-8 week post-castrated host mice. Significant relapse growth of tumors occurred at 3-4 week post-castration. **B,** qRT-PCR analysis of ∆R (∆NR3C4) and ∆KLK3. Results showed that mRNA levels of ∆R and ∆KLK3 were significantly elevated in castration-relapse VCaP-CRPC tumors. **C and D,** intratumoral androgen levels in VCaP-CRPC tumors as measured at 4-day and 8-week post-castration by LC-MS/MS. Results showed that the intratumoral levels of testosterone and DHT in VCaP-CRPC tumors dropped significantly at
4-day post-castration but rebounded at 8-week post-castration to levels almost close to that at pre-castration. **E**, qRT-PCR analysis of steroidogenic enzymes and LRH-1 in VCaP-CRPC tumors. Results showed that mRNA levels of LRH-1 and most key steroidogenic enzymes increased significantly at 4-day post-castration and dropped dramatically in the relapsed tumors, but still at higher levels than that at pre-castration. **F**, immunoblots of LRH-1 and two key steroidogenic enzymes (CYP17A1 and AKR1C3) expressed in VCaP-CRPC xenograft tumors. Results showed that protein levels of LRH-1, CYP17A1 and AKR1C3 remained unchanged or slightly increased at 4-day post-castration (4-day post) but increased significantly in relapse tumors (relapse) as compared to pre-castration (Pre). *, P < 0.05; **P < 0.01 versus VCaP tumors at pre-castration.

**Figure 3.** LRH-1 overexpression enhances androgen deprivation-insensitive growth and colony formation capacity in AR-positive LNCaP prostate cancer cells, and promotes *in vivo* castration-resistant tumorigenicity of LNCaP cells. **A**, growth responses of LNCaP-LRH-1 clones in culture conditions with normal FBS (left), bicalutamide (middle) and CS-FBS (right). LNCaP-LRH-1 clones grew at similar rates as the vector clones in culture condition with 10% normal FBS. However, LNCaP-LRH-1 clones were more resistant to culture with bicalutamide (5-100 µM, 4-day treatment) and 10% CS-FBS than vector clones. **B**, soft agar assay. Left panel: images of whole-well view of crystal violet-stained colonies formed by representative LNCaP-LRH-1 and vector clones. Right panel: graph shows the colony formation efficiencies. LNCaP-LRH-1 clones formed larger colonies and displayed higher colony formation efficiencies than vector clones. **C**, growth curve shows the growth patterns of tumors formed by LNCaP-LRH-1 and vector clones, first grown for 7 weeks in intact mice followed by another 2 weeks post-castration in the same hosts. LNCaP-LRH-1 clones formed larger tumors than vector clones in intact mice and continued to grow aggressively in castrated hosts, whereas tumors formed by LNCaP-vector clones became atrophied after
castration. **D**, upper panel, photograph shows the dissected tumors formed by LNCaP-LRH-1 and vector clones. Lower panel, measurement of wet weights of tumors formed by LNCaP-LRH-1 and vector clones in castrated mice at 9-week post-inoculation. **E and F**, androgen measurements by LC-MS-MS in tumors formed by LNCaP-LRH-1 clones and pellets of cultured LNCaP-LRH-1 cells. Significantly higher levels of testosterone and DHT were detected in both LNCaP-LRH-1-derived tumors and cultured LNCaP-LRH-1 clones, as compared to their vector counterparts. *, $P < 0.05$; ** $P < 0.01$ versus vector control.

**Figure 4.** Up-regulation of steroidogenic enzymes and direct transactivation of *CYP17A1* gene by LRH-1 in prostate cancer cells. **A-D**, qRT-PCR analyses. **A**, significant up-regulation of both *CYP17A1* and *LRH-1* were shown in bicalutamide-resistant LNCaP-BC cells as compared to their parental LNCaP cells. **B**, mRNA transcript levels of six key steroidogenic enzymes (*CYP17A1, STAR, HSD3B1, HSD3B2, CYP11A1* and *SRD5A2*) were significantly up-regulated in LNCaP-LRH-1 clones grown with normal FBS. **C**, increased mRNA expression of *CYP17A1* was also shown in AR-negative DU145-LRH-1 clones. **D**, *CYP17A1* mRNA levels in LNCaP-LRH-1 cells could be further increased by treatment with a LRH-1 agonist RJW100 in a dose-dependent manner. **E**, CYP17A1-luciferase reporter assay. Left, schematic diagrams of wild-type (WT) LRH-1 and its truncated mutants with deletions of different functional domains. The starting and ending positions of amino acid residues of LRH-1 are indicated. Right, CYP17A1-Luc reporter could be transactivated dose-dependently by the WT LRH-1 but not by truncated mutants (LRH-1ΔZF1, -ΔCTE, -ΔFtz-f1, and -ΔLBD). LRH-1-mediated transactivation of CYP17A1-Luc reporter could be further potentiated by LRH-1 agonist RJW100. **F**, ChIP assay of *CYP17A1* promoter. Upper panel, diagram shows the locations of the putative LRH-1 binding sites in the *CYP17A1* promoter and locations of primers used for ChIP assay. Lower panel, ChIP results showed that a 183-bp DNA fragment of *CYP17A1* gene promoter could be PCR-amplified in
immunoprecipitated DNA extracted from LRH-1-transfected HEK293 cells, but not in cells transfected with LRH-1ΔZF1 or empty vector and non-immune IgG-treated DNA. *, $P < 0.05$; ** $P < 0.01$ versus parental LNCaP or vector control.

