Identifying myosin heavy chain 11 as a predictive biomarker of prostate cancer progression and antiandrogen resistance

CONG CHEN*, ZHI LI*, JIANLIANG SHEN, GUILIN XIE and LIANGMING PAN

Department of Urology, Tinglin Hospital of Jinshan District, Shanghai 201505, P.R. China

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Abstract. Prostate cancer (PCa) poses a serious threat to the health of older men, with incidence rates steadily increasing worldwide. Antiandrogen drugs can effectively prolong survival in patients with PCa; however, resistance often develops after prolonged treatment and the mechanisms underlying this resistance remain to be elucidated. In the present study, genes that may serve key roles in antiandrogen drug resistance in PCa were investigated. Using the GSE211781 dataset from the Gene Expression Omnibus database, the present study analyzed RNA-sequencing data from lymph node carcinoma of the prostate (LNCaP) cell lines resistant to three antiandrogen drugs: Bicalutamide, enzalutamide and apalutamide. The present study identified 54 differentially expressed genes common to all three resistant lines, of which nine hub genes were confirmed using protein-protein interaction network analysis. Among these, myosin heavy chain 11 (*MYH11*) emerged as a key gene associated with both PCa progression and patient prognosis. Functional assays in C4-2 and LNCaP cells further indicated that MYH11 modulates sensitivity to bicalutamide and enzalutamide. Collectively, the present study findings suggest that *MYH11* may serve as a potential predictive biomarker of PCa development and antiandrogen drug resistance in the future.

Introduction

With increasing life expectancy, the morbidity of prostate cancer (PCa) has risen rapidly. According to data from the American Center for Cancer Research, PCa is now the most common malignancy with 29% incidence rate and the second leading cause of cancer-associated mortality with 11% death rate among men in the United States in 2024 (1). Its incidence is also increasing rapidly in China, where PCa has become the fastest-growing malignancy with an incidence rate of 0.02% in 2022 among men (2). Effective treatments such as ablative radiotherapy and radical prostatectomy can markedly prolong survival (3). Antiandrogen drugs can also effectively treat locally advanced and metastatic PCa (4). By decreasing androgen receptor (AR) expression (5), these drugs alleviate clinical symptoms such as bone pain, difficulty with urinating and extend survival (6). However, drug resistance inevitably develops and the mechanisms underlying antiandrogen resistance remain to be elucidated.

Antiandrogen therapy reduces androgen activity through drugs (7), such as bicalutamide (BIC), enzalutamide (ENZ) and apalutamide (APN) (8). BIC is a non-steroidal antiandrogen that inhibits the binding of testicular and adrenal androgens to AR (9). ENZ prevents nuclear translocation of activated AR, binding to androgen response elements and coactivator recruitment, thereby suppressing PCa proliferation (10). APN, another non-steroidal antiandrogen, binds directly to the AR ligand-binding domain and inhibits AR translocation, DNA binding and AR-mediated transcription (11). These drugs can inhibit PCa progression by decreasing AR expression. Although these drugs provide clinical benefit, resistance eventually develops and the underlying mechanisms require further study.

Genetic alterations are increasingly recognized as key contributors to both PCa development and drug resistance. For instance, microseminoprotein-β expression changes may influence PCa risk in obese individuals (12), while alterations in cyclin B1 and golgin A8 family member B have been associated with chemotherapy resistance (13,14). Bcl-2-binding component 3 mutations contribute to olaparib resistance (15) and CDK6 dysregulation has been associated with ENZ resistance (16). Collectively, these studies underscore the key role of genetic changes in PCa development and therapeutic resistance, suggesting that additional genetic factors may contribute to antiandrogen resistance.

The present study attempted to use bioinformatic methods to find the hub genes which are important for PCa resistance to antiandrogen drugs and verified their function using different types of PCa cell lines. RNA-sequencing data of lymph node carcinoma of the prostate (LNCaP) cells resistant to BIC, ENZ and APN (GSE211781 dataset) were obtained to find the hub

Correspondence to: Dr Guilin Xie or Dr Liangming Pan, Department of Urology, Tinglin Hospital of Jinshan District, 80 North Siping Road, Shanghai 201505, P.R. China
E-mail: 18862155767@163.com
E-mail: plm_8750@sina.com

*Contributed equally

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genes. RNA-sequence data from public databases such as Gene Expression omnibus (GEO), The Cancer Genome Atlast (TCGA) and Chinese Prostate Cancer Genome and Epigenome Atlas (CPGEA) were used to analyze the function of hub genes in affecting PCa progression and prognosis. Finally, the role of hub genes in influencing PCa cells sensitive to antiandrogen drugs were verified in two types of PCa cell lines: LNCaP and C4-2.

Materials and methods

Data source. The GEO database (https://www.ncbi.nlm.nih.gov/) is a publicly available repository containing microarray data for various diseases (17). Meanwhile, TCGA (http://cancergenome.nih.gov/) is a large public database that provides sequencing and clinical data for multiple cancer types (18). Gene expression data for prostate adenocarcinoma in TCGA database (TCGA-PRAD) was searched. The Chinese Prostate Cancer Genome and Epigenome Atlas (CPGEA; http://www.cpgea.com/) includes microarray data from Chinese patients with PCa (19). Gene expression and clinical data were obtained from these databases.

Data handling. The GSE211781 dataset (20) consists of RNA-sequencing data from LNCaP PCa cells resistant to antiandrogen drugs, including BIC, ENZ and APN, and was used to identify DEGs associated with drug resistance. In addition, the GSE136129 and GSE81796 datasets (21,22), which contain RNA-sequencing data from C4-2 PCa cells resistant to ENZ, were also collected. TCGA provides clinical information and microarray data from 497 patients with PCa and 52 healthy controls. The CPGEA database contains microarray data from 136 paired tumor and adjacent normal tissues from Chinese patients with PCa. All primary data from these public databases were normalized using the R package 'limma' (version, 3.40.2; R Development Core Team) (23).

Survival analysis. Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) an online tool, was used to evaluate the effects of genes on patient survival based on TCGA data (24). Disease-free survival (DFS) was assessed using GEPIA.

Identifying antiandrogen drug resistance genes (ADRGs) and constructing the PPI network. ADRGs in the GSE211781 dataset were identified based on the following criteria: i) Adjusted $P<0.05$ and $\log_2$ fold-change (FC) >1; and ii) expression changes observed in response to all three antiandrogen drugs.

