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ORIGINAL PAPER

SOX4 is activated by C-MYC in prostate cancer

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Abstract

Although MYC proto-oncogene (C-MYC) amplification has been consistently reported to be a potential marker for prostate cancer (PCa) progression and prognosis, the clinicopathological and prognostic significance of C-MYC protein expression remains controversial. Overexpression of SOX4 has been shown to play important roles in multiple cancers including PCa. However, the link between these two critical genetic aberrations is unclear. In the current study, we showed that C-MYC was overexpressed in 16.2% (17/105) of Chinese patients with localized PCa. Overexpression of C-MYC was significantly associated with high Gleason scores ($P=0.012$) and high Ki67 labeling index ($P=0.005$). C-MYC overexpression was correlated with cancer-related mortality and suggested to be an unfavorable prognostic factor in Chinese PCa patients ($P=0.018$). Overexpression of C-MYC is associated with SOX4 overexpression in PCa tissues. Notably, SOX4 is a direct target gene of C-MYC; C-MYC activates SOX4 expression via binding to its promoter. In addition, Co-IP analysis demonstrated a physical interaction between C-MYC and SOX4 protein in PCa cells. Clinically, C-MYC+/SOX4+ characterized poor prognosis in a subset of PCa patients. In total, C-MYC overexpression may contribute to PCa progression by activating SOX4. Our findings highlight an important role of C-MYC/SOX4 in PCa progression in a subset of PCa patients.

Keywords Prostate cancer · C-MYC · SOX4 · Overexpression · Prognosis

Introduction

Prostate cancer (PCa) is a common, heterogeneous disease with marked variability in progression [1]. Currently, the established prognostic factors (Gleason score, pathological stage and serum PSA) cannot precisely distinguish indolent PCas from highly aggressive cancers [1, 2]. Thus, novel prognostic biomarkers are urgently needed for PCa diagnosis and management.

C-MYC is a regulatory protein of transcription that regulates multiple cellular processes including cell proliferation, cell-cycle progression, transcription, differentiation, apoptosis, and cellular motility [3]. C-MYC is overexpressed in large varieties of tumor types and plays an important role in tumor initiation and progression [4]. In PCa, elevated C-MYC expression has been found in PCa tissues [5–7] and is associated with biochemical recurrence after radical prostatectomy [7]. The overexpression of C-MYC, in many cases, is associated with somatic genetic alterations including translocations and gene amplification [4]. C-MYC amplification has been consistently reported to be an independent potential marker for disease progression and prognosis in

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PCa [8, 9]. However, the relationship of C-MYC overexpression and C-MYC amplification as well as its correlation with clinicopathological parameters, disease progression, and prognosis remains controversial [9–12].

The sex determining region Y-box 4 (SOX4) gene is a developmental regulatory protein of transcription involved in the regulation of many key cellular processes, including apoptosis, cell-cycle control, microRNA processing, differentiation, and growth factor signaling [13]. Large-scale gene expression studies have identified the overexpression of the SOX4 gene in a variety of human cancers, including prostate [14], breast [15], lung [16], and colon cancers [17]. SOX4 has been recognized as one of the 64 "cancer signature" genes, suggesting that SOX4 plays a fundamental role in tumorigenesis and tumor progression [18]. Previously, we have reported that SOX4 may initiate a transcriptional program that enables the epithelial–mesenchymal transition (EMT) phenotype [19]. Tiwari et al. also reported that SOX4 is a master regulator of EMT by controlling EZH2 expression and epigenetic reprogramming [20, 21]. More importantly, SOX4 might contribute to metastasis by cooperating with other important oncogenes to promote PCa metastasis [22, 23]. Most recently, we have demonstrated a link between SOX4 and ERG gene in the development of a subset of PCa patients [22], and that SOX4 may serve as a prognostic marker for Chinese PCa patients [19]. Therefore, these data highlight the role of SOX4 in PCa progression and metastasis.

In this study, we show that SOX4 is a direct target of C-MYC. Clinically, the overexpression of C-MYC is significantly associated with SOX4 expression and C-MYC+/SOX4+ characterizes poor prognosis in a subset of PCa patients. Collectively, our data suggest a cooperative role of C-MYC and SOX4 in PCa.

