**NCOR1 may be a potential biomarker of a novel molecular subtype of prostate cancer**

Lu Tang¹, Lixia Zhang², Lei Liu¹, Liping Dong¹, Yuan Dong¹, Wenhe Zhu¹ and Huiyan Wang¹

1 Jilin Collaborative Innovation Center for Antibody Engineering, Jilin Medical University, Jilin, China
2 School of Landscape, Jiangxi Agricultural University, Nanchang, China

**Keywords**
- molecular subtype; NCOR1; prostate cancer

**Correspondence**
W. Zhu and H. Wang, Jilin Medical University, Jilin 132013, China
E-mails: huolizwh@163.com (WZ); jilmpcwhy@163.com (HW)

Lu Tang and Lixia Zhang contributed equally to this article

(Received 25 June 2020, revised 18 September 2020, accepted 12 October 2020)

doi:10.1002/2211-5463.13004

Prostate cancer (PCa) is the most frequently diagnosed male cancer. An earlier study of a cohort of 333 primary prostate carcinomas showed that 74% of these tumors fell into one of seven subtypes of a molecular taxonomy defined by specific gene fusions (ERG, ETV1/4 and FLI1) or mutations (SPOP, FOXA1 and IDH1). Molecular subtypes may aid in distinguishing indolent cases from aggressive cases and improving management of the disease. However, molecular features of PCa outside the seven subtypes are still not well studied. Here we report molecular features of PCa cases without typical features of the established subtypes. We performed comprehensive genomic analysis of 91 patients, including 54 primary and 37 metastatic cases, by whole-exome sequencing.

TP53, SPOP, FOXA1, AR (androgen receptor) and a TMPRSS2–ERG fusion emerged as the most commonly altered genes in primary cases, whereas AR, FOXA1, PTEN, CDK12, APC and TP53 were the most commonly altered genes in metastatic cases. Nuclear receptor corepressor (NCOR1) genomic alterations have been identified in 5% of cases, which are nontypical molecular features of PCa subtypes. A novel NCOR1 c.2182G>C (p.Val728Leu) was identified in tumor. RT-PCR was used to show that this mutation caused loss of NCOR1 exon 19 and might be oncogenic in PCa. NCOR1 is involved in maintenance of mitochondrial membrane potential in PCa cells, and loss of NCOR1 might contribute to PCa progression. Therefore, NCOR1 may be a potential molecular marker of a subtype of PCa.

**Abbreviations**
- ΔΨm, measurement of mitochondrial membrane potential; CRPC, recurrent castration-resistant prostate cancer; DCFH-DA, 2',7'-dichlorofluorescin diacetate; FFPE, formalin-fixed paraffin-embedded; EMT, epithelial mesenchymal transition; H&E, hematoxylin and eosin staining; HSF, human splicing finder; NC, negative control; NCOR1, Nuclear receptor corepressor; PCa, prostate cancer; ROS, reactive oxygen species; SNV, single-nucleotide variant; SSF, splicing site finder; TCGA, The Cancer Genome Atlas; VUS, variant with unknown significance.
molecular features might potentially be applied to establish further risk stratification to help distinguish indolent from aggressive PCa, as well as commitment to precision medicine.

Molecular subtypes of PCa are further risk stratifications based on molecular and genetic profiles. A study of a cohort of 333 primary prostate carcinomas showed that 74% of these tumors fell into one of seven subtypes of a molecular taxonomy defined by specific gene fusions (ERG, ETV1/4 and FLII) or mutations (SPOP, FOXA1 and IDH1) [3]. Fusion of the ETS family of genes is the most common recurrent rearrangement in PCa. TMPRSS2–ERG fusion accounted for 40–50% of the patients diagnosed with PCa, while TMPRSS2 fused to ETV4, ETV5 and ETV1 has also contributed to 1–5% of PCas [4,5]. Three subtypes were classified by mutations in FOXA1, SPOP and IDH1 [3,6,7]. Upon progression of localized PCa to metastatic disease, PTEN and TP53 are the two genes that contribute the most driver mutations for PCa to be aggressive and metastatic [8]. RBP loss (28%), amplification of MYC (10%), mutations in ATM (19%) and BRCA2 (~7%) are less common genetic alterations in the progression of PCa [9].

