TRIM36, a novel androgen-responsive gene, enhances anti-androgen efficacy against prostate cancer by inhibiting MAPK/ERK signaling pathways

Jie Li[1], Chao Liang[1], Shangqian Wang[1], Meilin Bao[2], Shifeng Su[1], Bianjiang Liu[1], Gong Cheng[1], Chao Qin[1], Pengfei Shao[1], Qiang Lv[1], Ninghong Song[1], Lixin Hua[1], Zhihong Zhang[2], Zengjun Wang[1]

[1] Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China [2] Department of Pathology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

Introduction

Background: Hormone therapy drugs, such as bicalutamide and enzalutamide, directed against prostate cancer focus on androgen receptor (AR) signaling and are initially effective, but the disease progresses to lethality as resistance to these drugs develops. A method to prolong the drug response time and improve the drug efficacy is still unavailable. In this study, we investigated the functional analysis and androgen regulation of TRIM36 and its underlying mechanisms enhancing anti-androgen efficacy against prostate cancer (PCa).

Methods: TRIM36 expression has been detected by mRNA microarray analysis, quantitative reverse transcription (qRT-PCR), Western blotting and Liquid chromatography-Mass Spectrum (LC-MS/MS) in matched prostate cancer and adjacent normal tissues, and prostate cell lines RWPE-1, C4-2, LNCaP, DU145, PC3. A total of 95cases of prostate cancer after radical prostatectomy were analysed in a tissue microarray (TMA) for TRIM36 and androgen receptor (AR) protein expression. Prostate cancer cells stably expressing and shRNAs knockdown TRIM36 were used for CCK-8 assay, clone formation assay and xenograft with or without ADT drugs. Androgen regulation was examined by ChIP, dual-luciferase reporter assay, qRT-PCR and Western blot analysis.

Results: In this study, we found that 63.4% (64/95) of Pca in TMA expressed the TRIM36 protein. Interestingly, patients with negative TRIM36 expression had a shorter biochemical recurrence-free survival. TRIM36 expression was significantly associated with the Gleason score ($P < 0.005$), delayed prostate cancer cell cycle progression and inhibited cell proliferation in vitro and in vivo, and these effects were mediated via inhibition of the MAPK/ERK phosphorylation pathway. Remarkably, we found that rescuing the expression of TRIM36 during anti-androgen therapy could improve the drug efficacy.

Conclusions: Collectively, TRIM36 is a novel androgen-responsive gene, and it dramatically enhanced the efficacy of anti-androgen drugs against prostate cancer.

Methods

Tissue microarray (TMA) of 95 Pca and definition of TRIM36 expression

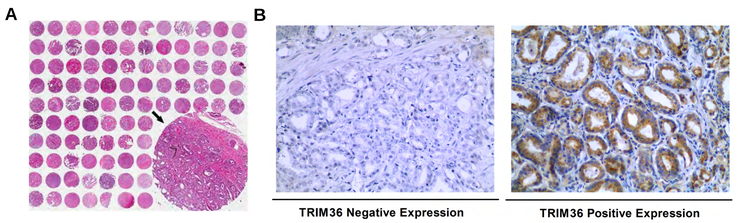

Figure 1. Tissue microarray (TMA) of 95 Pca and definition of TRIM36 expression
A: H&E of Tissue microarray (TMA) of 95 Pca
B: Different immunohistochemistry results for TRIM36 expression in a tissue microarray. Left: Negative TRIM36 expression. Right: Strong TRIM36 expression.

Function of TRIM36 in PCa cell lines.