Materials and methods

Patients and tissue microarray (TMA) construction

A total of 126 PCa patients (Qilu cohort) diagnosed between 2007 and 2014 participated in our study. The first cohort consisted of 105 men with localized PCas who have undergone radical prostatectomy as monotherapy. The follow-up data were available from 94 patients, ranging from 3 to 115 months (mean 35 months). The second cohort included patients with CRPC treated by transurethral resection of the prostate to relieve symptomatic obstruction due to the locally advanced disease ($n=21$). The initial treatment for patients was either observation or surgery. Morphology was validated by two pathologists (B. H. and H. Y. D.). Written informed consent was obtained from each patient. The study protocol was approved by the Institutional Review Board of Medicine School of Shandong University.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed by the standardized labeled streptavidin biotin kit (Dako Cytomation, USA) according to the manufacturer's instructions. The slides were incubated with rabbit polyclonal anti-SOX4 antibody (1:100 dilution, Abcam, UK), mouse monoclonal anti-C-MYC antibody (1:200 dilution, Abcam, UK), or mouse monoclonal anti-Ki67 (1:100 dilution, Dako, USA), and was blindedly evaluated by two pathologists (B.H. and H.Y.D.) independently. Sections with evaluation disagreement between the reviewers were re-reviewed until a consensus was reached. The scoring system to validate C-MYC, SOX4, and Ki67 expression was described elsewhere [19].

Fluorescence in situ hybridization (FISH)

FISH analysis of abnormalities in C-MYC gene copy was carried out with the C-MYC/CEP8 probe mix (Dako, Denmark) according to the manufacturer's protocol, and the scoring system used was previously described [8]. A minimum of 50 cancer cells under each visual field were recorded. Cases lacking tumor tissues in both two cores were excluded, and cancer tissues with very weak or no signals were recorded as insufficiently hybridized. Amplification of the C-MYC locus was defined as a C-MYC/CEP8 ratio of higher than 2.0.

Cell lines and plasmids

The HEK293T (CRL-3216) cell line and human PCa cell lines (LNCaP and VCaP) were obtained from American Type Culture Collection (Rockville, USA) and cultured according to the manufacturer's recommendations.

Cell transfection

The plasmids and siRNA were transfected into PCa cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The pcDNA3.3-C-MYC plasmid (Addgene plasmid #26818) was obtained as a gift from Dr. Derrick Rossi at Harvard Medical School.

RNA extraction and quantitative real-time PCR (RT-PCR)

RNA extraction and RT-PCR were performed as previously described [19]. Total RNA was extracted with Trizol reagents following the manufacturer's instructions (Invitrogen).

GAPDH was used as an internal loading control. The details of the primers for each gene are listed in Supplementary Table 1.

Western blot and co-immunoprecipitation (Co-IP) analysis

Western blot was carried out as previously described [19]. The membrane was incubated with primary antibodies for C-MYC (1:1000; CST, USA), SOX4 (1:1000; Abcam, UK), E-cadherin (1:1000, CST, USA), and Vimentin (1:1000, CST, USA). Actin (1:1000, ZSGB-BIO, China) was used as a loading control. Three independent experiments were performed.

Immunoprecipitations were performed with Protein A/G Agarose beads (Beyotime, China) to assess the interaction between C-MYC and SOX4 according to manufacturer's instructions. After harvesting the cells, the supernatants were incubated overnight at 4 °C with Protein G-Sepharose beads (Sigma) conjugated with rabbit anti-SOX4, anti-Flag, and anti-C-MYC antibody. The samples were then run through gradient SDS-polyacrylamide gels and transferred to membranes that were probed with anti-C-MYC or anti-SOX4 antibodies.

Table 1 Association of clinicopathological variables and molecular biomarkers with C-MYC overexpression

Variables	C-MYC protein expression		*P*
	Negative and weak (/%)	Moderate and strong (/%)	
All cases	80 (84.2)	15 (15.8)	
Age (years)			
≤65	14 (77.8)	4 (22.2)	0.474
>65	66 (85.7)	11 (14.3)	
Pre PSA (ng/ml)			
<4	4 (80.0)	1 (20.0)	0.501
4–10	5 (71.4)	2 (28.6)	
>10	61 (87.1)	9 (22.9)	
Gleason score			
<7	1 (100.0)	0 (0.0)	0.012
7	40 (93.0)	3 (7.0)	
>7	33 (73.3)	12 (26.7)	
Pathological tumor stage			
≤pT2	53 (86.9)	8 (13.1)	0.287
≥pT3	17 (77.3)	5 (22.7)	
Clinical tumor stage			
≤cT2	54 (90.0)	6 (10.0)	0.056
≥cT3	19 (73.1)	7 (26.9)	
Metastasis			
No	55 (87.3)	8 (12.7)	0.307
Yes	17 (77.3)	5 (22.7)	
Ki67			
<10%	74 (89.2)	9 (10.8)	0.002
≥10%	6 (50.0)	6 (50.0)	
C-MYC amplification			
Not amplified	75 (94.9)	4 (5.1)	<0.001
Amplified	5 (31.3)	11 (68.7)	
ERG			
Not rearranged	62 (86.1)	10 (13.9)	0.215
Rearranged	9 (69.2)	4 (30.8)	
SOX4			
Negative and weak	63 (87.5)	9 (12.5)	0.022
Moderate and strong	6 (54.5)	5 (45.5)	