Molecular subtypes may result in distinguishing indolent cases from aggressive cases and improving management of the disease. However, molecular features of PCa outside the seven subtypes are still not well studied. Here we reported molecular features of PCa cases without typical features of the established subtypes.

**Materials and methods**

**Patient samples and sequencing analysis**

Patient samples were derived from patients with high-grade (Gleason score 6 or higher) or metastatic biopsies. All subjects included in this study provided informed written consent for research use of tumor tissue with institutional review board approvals or appropriate waivers. Formalin-fixed paraffin-embedded (FFPE) tissue specimens or flash-frozen needle biopsies and matched normal samples underwent nucleic acid extraction as described. Extracted DNA underwent whole-exome library construction and somatic mutation analysis as described previously [10]. BAM files were aligned to the hg19 human genome build. Copy number aberrations were quantified and reported for each gene as previously described [11]. Single-nucleotide variants (SNVs) and small indels were identified using VarScan2 with the minimum variant allele frequency threshold set at 0.01, and the P value threshold for calling variants was set at 0.05 to generate variant call format files [12]. All SNVs and indels were annotated with ANNOVAR [13].

**RNA extraction and RT-PCR**

Formalin-fixed paraffin-embedded tissues were used to extract tumor RNA. Tumor area was circled by a pathologist in the slide with hematoxylin and eosin staining. RNA was extracted from tissue without hematoxylin and eosin staining with RNeasy FFPE Kit from QIAGEN (Hilden, Germany) according to its protocol. RT-PCR was performed to amplify Nuclear receptor corepressor (NCO RI) exon 19 with primers 5'-CAGCAGAGAAATGAGGAAA-3' and 5'-GGTGCGGTGCTTCAGTA-3'. A fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control was amplified with primers P12 (5'-GACGTCAGCGCATCTTCTT-3') and P13 (5'-CAA TACGACAAAATCCGGTGAC-3'). The following PCR conditions were used: 98 °C 2 min; 98 °C 10 s, 55 °C 30 s, 72 °C 1 min, 30 cycles; final extension, 72 °C 5 min.

**Cell culture, RNA interference and western blot**

Human PCa lymph node carcinoma of the prostate (LNCaP) cells were grown in RPMI 1640 supplemented with 10% heat-inactivated FBS, 1% penicillin-streptomycin in 25-cm² polystyrene flasks and maintained at 37 °C in a humidified atmosphere with 5% CO₂. Casodex-resistant LNCaP-CR cells were established after prolonged exposure of LNCaP cells to high concentration (100 mm) of Casodex. LNCaP-CR cells were maintained in RPMI 1640 containing 10% charcoal-stripped heat-inactivated FBS, 1% penicillin–streptomycin.

The NCO R1 siRNA and control siRNA (NC) were purchased from GenePharma (Shanghai, China): NCO R1 siRNA, 5'-GCAGAUAUUCAAAUAUUTT-3' and 5'-AUAAUUUGGACAUAACUGTTT-3'; NC, 5'-UUC UCCGAACGUGUACACGUTT-3' and 5'-ACGUGACAC GUUUCGAGAATT-3'. For RNA interference experiments, 5 x 10⁵ LNCaP and LNCaP-CR cells were seeded with 2 mL of RPMI containing 10% FBS on six-well plates for 48 h. Then NCO R1 siRNA (1 μg siRNA/well) was transfected using siRNA-Mate plus (GenePharma) into cells for 8 h, and then the medium was replaced with fresh medium. Cells were harvested after 48 h, and the RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), reverse transcribed to cDNA and amplified by PCR. The NCO R1 mRNA expression was determined by RT-PCR. Total protein was prepared by using radio immunoprecipitation assay buffer. Thirty micrograms of whole-cell extracts was subjected to SDS/PAGE and transferred to a nitrocellulose membrane. After 1-h block with 5% nonfat dry milk, the membrane was incubated with primary antibodies [NCO R1 (sc-515934)], GAPDH (sc-365062) from Santa Cruz Biotechnology, Inc., Shanghai, China) for overnight at 4 °C with the concentration of 0.2–0.4 mg/mL. Then the membrane was incubated for...
1 h with the horseradish peroxidase-conjugated secondary antibody (Amersham Biosciences, Waltham, MA, USA) at 1:5000 dilutions. Equal loading and transfer were confirmed by repeat probing for GAPDH. The bands were detected by an enhanced chemiluminescence kit (Amersham Biosciences).