A PPI network of these ADRGs was constructed using the STRING database. Raw PPI data were downloaded from STRING (http://string-db.org/) (25) and reconstructed with Cytoscape (version, 3.9.1; https://cytoscape.org/) software (26). Hub genes were identified as those exhibiting the highest connectivity within the network (16).

Cell culture and transduction. The C4-2 and LNCaP PCa cell lines were purchased from the Chinese Academy of Sciences Cell Bank. Cell culture and transduction were performed as previously described (27). Briefly, cells were maintained in RPMI 1640 medium (cat. no. R8758; MilliporeSigma) supplemented with 10% FBS (cat. no. 10091; Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere with 5% CO_2 and 95% air at 37˚C. Antibiotics were not used and cells were passaged when density reached 80%. All lines were tested using a mycoplasma detection kit (cat. no. C0298; Beyotime Institute of Biotechnology) to confirm that the cells are free of mycoplasma contamination (cat. no. MP0030; MilliporeSigma; Merck KGaA).

Cell transduction. To generate PCa cells with MYH11 knockdown, the 2nd generation system was utilized comprising the packaging plasmids pMD2.G (plasmid cat. no. 12259; Addgene, Inc.) and psPAX2 (plasmid cat. no. 12260; Addgene, Inc.). For each 6-well plate, 5.5 μg pLVX-shMYH11 and 2.0 μg pMD2.G was used to transfect cells for 12 h at 37˚C by Lipofectamine™ 2000 (cat. no. 11668019; Thermo Fisher Scientific, Inc.) and polyethyleneimine (PEI; cat. no. 408700; MilliporeSigma; Merck KGaA), according to the manufacturers' protocol. Short hairpin RNA (shRNA) targeting MYH11 and a negative control (shControl) lentivirus were constructed by Hebei Youze Biotechnology Co., Ltd. Lentiviral packaging plasmids were transduced into 293T cells using PEI for 12 h at 37˚C. The culture medium was replaced at 24 h post-transduction and supernatants were collected at 48 h. These were then used to infect PCa cells with multiplicity of infection of 2 at 37˚C for 48 h. Following transduction, cells exhibiting stable integration were selected using puromycin (cat. no. P8833; Sigma-Aldrich; Merck KGaA) at 1.0 μg/ml for 5 days. The shMYH11 sequences were sense (S), 5'-GCA AATTCATCCGCATCAACT-3' and antisense (AS), 3'-AGT TGATGCGGATGAATTTGC-5', and the shControl sequences were S, 5'-GCTCACACGGATGTAGG-3' and AS, 3'-CCT ACATCCGTGTGAGCA-5', which do not affect gene expression in these cells.

Drug treatment. BIC (cat. no. S1190) and ENZ (cat. no. S1250) were purchased from Selleck Chemicals. Untreated C4-2 and LNCaP cells were exposed to 40 μM BIC or ENZ for 10 days before subsequent experiments. After transduction with lentivirus, cells were treated with 80 μM BIC or ENZ for 4 days at 37˚C for further experiments. In addition, the control cells were treated with 0.5% DMSO for 4 days at 37˚C.

Cell proliferation assay. Cell proliferation experiments were performed 48 h after transduction, as previously described (26). Proliferation was assessed using the Cell Counting Kit-8 kit (CCK-8; cat. no. CA1210; Beijing Solarbio Science & Technology Co., Ltd.). Cells were seeded in 96-well plates (3,000 cells/well) and cultured in 200 μl RPMI 1640 medium supplemented with 10% FBS for 0, 24, 48 and 72 h. The cells were treated with BIC or ENZ at 40 or 80 μM according to experimental requirements. After incubation, cells were treated with CCK-8 reagent for 1 h following the manufacturer's instructions and absorbance was measured at 450 nm using a multimode reader (cat. no. LB942; Titertek-Berthold).

Western blotting. Western blotting experiments were performed as previously described (28). Total proteins were extracted from samples and cell lines using RIPA lysis buffer with a protease inhibitor cocktail (MilliporeSigma; Merck KGaA). Then, the protein concentration was detected by

BCA using a BCA kit (cat. no. P0012; Beyotime Institute of Biotechnology). Then, protein samples were handled by Dual Color Protein Loading Buffer (Thermo Fisher Scientific, Inc.). Equal amounts of protein (24 μg per lane) were added and separated via SDS-PAGE (7.5 and 10% gels) and then transferred to nitrocellulose membranes (cat. no. 71078; Merck KGaA). Membranes were blocked with Protein-Free Rapid Blocking Buffer at 37˚C for 1 h (cat. no. 37584; Thermo Fisher Scientific, Inc.) and incubated overnight at 4˚C with primary antibodies against MYH11 (1:1,000; cat. no. ab124679; Abcam) and GAPDH (1:1,000; cat. no. ab9482; Abcam). The next day, membranes were washed three times for 10 min each with 1X TBST with 0.1% Tween, then incubated at room temperature for 1 h with a matched secondary antibody HRP-labeled Goat Anti-Human IgG (H+L) (cat nos. A0216 and A0208; Beyotime Institute of Biotechnology). Signals were visualized using X-ray exposure (Abcam). Protein band intensities were quantified using ImageJ software (version no. 1.54h; National Institutes of Health) and bar charts were generated to present the results.

Statistical analysis. Data were obtained from at least three independent experiments and are presented as mean ± SD. Differences between two groups were assessed using an unpaired, two-tailed Student's t-test and differences among ≥3 groups were analyzed with one-way ANOVA followed by the Scheffe post hoc test to account for unequal group sizes. The box plots were made using an online tool: Sangerbox (http://sangerbox.com/index.html). All statistical analyses were performed with SPSS (version 22.0; IBM Corp.) and data from public databases were analyzed using R software (version 4.0.3; R Development Core Team) with appropriate packages. P<0.05 was considered to indicate a statistically significant difference.