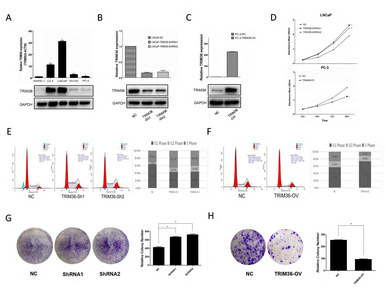

Figure 5. Function of TRIM36 in PCa cell lines.
a Expression of TRIM36 in prostate cancer cell lines. Top: Relative mRNA expressions of TRIM36 in Pca cell lines (C4–2, LNCAP, DU145, and PC-3) and the normal human prostate epithelial cell line RWPE-1. Bottom: Relative protein expression of TRIM36 in PCa cell lines (C4–2, LNCAP, DU145 and PC-3) and RWPE-1. b, c The knockdown and overexpression of TRIM36 were confirmed. b The knockdown and overexpression of TRIM36 in LNCAP and PC-3 cell lines were confirmed with RT-PCR to detect the relative mRNA expression of TRIM36 (*P < 0.05). c The knockdown and overexpression of TRIM36 in LNCAP and PC-3 cell lines were confirmed with western blot to detect the protein expression of TRIM36 (*P < 0.05). d–h TRIM36 regulates cell cycles and proliferation. d The knockdown of TRIM36 significantly promoted cell growth in the LNCAP line, while the overexpression of TRIM36 significantly inhibited cell growth in the PC-3 line. e–f The knockdown of TRIM36 prevented G0/G1 phase cell cycle arrest in the LNCAP line, while the overexpression of TRIM36 resulted in G0/G1 phase cell cycle arrest in the PC-3 line. g, h The knockdown of TRIM36 obviously increased colony formation efficiency in the LNCAP line, while the overexpression of TRIM36 decreased colony formation efficiency in the PC-3 line

Results

Androgen-responsive expression of TRIM36 in prostate cancer cell

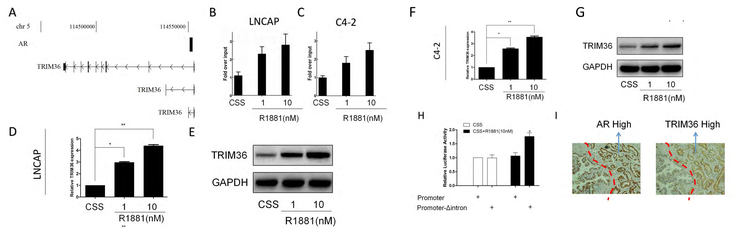

Figure 2. Expression of TRIM36 is upregulated by androgen stimulation in LNCAP cells. a Genomic view of the TRIM36 gene in the UCSC genome browser. ChIP-chip analysis identified an ARBS in the first intron region of TRIM3616. b–c Validation of ligand-dependent androgen receptor recruitment to the TRIM36 ARBS using a ChIP assay. LNCAP (b) and C4–2 (c) cells were treated with R1881 (1/10 nM) or CSS for 24 h. d–g Induction of TRIM36 by androgen treatment in LNCAP cells. d, f RT-PCR revealing androgen-dependent upregulation of TRIM36 mRNA in LNCAP and C4–2 cells. LNCAP cells were treated with R1881 (1/10 nM) or CSS. The TRIM36 mRNA levels are plotted relative to those of the CSS control. e, g Androgen mediated induction of TRIM36 protein expression in LNCAP and C4–2 cells. Protein levels were analyzed by Western blot analysis. GAPDH was used as a loading control. TRIM36 protein levels were quantified by densitometry and normalized to GAPDH levels. h LNCAP cells were transfected with TRIM36-promoter reporter plasmid or TRIM36-promoter + Δintron reporter plasmid, and treated with R1881 (10 nM) or CSS for 24 h. TRIM36 promoter activity was significantly increased in the TRIM36 promoter + Δintron group with R1881 (10 nM) in a dual-luciferase assay. i The TRIM36 gene is overexpressed in prostate cancer tissues with the same pattern of AR expression in the nucleus

Anti-androgen therapy reduces TRIM36 expression

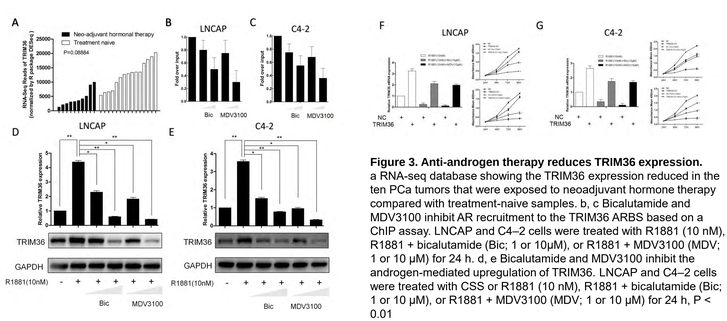

Figure 3. Anti-androgen therapy reduces TRIM36 expression. a RNA-seq database showing the TRIM36 expression reduced in the ten PCa tumors that were exposed to neoadjuvant hormone therapy compared with treatment-naive samples. b, c Bicalutamide and MDV3100 inhibit AR recruitment to the TRIM36 ARBS based on a ChIP assay. LNCAP and C4–2 cells were treated with R1881 (10 nM), R1881 + bicalutamide (Bic; 1 or 10μM), or R1881 + MDV3100 (MDV; 1 or 10 μM) for 24 h. d, e Bicalutamide and MDV3100 inhibit the androgen-mediated upregulation of TRIM36. LNCAP and C4–2 cells were treated with CSS or R1881 (10 nM), R1881 + bicalutamide (Bic; 1 or 10 μM), or R1881 + MDV3100 (MDV; 1 or 10 μM) for 24 h, P < 0.01