Values not available for all 95 cases

Chromatin immunoprecipitation assay (ChIP)

ChIP assay was performed according to the manufacturer's protocol (ChIP Assay Kit, Millipore, USA). The purified chromatin was immunoprecipitated by 6 μg of mouse monoclonal anti-C-MYC (Abcam, UK) or mouse anti-IgG (Beyotime, China). The purified chromatin was assessed by RT-PCR. The primers used are described in Supplementary Table 1.

Dual-luciferase assay and reporter constructs

Constructs of the SOX4 luciferase reporter or the mutants were co-transfected with C-MYC expression plasmids into 293T cells for 48 h. Luciferase activity was measured using the dual-luciferase reporter assay system (Promega). The pRL-TK Renilla luciferase reporter vector was used as an internal control (Promega). Renilla luciferase activity was used to normalize luciferase activity.

Cell migration and invasion assays

Cell migration experiments and invasion assays were performed as previously described [19]. Each assay was performed three times.

Bioinformatics analysis

The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering Cancer Center (MSKCC) datasets were downloaded from (http://gdac.broadinstitute.org/). Datasets of GSE35988, GSE33316, GSE120006, GSE70769, and GSE74367 were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo). The ChIP-seq data GSM1907205, GSM1234499, and GSM2807320 were downloaded from the Cistrome database (http://cistrome.org). Gene Set Enrichment Analysis (GSEA) was performed according to the manufacturer's instructions (http://software.broadinstitute.org/gsea/index.jsp). For GSEA, patients of the

TCGA and GSE70769 cohort were grouped by the median of C-MYC expression. ChIPseeker packages and pheatmap packages (R version) were utilized as previously described.

Statistical analysis

The data were analyzed statistically with SPSS 19.0 (Chicago, IL, USA). Two-sided Student's *t* test and the Mann–Whitney test were used for statistical comparisons. Correlations between C-MYC protein expression and clinicopathological parameters were evaluated by Spearman's test. The Kaplan–Meier method was applied for the analysis of follow-up data.

Results

Genetic aberrations and expression of C-MYC in PCa patients

IHC and FISH were performed to characterize the expression and genetic abnormalities of C-MYC in Chinese PCa patients Overall, *C-MYC* amplification was identified in 16.8% (16/95) of the cases. Representative IHC and FISH images for C-MYC are shown in Fig. 1a, b. *C-MYC* amplification was also significantly associated with C-MYC overexpression and there was a 90.5% concordance between *C-MYC* amplification and protein expression (Table 1). In total, 11 of 16 *C-MYC* amplified cases showed C-MYC overexpression by IHC, whereas four C-MYC IHC-positive cases had no *C-MYC* amplification. We also demonstrated that C-MYC was overexpressed in 17 of 105 patients (16.2%) with localized PCa. In contrast,