Results

Identifying nine hub genes among 54 ADRGs from the GSE211781 dataset. The present study first analyzed the GSE211781 dataset to identify genes key for PCa resistance to antiandrogen drugs. RNA-sequencing data from LNCaP cells resistant to BIC, ENZ and APN were examined. In APN-resistant cells, 68 genes were upregulated and 56 were downregulated. In BIC-resistant cells, 34 genes were upregulated and 27 were downregulated. In ENZ-resistant cells, 70 genes were upregulated and 48 were downregulated. Altogether, 54 genes were differentially expressed across all three resistant cell lines, which were classified as ADRGs in the present study (Fig. 1A). Table I summarizes the expression levels of the nine hub genes identified among these 54 ADRGs and complete expression data for all genes are presented in Table SI. A PPI network generated with Cytoscape further revealed nine hub genes (*NTS, GCG, CD200, IL1B, BCHE, MYH11, CCDC80, COL5A1* and *COL27A1*) among these ADRGs, each demonstrating strong connectivity with other genes in the network (Fig. 1B).

Hub genes from ADRGs influence PCa occurrence. The present study next investigated whether these nine hub genes were associated with PCa occurrence. GEPIA analysis of

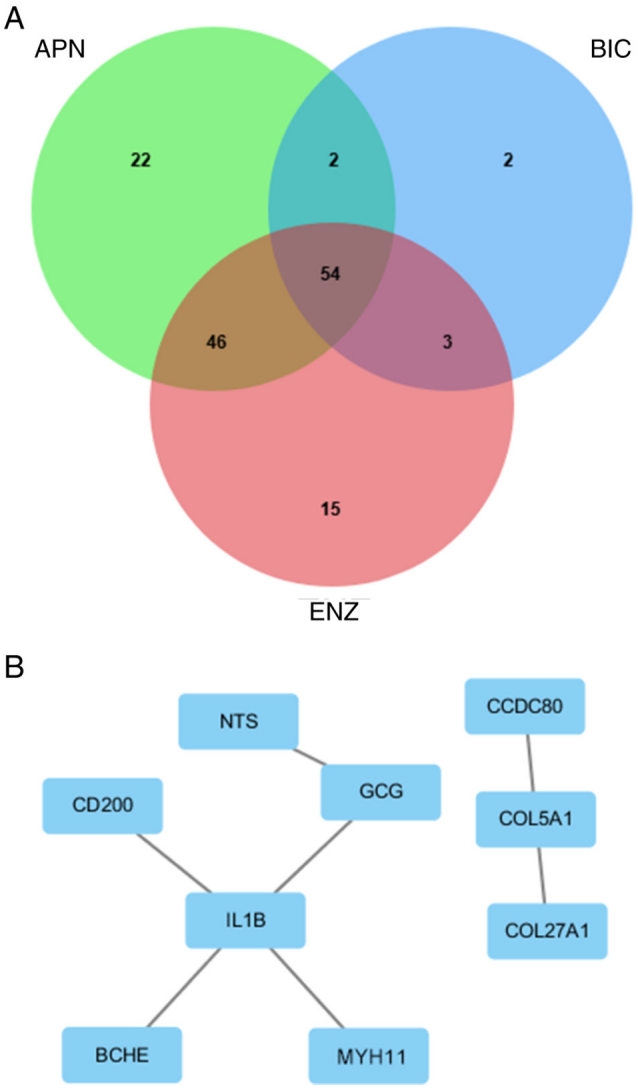

Figure 1. A total of nine hub genes among 54 ADRGs are key for resistance to antiandrogen drugs in LNCaP cells. (A) A total of 54 ADRGs were identified in LNCaP cells resistant to BIC, ENZ and APN. (B) Overall, nine hub genes were identified from the PPI network. ADRGs, antiandrogen drug resistance genes; LNCaP, lymph node carcinoma of the prostate; BIC, bicalutamide; ENZ, enzalutamide; APN, apalutamide; PPI, protein-protein interaction.

TCGA-PRAD data was used to compare hub gene expression between PCa samples and healthy prostate tissues. Among the nine hub genes, *BCHE* ($P=2.3x10^{-12}$), *CD200* ($P=5.8x10^{-17}$) and *MYH11* ($P=2.3x10^{-17}$) were significantly downregulated in PCa samples compared with healthy tissues (Fig. 2).

Hub genes influence PCa occurrence in Chinese patients. Since TCGA predominantly represents Western populations, the present study also investigated gene expression in a Chinese cohort using microarray data from the CPGEA database. Furthermore, 8 out of the 9 hub genes (excluding *NT*; P=0.86) were significantly differentially expressed in Chinese patients with PCa (Fig. 3). These findings suggested that the identified hub genes are key to PCa occurrence in Chinese populations.

Hub genes influence PCa progression. Tumor, lymph node, metastasis (TNM) staging is widely used to evaluate tumor

Table I. Hub genes of 54 ADRGs among BIC, ENZ and APN in GSE211781 dataset.

	BIC		ENZ		APN	
Gene name	Log_2 FC	P-value	Log_2 FC	P-value	Log_2 FC	P-value
CD200	2.0458783	$5.93x10^{-19}$	1.59821854	$4.63x10^{-12}$	3.0572163	$9.50x10^{-21}$
GCG	1.3191416	$5.76x10^{-14}$	1.11484425	$1.26x10^{-8}$	3.9088046	$4.55x10^{-12}$
NTS	2.7808322	$3.18x10^{-10}$	3.02708496	$8.10x10^{-7}$	2.7487693	$2.77x10^{-10}$
IL1B	-2.409564	$8.70x10^{-8}$	-1.6599581	$1.08x10^{-5}$	-1.1746371	$1.38x10^{-8}$
CCDC80	-1.814258	$5.51x10^{-6}$	-1.9233845	$1.06x10^{-4}$	-1.6778532	$3.09x10^{-7}$
BCHE	1.3011477	$2.06x10^{-4}$	2.91307066	$8.68x10^{-4}$	2.4957071	$7.11x10^{-6}$
MYH11	1.4286021	$6.93x10^{-4}$	1.08698911	$3.41x10^{-3}$	2.3045734	$4.55x10^{-5}$
COL5A1	-3.935565	$1.43x10^{-3}$	-3.6223903	$5.24x10^{-3}$	-1.5384003	$1.06x10^{-4}$
COL27A1	-1.532002	$2.39x10^{-3}$	-1.1766088	$1.06x10^{-2}$	-2.6972333	$2.81x10^{-4}$

BIC, bicalutamide; ENZ, enzalutamide; APN, apalutamide; ADRGs, antiandrogen drug resistance genes; FC, fold-change; CD200, cluster of differentiation 200; BCHE, butyrylcholinesterase; CCDC80, coiled-coil domain containing 80; COL5A1, collagen type V α 1 chain; COL27A1, collagen type XXVII α 1 chain; GCG, glucagon, IL1B, IL-1 β; MYH11, myosin heavy chain 11; NTS, neurotensin.

severity (29). Therefore, the present study examined whether hub genes associated with antiandrogen resistance also influenced PCa progression. RNA-sequencing data and clinical information were obtained from TCGA. Since metastasis data were unavailable for most patients, only T and N stages were analyzed. Hub gene expression was not associated with the T stage (Fig. 4A). However, *MYH11* expression was significantly reduced in patients with lymph node metastasis (P=0.03; Fig. 4B). Thus, in addition to its role in PCa occurrence, MYH11 also appears to affect disease progression.