Fig. 1. Landscape of genomic alterations. (A) Site distribution of the metastatic cases. (B) Frequency of alteration by gene in primary cases. (C) Frequency of alteration by gene in metastatic cases. (D) Frequency of alteration by pathway. EMT, epithelial mesenchymal transition.
Reactive oxygen species analysis

A Reactive Oxygen Species (ROS) Assay kit (Beyotime Institute of Biotechnology, ShangHai, China) was used for active ROS detection using the fluorescent probe 2',7'-dichlorofluorescin diacetate (DCFH-DA). The DCFH-DA reagent must be diluted to 10 \( \mu \)M in serum-free medium before use. Following the experimental treatments, 10 \( \mu \)M DCFH-DA was added to cells with subsequent incubation for 30 min at 37 \(^\circ\)C in a humidified incubator, and cells were washed three times with serum-free medium and collected. Then the relative fluorescence intensities were detected.

Measurement of mitochondrial membrane potential

Measurement of mitochondrial membrane potential (\( \Delta \Psi m \)) was evaluated by JC-1 probe (Beyotime Institute of Biotechnology). LNCaP or LNCaP-CR cells were cultured in six-well plates. Then NCOR1 siRNA (1 \( \mu \)g siRNA/well) was transfected. After transfection, the cells were incubated with an equal volume of JC-1 staining solution (5 \( \mu \)g/mL) at 37 \(^\circ\)C for 20 min and rinsed twice with PBS. Nontreated LNCaP cells were used as negative controls. CCCP-treated LNCaP cells were used as the positive control. Mitochondrial membrane potentials were monitored by flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Ethical statement

This study was approved by the Ethical Committee of Jilin Medical University. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Statistical analysis

The quantitative data were shown as the mean ± SD. Statistical analysis was performed using the srs.s 17.0 software (SPSS Inc., USA). The difference between groups was analyzed by one-way ANOVA. A \( P \) value < 0.05 was considered to be statistically significant.

Results

Landscape of genomic alterations

Whole-exome sequencing was successfully performed on 91 tumors, as well as matched peripheral blood DNA. Of the 91 biopsies, 54 samples are primary tumors derived from patients who were diagnosed with PCa by transurethral resection of the prostate and having a high grade (Gleason score 6 or higher). Of the 37 metastatic biopsies, 38% were lymph node, 15% were bone, 12% were liver and 5% were lung biopsies (Fig. 1A). Median age at diagnosis with PCa was 61 years for primary cases and 62 for metastatic cases. Median age at biopsy of the profiled sample was 64 years for primary cases and 67 for metastatic cases (Table 1).

The frequency of genomic alterations, including SNVs, small deletion and insertion (indel), copy number variation and structural variants, was analyzed in both primary and metastatic cases. \( TP53 \), \( SPOP \), \( FOXA1 \), \( AR \) and \( TMPRSS2-ERG \) fusion emerged as the most commonly altered genes in primary cases (Fig. 1B). In metastatic cases, \( AR \), \( FOXA1 \), \( PTEN \), \( CDK12 \), \( APC \) and \( TP53 \) were the most commonly altered genes (Fig. 1C). Consistent with prior reports, a high frequency of genomic alterations in androgen receptor (AR), namely, amplifications and mutations, was confirmed, especially in metastatic cases, with more than 20% of AR amplification (Fig. 1C). More than 30% of cases harbor at least one alteration in kinase signaling, cell-cycle, epigenetic or DNA repair pathway genes (Fig. 1D).

\( NCOR1 \) as a biomarker in prostate cancer subtypes

\( NCOR1 \) is a well-studied corepressor of nuclear receptors, including androgen receptor, involved in repression of their respective target genes. Studies have shown that the PCa response to castration therapies is dependent on functional AR–NCOR1 complexes, and NCOR1 protein levels decline with PCa progression in patients with PCa [14,15]. In an analysis of recent PCa projects (after 2019) in The Cancer Genome Atlas...
(TCGA) database, including three TCGA projects, Metastatic Prostate Adenocarcinoma (SU2C/PCF Dream Team), Prostate Adenocarcinoma (Memorial Sloan Kettering Cancer Center), Prostate Adenocarcinoma (Memorial Sloan Kettering Cancer Center/Dana-Farber Cancer Institute) and The Metastatic PCa Project (Provisional, November 2019), there were a total of 4322 prostate cases. Among them, there were 85 cases with NCOR1 mutations, with an average of 2.5% (Fig. 2A).