**Figure 5.** Effects of CYP17A1 inhibitor abiraterone on AR signaling and growth sensitivity of LNCaP-LRH-1 cells to androgen deprivation and antiandrogen. **A,** immunoblot analysis of AR and PSA in LNCaP-LRH-1 cells. No significant changes in AR (nuclear and total cellular) and PSA were shown in both LNCaP-LRH-1 and -vector transduced clones cultured with normal FBS. However, higher levels of nuclear AR and cellular PSA were detected in LNCaP-LRH-1 clones cultured with CS-FBS (androgen-deprivation). **B-D,** qRT-PCR analysis of three AR-regulated genes in LNCaP-LRH-1 clones grown in medium with CS-FBS. *KLK3* (PSA), *KLK2* and *NKX3-1* showed up-regulation in abiraterone-untreated LNCaP-LRH-1 clones, as compared to vector clones. Upon abiraterone treatment, the transcript levels of these AR-regulated targets showed significant down-regulation in both LNCaP-LRH-1 and vector clones in a dose-dependent manner, with more significant in LRH-1 clones. **E and F,** cell growth responses of LNCaP-LRH-1 cells to abiraterone and combined abiraterone-bicalutamide treatments. In single abiraterone or combined abiraterone-bicalutamide treatments, abiraterone could dose-dependently suppress the cell survival of LNCaP-LRH-1 clones, but showed no effect on the vector clone. **G,** cell growth response of bicalutamide-resistant LNCaP-BC cells to abiraterone. Abiraterone could dose-dependently suppress the cell survival of LNCaP-BC cells cultured in CS-FBS condition. *, $P < 0.05$; ** $P < 0.01$ versus vector control.

**Figure 6.** Suppression of LRH-1 enhances the sensitivity of VCaP prostate cancer cells to androgen-deprivation. **A-D,** knockdown of LRH-1 in VCaP cells by siLRH-1. **A,** immunoblot validation of decreased LRH-1 levels in siLRH-1-transfected VCaP cells. **B,** qRT-PCR analysis of three key steroidogenic enzymes in siLRH-1-transfected VCaP cells.
mediated knockdown of LRH-1 could suppress the expression levels of CYP17A1, HSD3B1, HSD3B1 and AKR1C3 in VCaP cells. C and D, cell growth responses of VCaP cells with or without siLRH-1 knockdown towards single abiraterone or combined abiraterone-bicalutamide treatments as assayed by MTT. siLRH-1 knockdown could further suppress the cell growth of VCaP cells upon single abiraterone (8 µM) or combined abiraterone-bicalutamide treatments. E-H, in vitro treatment with the LRH-1 inverse agonist ML-180. E, qRT-PCR analysis showed that the expression levels of three key steroidogenic enzymes (CYP17A1, HSD3B1, STAR and AKR1C3) were suppressed in VCaP cells upon ML-180 treatment in a dose-dependent manner. F, ML-180 treatment could dose-dependently suppress the LRH-1-driven transactivation of CYP17A1-Luc reporter in HEK293 cells. G and H, MTT assay. In vitro ML-180 treatment could dose-dependently suppress the cell growth of VCaP and LNCaP-BC cells grown under CS-FBS condition. *, P < 0.05; ** P < 0.01 versus scramble-transfected cells or parental cells. I, schematic diagram illustrating the role of LRH-1 in the promotion of intratumoral androgen biosynthesis and reactivation of AR signaling in castration-resistant prostate cancer via its direct transactivation of some key steroidogenic enzyme genes.
Figure 1
Figure 3

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Figure 5

(A) Western blot analysis of LNCaP cells treated with different conditions.

(B) Bar graph showing the relative mRNA levels of KLK3 (PSA) in LNCaP-vector and LNCaP-LRH-1 treated with abiraterone.

(C) Bar graph showing the relative mRNA levels of KLK2 in LNCaP-vector and LNCaP-LRH-1 treated with abiraterone.

(D) Bar graph showing the relative mRNA levels of NKK3-1 in LNCaP-vector and LNCaP-LRH-1 treated with abiraterone.

(E) Bar graph showing the relative cell survival (MTT, A570) in LNCaP-vector and LNCaP-LRH-1 treated with abiraterone.

(F) Bar graph showing the relative cell survival (MTT, A570) in LNCaP-vector and LNCaP-LRH-1 treated with bicalutamide and abiraterone.

(G) Bar graph showing the relative cell survival (MTT, A570) in LNCaP and LNCaP-BC32 treated with abiraterone.
Figure 6
Nuclear receptor LRH-1 functions to promote castration-resistant growth of prostate cancer via its promotion of intratumoral androgen biosynthesis

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