MYH11 influences DFS in patients with PCa. Next, the present study evaluated whether hub genes were associated with patient survival. GEPIA analysis of TCGA data was used to assess correlations between hub gene expression and DFS. Only *COL5A1* (P=0.011) and *MYH11* (P=0.02) expression significantly influenced DFS in patients with PCa (Fig. 5). These results indicated that *MYH11* impacts both PCa progression and patient survival.

MYH11 is central to PCa cells resistance to antiandrogen drugs in vitro. The present study observed that MYH11 was upregulated in LNCaP cells resistant to BIC, ENZ and APN, and that it influenced PCa occurrence, progression, and prognosis based on multiple databases. Therefore, we hypothesized that *MYH11* serves a key role in PCa development and resistance to antiandrogen drugs. To further investigate this, the present study collected two additional datasets, GSE189129 and GSE81796, which include RNA-sequencing data from C4-2 cells resistant to ENZ. Consistent with results from LNCaP cells, *MYH11* expression was also significantly increased in ENZ-resistant C4-2 cells (GSE136129; $P=5.47x10^{-7}$ and GSE81796; P=0.0274; Fig. S1). These findings suggested that *MYH11* may be key in mediating resistance to antiandrogen drugs in PCa cells.

The present study next validated this function experimentally. A shMYH11 lentivirus was constructed and transduced into C4-2 cells, which were then treated with BIC or ENZ. First, the present study identified MYH11 expression significantly increased rapidly in C4-2 cells exposed to 40 μM BIC or ENZ for 10 days (BIC group, P=0.0007; ENZ group, P=0.006; Fig. 6A and B). Proliferation assays demonstrated a significant reduction in cell proliferation after treatment with different drugs (BIC group, P=0.0320; ENZ group, P=0.0390; Fig. 6C and D). Knockdown with shMYH11 lentivirus significantly reduced MYH11 protein expression in C4-2 cells compared with shControl cells (P=0.0001; Fig. 6E). When these cells were subsequently treated with 80 μM BIC or ENZ, became more sensitive to the drugs, indicating higher rates of cell death compared with the cells treated by DMSO (BIC group, P=0.0160; ENZ group, P=0.0120; Fig. 6F and G). Since MYH11 was initially identified from LNCaP cells and these differ biologically from C4-2 cells, the present study also performed parallel analyses in LNCaP cells. In LNCaP cells, treatment with BIC or ENZ significantly increased MYH11 expression and reduced proliferation (BIC group, P=0.0001; ENZ group, P=0.0001; Fig. S2A-D). The shMYH11 lentivirus also significantly decreased MYH11 expression in LNCaP cells (P=0.0001; Fig. S2E), while shMYH11 knockdown sensitized cells to drug treatment (BIC group, P=0.0180; ENZ group, P=0.0160; Fig. S2F and G). Together, these results indicate that MYH11 is key to determining PCa cell sensitivity to antiandrogen drugs.

Discussion

The increasing life expectancy of men has contributed to rising PCa morbidity (30). Improvements in health awareness and the widespread use of prostate-specific antigen screening have facilitated early detection (31). Nonetheless, aggressive treatment is warranted to achieve improved therapeutic effects. Among the available treatments, androgen deprivation therapy (ADT), which lowers androgen levels or decreases AR expression, has demonstrated therapeutic efficacy and can prolong survival (6). However, most patients (86%) develop resistance to ADT ≤2 years (32), highlighting the need to elucidate the mechanisms underlying antiandrogen drug resistance.