In our cohort, five cases (four primary cases and one metastatic case) were identified with NCOR1 alterations of four missense mutations and one nonsense mutation (Table 2). The nonsense mutation c.6953C>G p.S2318* was present in a primary case. This case was also identified with the oncogenic TP53 c.672+1G>T. Although there are no literature and data on the nonsense mutation c.6953C>G p.S2318*, based on its truncated effect on NCOR1, this mutation might be likely oncogenic.

In case 33, NCOR1 c.59A>C p.Y20S co-occurred with likely oncogenic TP53 c.799C>T p.R267W. Given the fact that of high minor allele frequency (2.24%), NCOR1 c.59A>C p.Y20S likely would be benign. However, with lack of population data and functional
data, both \textit{NCOR1} c.3056C>T p.P1019L and \textit{NCOR1} c.1375C>T p.R459C will be considered as variant with unknown significance (VUS).

The mutation \textit{NCOR1} c.2182G>C (p.Val728Leu) was identified as a somatic mutation in the tumor sample of patient P6 (Fig. 2B,C). In patient P6, the estimated tumor mutation burden for the sample is 1.8 mutations per megabase (mt/Mb). This patient’s cancer does not fall into any subtypes based on molecular features. Neither gene fusions (\textit{ERG}, \textit{ETV1}/4 and \textit{FLI1}) nor mutations (\textit{SPOP}, \textit{FOXA1} and \textit{IDH1}) had been identified. There is no driver mutation for PCa in the patient. Loss of heterozygosity was present in the sample. The mutation \textit{NCOR1} c.2182G>C (p.Val728Leu) has not been reported in the literature and in the COMIC database. The sequence changed Val to Leu at position 728. There is a small physicochemical difference between Val and Leu, and \textit{in silico} analysis predicted this to be tolerated on protein structure and function. Further analysis of gene structure revealed that \textit{NCOR1} c.2182G is the last nucleotide of exon 19 (NM_006311.3). \textit{NCOR1} c.2182G>C probably affects mRNA splicing. \textit{In silico} prediction tools were applied to analyze the potential effect on mRNA splicing by the mutation. splicing site finder (SSF), NNSPLICE, NetGene2 and GeneSplicer predict to

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Case & TMB & \textit{NCOR1} & Classification & Other alterations (oncogenic/likely oncogenic) \\
\hline
P6 & 1.8 & c.2182G>C (p.Val728Leu) & Likely oncogenic? & \\
\hline
P16 & 2.2 & c.3056C>T p.P1019L & VUS & \\
\hline
P27 & 1.6 & c.6953C>G p.S2318* & Likely oncogenic & TPS3 c.672+1G>T \\
\hline
P33 & 2.0 & c.59A>C p.Y205C & Likely benign & TPS3 c.799C>T \\
\hline
M1 & 2.0 & c.1375C>T p.R459C & VUS & AR amplification: 3.9 \\
\hline
\end{tabular}
\caption{Summary of the five cases with \textit{NCOR1} genomic alterations. TMB, tumor mutation burden.}
\end{table}

Fig. 3. Knockdown of \textit{NCOR1} results in increased ROS level in PCa cells. (A) RT-PCR showed that \textit{NCOR1} was silenced in LNCaP and LNCaP-CR. (B) \textit{NCOR1} was silenced with siRNA in LNCaP and LNCaP-CR by western blotting. (C) Knockdown of \textit{NCOR1} results in increased ROS level in PCa cells. Column 1, ROS level in LNCaP; column 2, \textit{NCOR1} was silenced by siRNA followed by examining ROS level; column 3, ROS level in LNCaP-CR; column 4, \textit{NCOR1} was silenced by siRNA followed by examining ROS level in LNCaP-CR. Data were indicated as mean ± SD (\(n = 6\)). *\(P < 0.05\) vs. LNCaP cells. The difference between groups was analyzed by one-way ANOVA.
totally abolish the WT donor site at c.2182. The variant is predicted to significantly damage this WT donor site by MaxEnt (54.5% decreased value). HSF also predicts to weaken this site by the variant (14.1% decreased value) (Fig. 2D). RT-PCR confirmed the abnormal mRNA splicing on the NCOR1 exon 19 in the patient’s tumor, suggesting NCOR1 c.2182G>C (p.Val728Leu) to be likely oncogenic (Fig. 2E).