Rescued TRIM36 increased the anti-androgen sensitivity

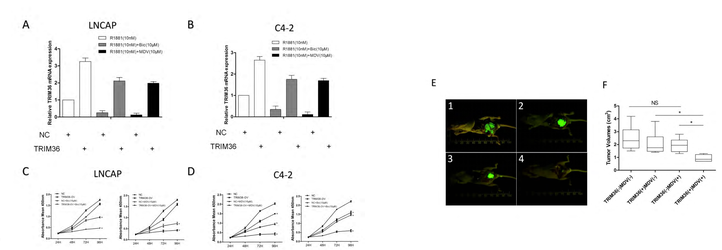

Figure 4. Rescued TRIM36 increased the anti-androgen sensitivity in vitro and in vivo.
a, b Validation of rescued TRIM36 expression after antiandrogen drugs in LNCAP and C4–2 cell. c, d The rescued TRIM36 alters the anti-androgen drugs efficacy by inhibiting prostate cancer cell growth. E C4–2 cells with or without expression of TRIM36 were labeled with GFP and injected orthotopically into nude mice. Two week after implantation, tumors were formed and visualized by fluorescence image. Representative fluorescence images of each group are shown (e1–e4). Sequential in vivo whole body fluorescence imaging of tumor progression in different groups. After another 3 weeks and a final fluorescence imaging, mice were sacrificed and tumors were removed (e5–e8). f Tumor sizes in the four groups after sacrifice and tumor removal. *P < 0.05, **P < 0.01

Clinical Outcome

Table1. Relationship of TRIM36 expression and clinicopathologic characteristics of patients

Variable	TRIM36 expression		
	Negative ($n = 31$)	Positive ($n = 64$)	*P* value
Age			0.605
<60	3	3	
60–70	14	28	
>70	14	33	
Preoperative PSA (ng/ml)			0.649
<10	7	20	
10–20	10	20	
>20	14	24	
Gleason score			**0.005**
≦6 or =3+4	12	44	
=4+3 or ≧8	19	20	
T stage			0.950
pT2	26	54	
pT3/ T4	5	10	
Biochemical recurrence			**0.001**
Negative	12	48	
Positive	19	16	

Bold values signify $P < 0.05$. *P* values were two-tailed and based on the Pearson chi-square test

Co-relationship between clinical factors and the expression of TRIM36 in prostate cancer

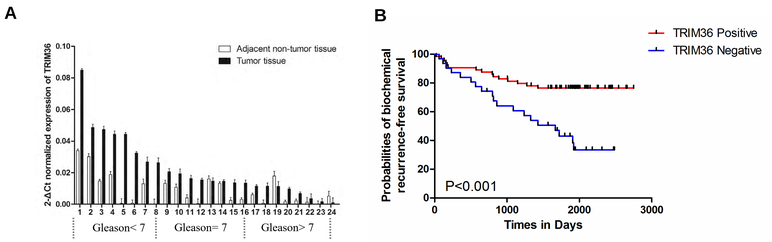

Figure 7. Co-relationship between clinical factors and the expression of TRIM36 in prostate cancer
A: Relative mRNA expression of TRM36 in PCa tissues with different Gleason scores compared with the corresponding non-tumor tissues. The TRIM36 mRNA level is higher in the PCa tissues with low Gleason scores.
B: Kaplan–Meier biochemical recurrence-free survival curves for PCa patients based on TRIM36 expression levels. Patients with positive TRIM36 expression had obviously longer survival times than those with negative TRIM36 expression (log-rank test, P < 0.001)

TRIM36 inhibits the MAPK/ERK phosphorylation pathway

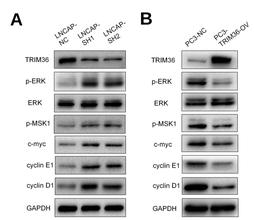

Figure 6. TRIM36 inhibits the MAPK/ERK phosphorylation pathway
The knockdown of TRIM36 promoted changes in MAPK/ERK phosphorylation pathway marker expressions with gains in p-ERK, p-MSK1, C-MYC and Cyclin E1, and Cyclin D1 in the LNCAP line. d In contrast, the overexpression of TRIM36 promoted changes with losses in p-ERK, p-MSK1, C-MYC and Cyclin E1, and Cyclin D1 in the PC-3 line. GAPDH was used as a loading control. *P < 0.05

Conclusion

Conclusions: Collectively, TRIM36 is a novel androgen-responsive gene, and it dramatically enhanced the efficacy of anti-androgen drugs against prostate cancer.