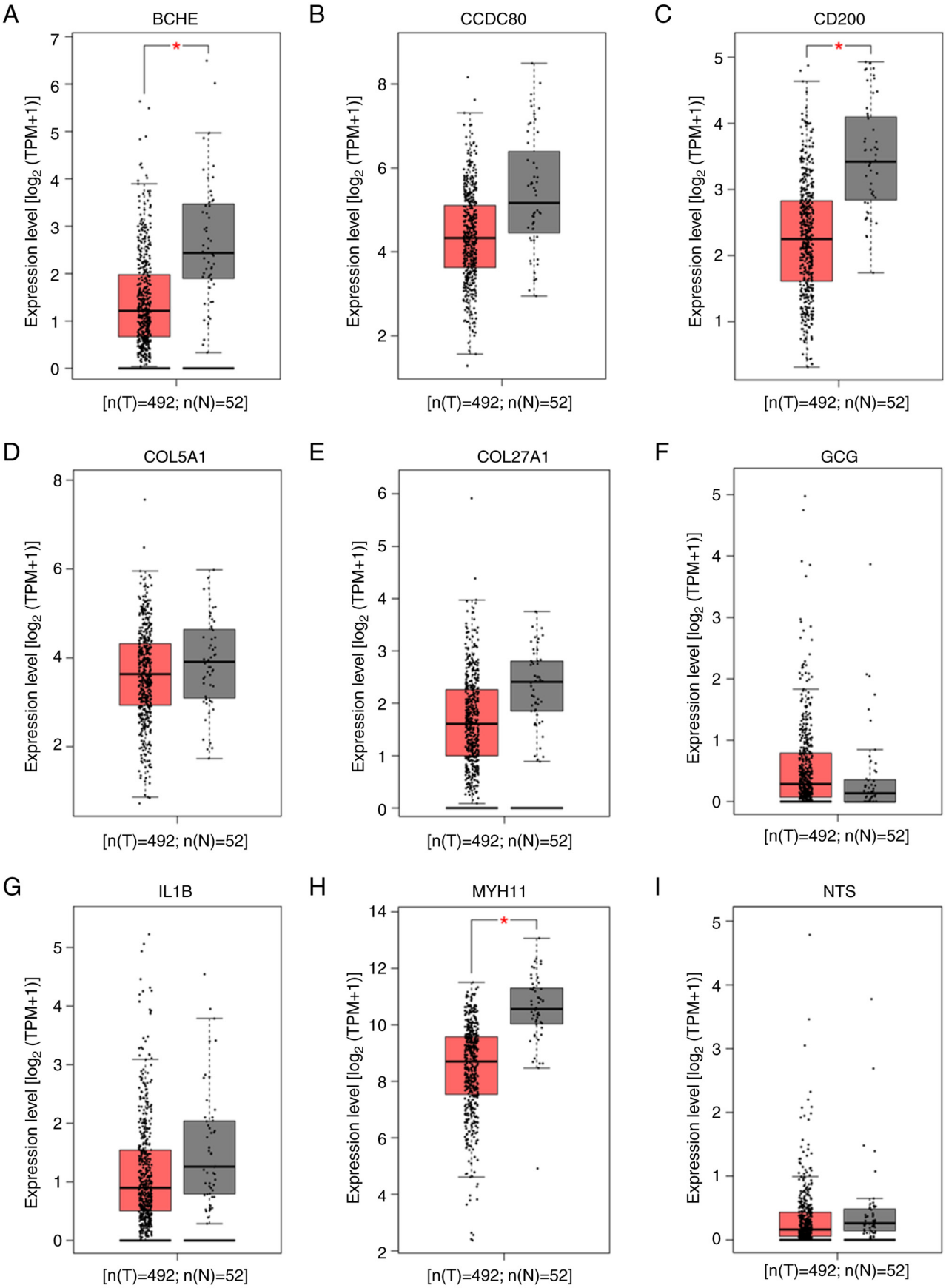

Figure 2. Expression levels of nine hub genes from ADRGs in PCa tissues and healthy prostate tissues analyzed using the GEPIA online tool based on TCGA. Expression levels of (A) BCHE, (B) CCDC80, (C) CD200, (D) COL5A1, (E) COL27A1, (F) GCG, (G) IL1B, (H) MYH11 and (I) NTS. *P<0.05. ADRGs, antiandrogen drug resistance genes; PCa, prostate cancer; GEPIA, Gene Expression Profiling Interactive Analysis; TGCA, The Cancer Genome Atlas; CD200, cluster of differentiation 200; BCHE, butyrylcholinesterase; CCDC80, coiled-coil domain containing 80; COL5A1, collagen type V α 1 chain; COL27A1, collagen type XXVII α 1 chain; GCG, glucagon; IL1B, interleukin 1β; MYH11, myosin heavy chain 11; NTS, neurotensin; PRAD, prostate adenocarcinoma; T, tumor; N, normal.

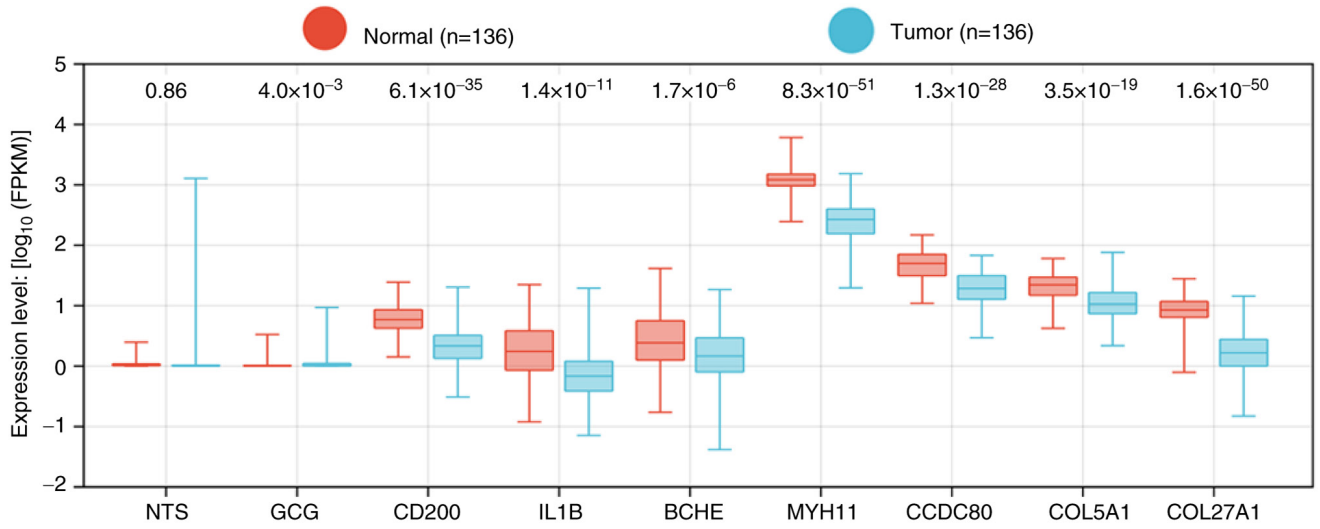

Figure 3. Expression levels of nine hub genes from ADRGs in the CPGEA database. CPGEA, Chinese Prostate Cancer Genome and Epigenome Atlas; ADRGs, antiandrogen drug resistance genes; NTS, neurotensin; GCG, glucagon; IL1B, interleukin 1β; BCHE, butyrylcholinesterase; CCDC80, coiled-coil domain containing 80; COL5A1, collagen type V α 1 chain; COL27A1, collagen type XXVII α 1 chain; MYH11, myosin heavy chain 11; CD200, cluster of differentiation 200.