**Regulation of energy metabolism by NCOR1 in PCa**

NCOR1 is a well-studied corepressor of nuclear receptors, including androgen receptor, involved in repression of their respective target genes. Loss of NCOR1 alters the bicalutamide-regulated gene expression profile [15]. Besides the role of NCOR1 as corepressor of AR in regulation of PCa progression, it is believed that NCOR1 is also involved in regulation of mitochondrial function, as well as energy metabolism [16]. It is interesting to examine the role of NCOR1 in maintenance of ROS and ΔΨm for PCa progression. ROS is a mediator of intracellular signals and plays an important role in causing apoptotic cell death. NCOR1 was silenced by siRNA followed by examining ROS in PCa cells LNCaP and LNCaP-CR (Fig. 3A,B). As shown in Fig. 3C, knockdown of NCOR1 increases ROS level in both LNCaP and LNCaP-CR. ΔΨm is an important index on mitochondria function. As shown in Figs 4 and S1, knockdown of NCOR1 increases ΔΨm in both LNCaP and LNCaP-CR, and overexpression of NCOR1 decreases ΔΨm. That indicated collapse of the mitochondrial membrane potential and eventually apoptosis, suggesting the potential role of NCOR1 as suppressor in cancer progression.

**Fig. 4.** Effect of NCOR1 on ΔΨm in PCa cells. Knockdown of NCOR1 results in increased ΔΨm of PCa cells. (A) ΔΨm in LNCaP. (B) NCOR1 was silenced by siRNA followed by examining ΔΨm in LNCaP. (C) ΔΨm in LNCaP-CR. (D) NCOR1 was silenced by siRNA followed by examining ΔΨm in LNCaP-CR.
Discussion

Recurrent castration-resistant PCa (CRPC) is the major cause of mortality of patients with PCa. Understanding the genetic alterations that underlie the development and progression of PCa to CRPC is the major goal of cancer genomics. Molecular characterization and classification of clinically relevant subtypes of PCa are likely to improve prognostic and predictive accuracy for patients. Here we reported comprehensive genomic analysis of 91 patients, including 54 primary and 37 metastatic cases, by whole-exome sequencing and characterization of NCOR1 as a molecular marker for a possible subtype of PCa.

NCOR1 is a steroid receptor coregulatory protein. This cofactor was shown to repress both agonist- and antagonist-dependent transcriptional activity on ARE-driven reporters in terms of AR transactivation [17]. Depletion of NCOR1 in PCa cells changed the expression of genes that play significant roles in PCa progression [15]. Five cases (four primary cases and one metastatic case) were identified with NCOR1 alterations of four missense mutations and one nonsense mutation. In one patient, typical genomic alterations of PCa were not observed except for the mutation NCOR1 c.2182G>C (p.Val728Leu). NCOR1 c.2182G is the last nucleotide of exon 19, which is in the WT donor site within the consensus splice-site regions. Donor and acceptor splice-site variants typically lead to exon skipping, truncated protein and a loss of protein function. The kinds of variant/mutation are normally pathogenic/likely pathogenic in germline and oncogenic/likely oncogenic in somatics. Whether NCOR1 is an oncogene or tumor suppressor in PCa is still inconclusive. Some studies provided direct evidence in vivo that NCOR1 could function as an oncogene via transcription regulation in a mouse model of thyroid cancer [18]. Given the fact of NCOR1 as AR suppressor in PCa progression and an oncogenic role in thyroid cancer, it is reasonable for NCOR1 to act as both oncogene and tumor suppressor.

Conclusion

Similar to FOXA1 in the AR pathway, genomic alterations in NCOR1 have been previously reported in primary PCAs and CRPC [6,7]. Besides its role in the AR pathway, NCOR1 plays a key role in mitochondrial gene regulation [19]. Our data showed that NCOR1 is critical in the maintenance of ΔΨm in PCa cells. Knockdown of NCOR1 increases ΔΨm and eventually results in cell apoptosis. NCOR1 has both nuclear and cytoplasmic fractions that have distinct cellular functions. Nuclear-cytoplasmic shuttling is regulated in part by NCOR1 phosphorylation. How nuclear and cytoplasmic fractions function in mitochondrial metabolism and PCa progression still remains elusive.