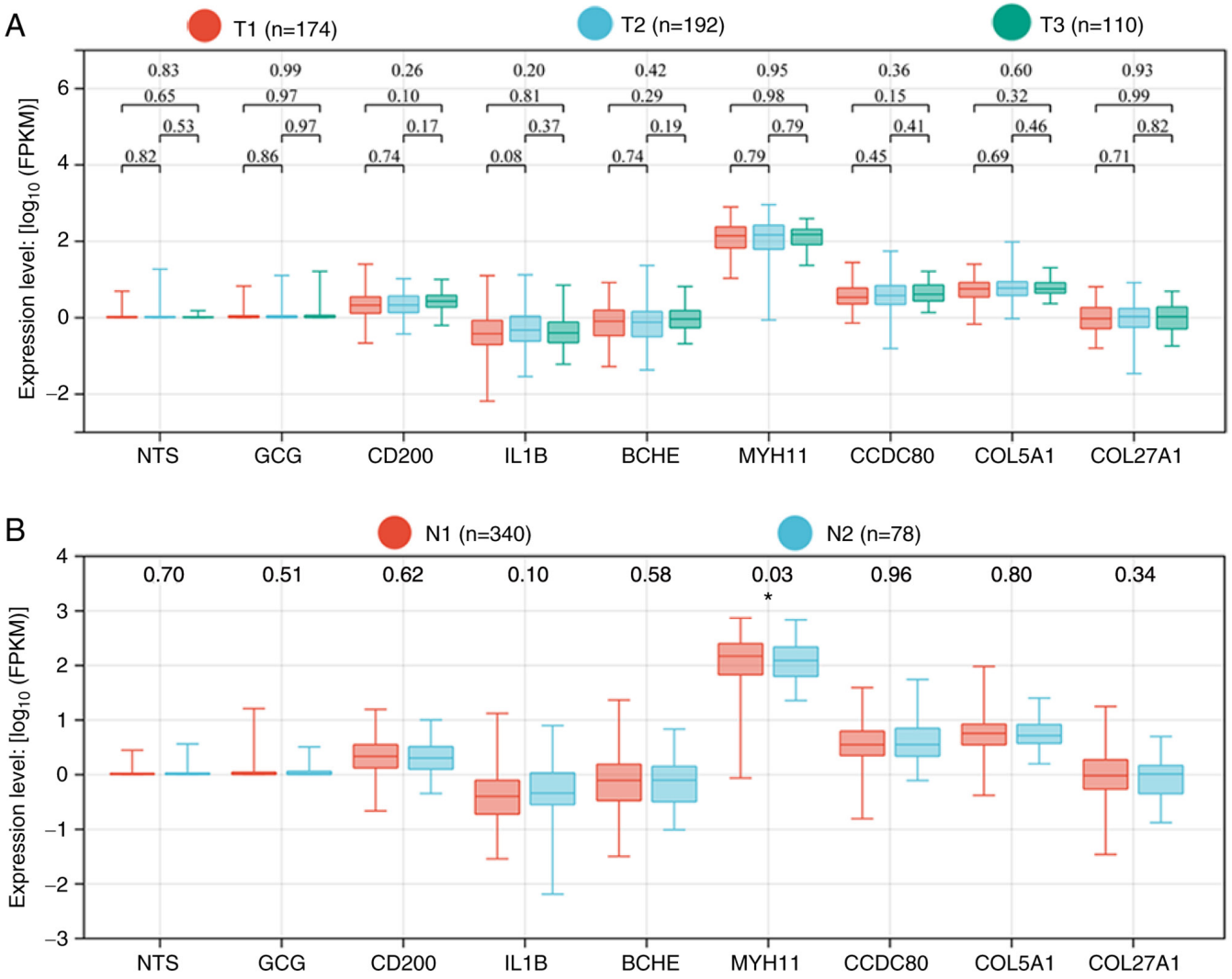

Figure 4. Expression levels of nine hub genes from ADRGs in patients with PCa and different tumor stages (data from TGCA). (A) T stage (B) N stage. *P<0.05. ADRGs, antiandrogen drug resistance genes; PCa, prostate cancer; TGCA, The Cancer Genome Atlas; T, tumor; N, lymph node; NTS, neurotensin; GCG, glucagon; IL1B, interleukin 1β; BCHE, butyrylcholinesterase; CCDC80, coiled-coil domain containing 80; COL5A1, collagen type V α 1 chain; COL27A1, collagen type XXVII α 1 chain; MYH11, myosin heavy chain 11; CD200, cluster of differentiation 200.

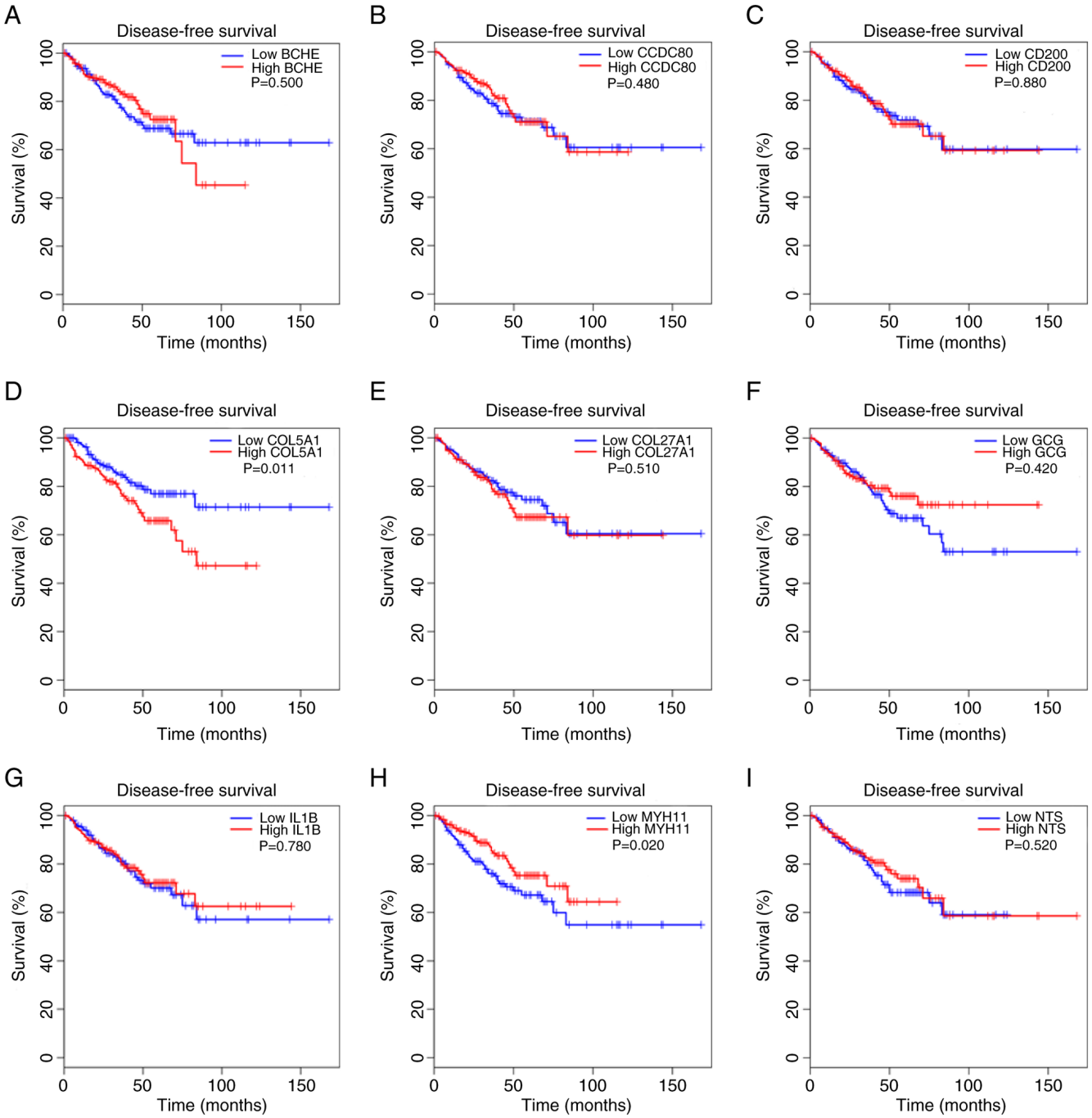

Figure 5. Association between the expression levels of nine hub genes from ADRGs and DFS status from the GEPIA online tool based on TCGA. (A) BCHE (B) CCDC80 (C) CD200 (D) COL5A1 (E) COL27A1 (F) GCG (G) IL1B (H) MYH11 (I) NTS. DFS, disease-free survival; ADRGs, antiandrogen drug resistance genes; GEPIA, Gene Expression Profiling Interactive Analysis; TGCA, The Cancer Genome Atlas; CD200, cluster of differentiation 200; BCHE, butyrylcholinesterase; CCDC80, coiled-coil domain containing 80; COL5A1, collagen type V α 1 chain; COL27A1, collagen type XXVII α 1 chain; GCG, glucagon, IL1B, interleukin 1 β; MYH11, myosin heavy chain 11; NTS, neurotensin.

BIC, ENZ and APN are three antiandrogen drugs with confirmed therapeutic benefits in PCa (8). These agents markedly prolong median survival in patients (33-35); however, resistance eventually develops and the mechanisms remain to be elucidated. Genetic alterations are considered to be a key contributing factor. For example, loss of the chromodomain helicase DNA binding protein 1 gene has been associated with antiandrogen resistance (36). Furthermore, changes in neuregulin 1 expression may alter the tumor microenvironment and promote resistance (37). Collectively, these findings emphasize the importance of genetic alterations in driving resistance to antiandrogen drugs.

Due to the role of genetic alterations in resistance, the present study aimed to identify potential hub genes involved in this process. RNA-sequencing analysis of the GSE211781 dataset identified nine hub genes among 54 ADRGs that may be key for resistance. Further analysis indicated that several of these genes also serve roles in PCa occurrence. *MYH11* emerged as a particularly key gene associated with PCa occurrence, progression and prognosis. Two PCa cell lines, LNCaP