Acknowledgements

We thank Dr Yirong Li from Memorial Sloan Kettering Cancer Center. This work was supported by Science & Technology Development of Jilin Province (20170414022GH), Jilin Collaborative Innovation Center for Antibody Engineering (20180623045TC) and Scientific Research Project of Education Department of Jilin Province (JJKH20180822KJ).

Conflict of interest

The authors declare no conflict of interest.

Data accessibility

Data will be available from the corresponding author upon reasonable request. Please note these are sensitive patient data.

Author contributions

WZ and HW conceived and designed the project; LT, LL, LD and YD acquired the data; LZ analyzed and interpreted the data; LZ wrote the paper.

References

1 Siegel RL, Miller KD and Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68, 7–30.
2 Al Olama AA, Kote-Jarai Z, Berndt SI, Conti DV, Schumacher F, Han Y, Benlloch S, Hazelett DJ, Wang Z, Saunders E et al. (2014) A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. Nat Genet 46, 1103–1109.
3 Cancer Genome Atlas Research Network (2015) The molecular taxonomy of primary prostate cancer. Cell 163, 1011–1025.
4 Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R et al. (2005) Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310, 644–648.
5 Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, Menon A, Jing X, Cao Q, Han B et al. (2007) Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 448, 595–599.
NCOR1 as a biomarker in prostate cancer subtypes

L. Tang et al.

6. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC et al. (2012) The mutational landscape of lethal castration-resistant prostate cancer. Nature 487, 239–243.

7. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, White TA, Stojanov P, Van Allen E, Stransky N et al. (2012) Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 44, 685–689.

8. Arora K and Barbieri CE (2018) Molecular subtypes of prostate cancer. Curr Oncol Rep 20, 58.

9. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G et al. (2015) Integrative clinical genomics of advanced prostate cancer. Cell 162, 454.

10. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M et al. (2010) The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20, 1297–1303.

11. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, Miller CA, Mardis ER, Ding L and Wilson RK (2012) VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 22, 568–576.

12. Bolger AM, Lohse M and Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2144–2140.

13. Robinson JT, Thorvaldsdottir H, Wenger AM, Zehir A and Mesirov JP (2017) Variant review with the integrative genomics viewer. Cancer Res 77, e31–e34.

14. Qi J, Tripathi M, Mishra R, Sahgal N, Fazli L, Ettinger S, Placzek WJ, Claps G, Chung LW, Bowtell D et al. (2013) The E3 ubiquitin ligase Siah2 contributes to castration-resistant prostate cancer by regulation of androgen receptor transcriptional activity. Cancer Cell 23, 332–346.

15. Lopez SM, Agoulnik AI, Zhang M, Peterson LE, Suarez E, Gandarillas GA, Frolov A, Li R, Rajapakse K, Coarfa C et al. (2016) Nuclear receptor corepressor 1 expression and output declines with prostate cancer progression. Clin Cancer Res 22, 3937–3949.

16. Lima TI, Valentim RR, Araujo HN, Oliveira AG, Favero BC, Menezes ES, Araujo R and Silveira LR (2018) Role of NCoR1 in mitochondrial function and energy metabolism. Cell Biol Int 42, 734–741.

17. Agoulnik IU, Krause WC, Bingman WE 3rd, Rahman HT, Amrikachi M, Ayala GE and Weigel NL (2003) Repressors of androgen and progesterone receptor action. J Biol Chem 278, 31136–31148.

18. Fozzatti L, Park JW, Zhao L, Willingham MC and Cheng SY (2013) Oncogenic actions of the nuclear receptor corepressor (NCOR1) in a mouse model of thyroid cancer. PLoS One 8, e67954.

19. Catic A, Suh CY, Hill CT, Daheron L, Henkel T, Orford KW, Dombkowski DM, Liu T, Liu XS and Scadden DT (2013) Genome-wide map of nuclear protein degradation shows NCoR1 turnover as a key to mitochondrial gene regulation. Cell 155, 1380–1395.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Overexpression of NCOR1 on ΔΨm of PCa cells. (A) Overexpression of NCOR1 in LNCaP detected by western blotting. (B) NCOR1 was overexpressed followed by examining ΔΨm in LNCaP-CR.