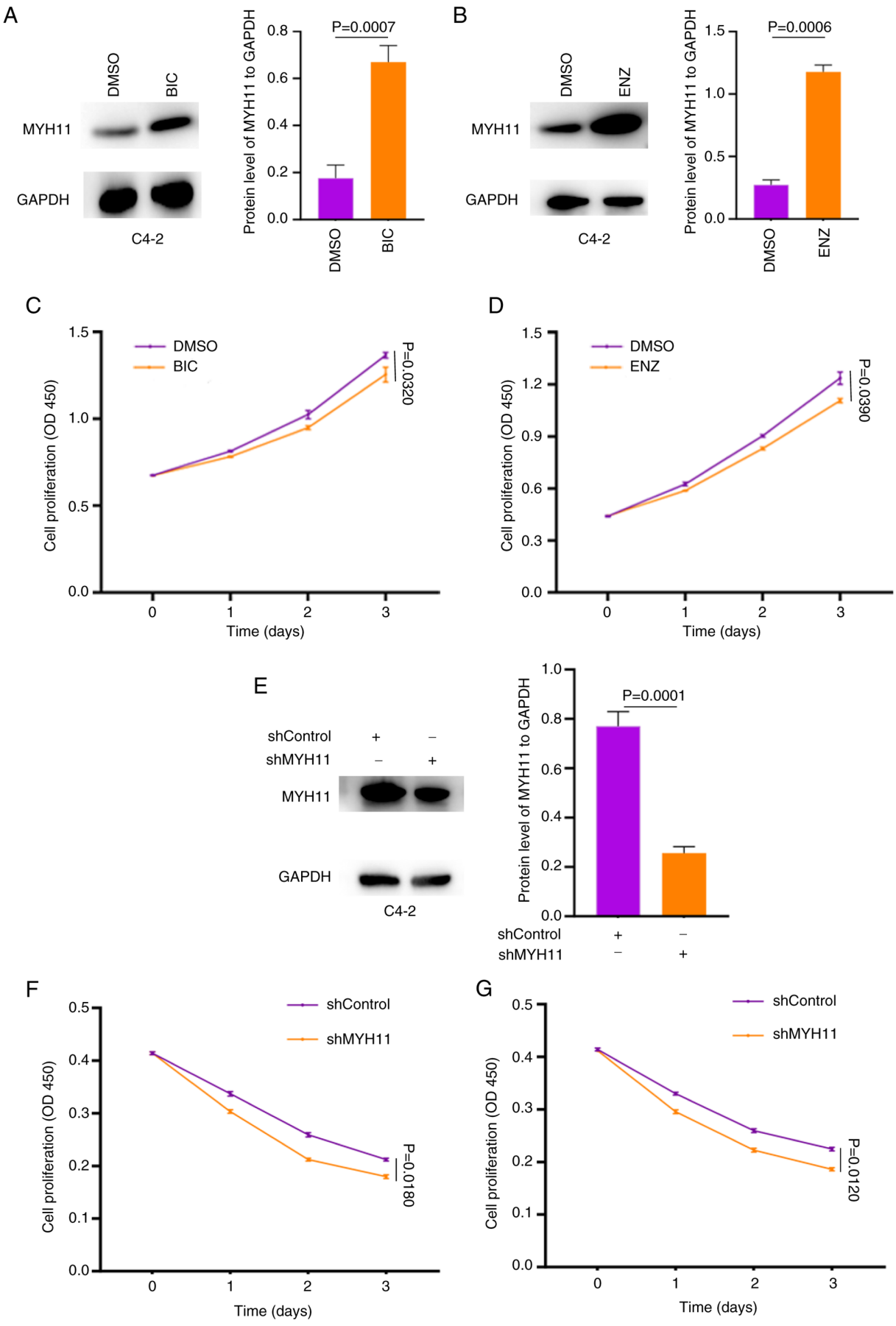

Figure 6. Role of MYH11 in C4-2 cells resistant to BIC and ENZ. (A) Protein levels of MYH11 in C4-2 cells treated by BIC. (B) Protein levels of MYH11 in C4-2 cells treated by ENZ. Proliferative ability of C4-2 cells after BIC (C) and ENZ (D) treatment. (E) Protein levels of MYH11 after C4-2 PCa cells are transduced with shMYH11 lentivirus. Proliferative ability of C4-2 cells after BIC (F) and ENZ (G) treatment after MYH11 knockdown. MYH11, myosin heavy chain 11; BIC, bicalutamide; ENZ, enzalutamide; APN, apalutamide; sh, short hairpin RNA.

and C4-2, were subsequently used to examine whether *MYH11* could influence cellular sensitivity to antiandrogen drugs. Since C4-2 cells were derived from bone metastasis of LNCaP in nude mice, they represent a castration-resistant line with distinct biological features (38). Here, the present study found that although the rate of cell proliferation slowed down slightly in the cells of *MYH11* knockdown compared with the normal control, there were still statistically significant differences for C4-2 cells (BIC group, P=0.016; ENZ group, P=0.012) and LNCaP cells (BIC group, P=0.018; ENZ group, P=0.016). So, the present study identified that *MYH11* modulated the sensitivity of both LNCaP and C4-2 cells to BIC and ENZ, suggesting that *MYH11* can be an indicator for predicting PCa to antiandrogen drugs resistance. However, the present study verified the function of MYH11 only *in vitro* and did not investigate underlying mechanisms. Due to the complexity of drug resistance, including the possibility of acquired mechanisms, these results require additional confirmation in future studies.

MYH11, encoded by the *MYH11* gene, is a smooth muscle myosin belonging to the myosin heavy chain family (39). Mutations in *MYH11* have been implicated in the development of several cancer types (40). For example, *MYH11* hypermethylation promotes gastric cancer progression (41) and the gene is key in acute myeloid leukemia (42,43). *MYH11* also serves as a biomarker for the prediction of lung cancer prognosis (39). Furthermore, MYH11 has been associated with PCa development: Somatic mutations in *MYH11* contribute to PCa occurrence and predict disease progression (44,45).

In the present study however, some unexpected phenomenon were observed: First, *MYH11* was downregulated in the tissues of patients with PCa but demonstrated increased expression in PCa cells treated with antiandrogen drugs. Several factors may explain this discrepancy. First, biological complexity and interindividual variability can produce different results in tissues compared with cell lines. Second, drug treatment can alter gene expression patterns. For instance, previous studies have reported that antiandrogen drugs initially suppress AR expression but eventually increase it through cellular adaptation (5,46). A similar mechanism may underlie the regulation of MYH11. Another phenomenon noted in the present study is that the cells without *MYH11* knockdown would still proliferate after treatment with BIC or ENZ but exhibited decreased proliferation after *MYH11* knockdown. This could be because in PCa cells with *MYH11* expression, proteins such as AR which affect the function of BIC or ENZ still have normal expression but after *MYH11* knockdown, the expression of these proteins may decrease. After a protein such as AR decreases, the cells would be more sensitive to antiandrogen drugs and proliferate at a decreased rate. Although, to the best of our knowledge, no direct studies have reported an association between MYH11 and AR in PCa, a previous study in penile tissue identified such a relationship (47), suggesting a possible association in other tissues. In the present study, drugs that inhibited AR expression also affected MYH11 expression, supporting the idea of a regulatory association between MYH11 and AR in PCa. Cells with *MYH11* knockdown decreased after treated by BIC or ENZ which further indicated that MYH11 may influence AR expression in PCa cells. These findings suggest that MYH11 could be a therapeutic target for drug-resistant PCa.

However, the present study had several limitations. First, the present study identified 54 ADRGs as potentially key for resistance to antiandrogen drugs in PCa and PPI network analysis highlighted nine hub genes. The functional roles of the remaining ADRGs, however, remain to be elucidated. Second, the present examined gene expression using RNA-sequencing data from different databases; however, these findings were not validated in clinical samples. The mechanism of MYH11 in PCa therefore requires further experimental investigation in the future. Third, although *MYH11* expression was generally lower in patients with PCa, higher *MYH11* expression patients were associated with improved DFS compared with patients with low *MYH11* level. This apparent contradiction warrants additional study. Lastly, the present functional analysis was performed using bioinformatics approaches and the mechanisms by which these genes influence PCa development and drug resistance remain to be elucidated.

Additional experiments should be carried out in the future to investigate the mechanism of MYH11, including its interactions with AR signaling. For example, one group of C4-2 cells may be treated with shMYH11 lentivirus and another group with ENZ to make these cells resistant to ENZ. Subsequently, RNA-sequencing could be performed and the differentially expressed genes could be obtained. A pathway analysis could be performed to identify the genes associated with both BIC and ENZ resistance. After identifying these genes, basic experiments such as PCR, western blotting should be performed to further identify the association of potential genes with MYH11. Furthermore, validation in additional PCa cell lines such as 22Rv1 and vertebral-cancer of the prostate is necessary to strengthen the generalizability of the findings in the future. Despite these limitations, the present study suggests that MYH11 serves a key role in resistance to antiandrogen drugs and may potentially serve as a predictive indicator for PCa development and therapeutic response in the future.

In summary, the present study identified nine hub genes among 54 ADRGs from the GSE211781 dataset, with *MYH11* emerging as a key indicator for the prediction of PCa occurrence, progression and also key for PCa cells resistant to antiandrogen drugs.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LP and JS designed the study and got the data from public databases. LP and ZL analyzed the data from pubic databases and wrote the paper. GX and CC conducted all the

experiments including cell culture, western blot and CCK-8 assay. CC contributed to the statistical analysis and revised the manuscript. CC and LP confirm the authenticity of all the raw data. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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