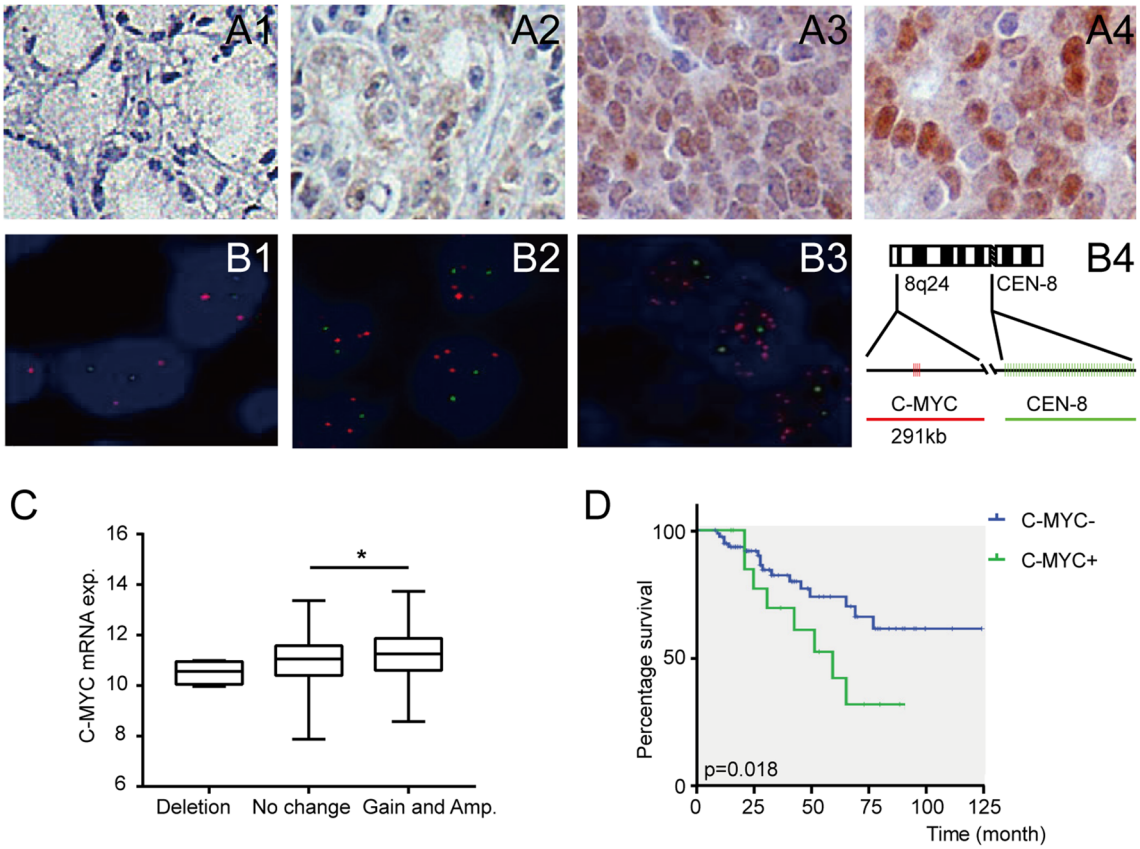

Fig. 1 C-MYC expression is correlated with C-MYC copy number variation and poor prognosis. **a** Representative IHC images of C-MYC expression in the Qilu cohort. **A1**, Negative; **A2**, Weak; **A3**, Moderate; **A4**, Strong. **b** Representative FISH images of C-MYC gene aberrations. **B1**, No amplification of C-MYC or chromosome 8 (C-MYC:CEP8 ratio = 1.06); **B2**, Low-level amplification of C-MYC (C-MYC:CEP8 = 2.32); **B3**, High-level amplification of C-MYC (C-MYC:CEP8 ratio = 4.84); **B4**: Schematic illustration of C-MYC FISH DNA Probe hybridization regions (C-MYC = red and CEP8 = green). **c** C-MYC expression in PCa cases with a different copy number variation in the TCGA cohort. **d** Kaplan–Meier survival analysis of PCa cases from the Qilu cohort stratified by high and low C-MYC expression levels ($P = 0.018$, Log-rank test). $^*P < 0.05$

among CRPC tumors, 12 (57.1%) showed moderate or strong staining for C-MYC. In addition, PCa cases with *C-MYC* gain or amplification expressed higher C-MYC according to the TCGA dataset (Fig. 1c).

C-MYC overexpression was significantly associated with high Gleason scores ($P=0.012$), high Ki67 index ($P=0.002$), and clinical tumor stage ($P=0.056$), but not with patient's age ($P=0.474$) or preoperative PSA levels ($P=0.501$). We next determined whether C-MYC overexpression may serve as a prognostic marker in PCa. As shown in Fig. 1d, C-MYC overexpression was significantly linked to cancer-related death in our cohort ($P=0.018$, Kaplan–Meier survival analysis).

Overexpression of C-MYC is associated with SOX4 overexpression in prostate cancer

Previously, we have found SOX4 may serve as a prognostic marker for Chinese PCa patients [19]. The result of GSEA in the TCGA dataset revealed that the SOX4 co-expression signature was significantly enriched for C-MYC expression (Fig. 2a). To investigate the relationship between C-MYC and SOX4, we performed in silico analysis using published datasets. As shown in Fig. 2b–d, the mRNA level of C-MYC and SOX4 presented a consistent pattern. We also found a significant correlation between C-MYC and SOX4 expression levels (Fig. 2e–h). Notably, the correlation was further validated by IHC in the Qilu cohort. Notably, SOX4

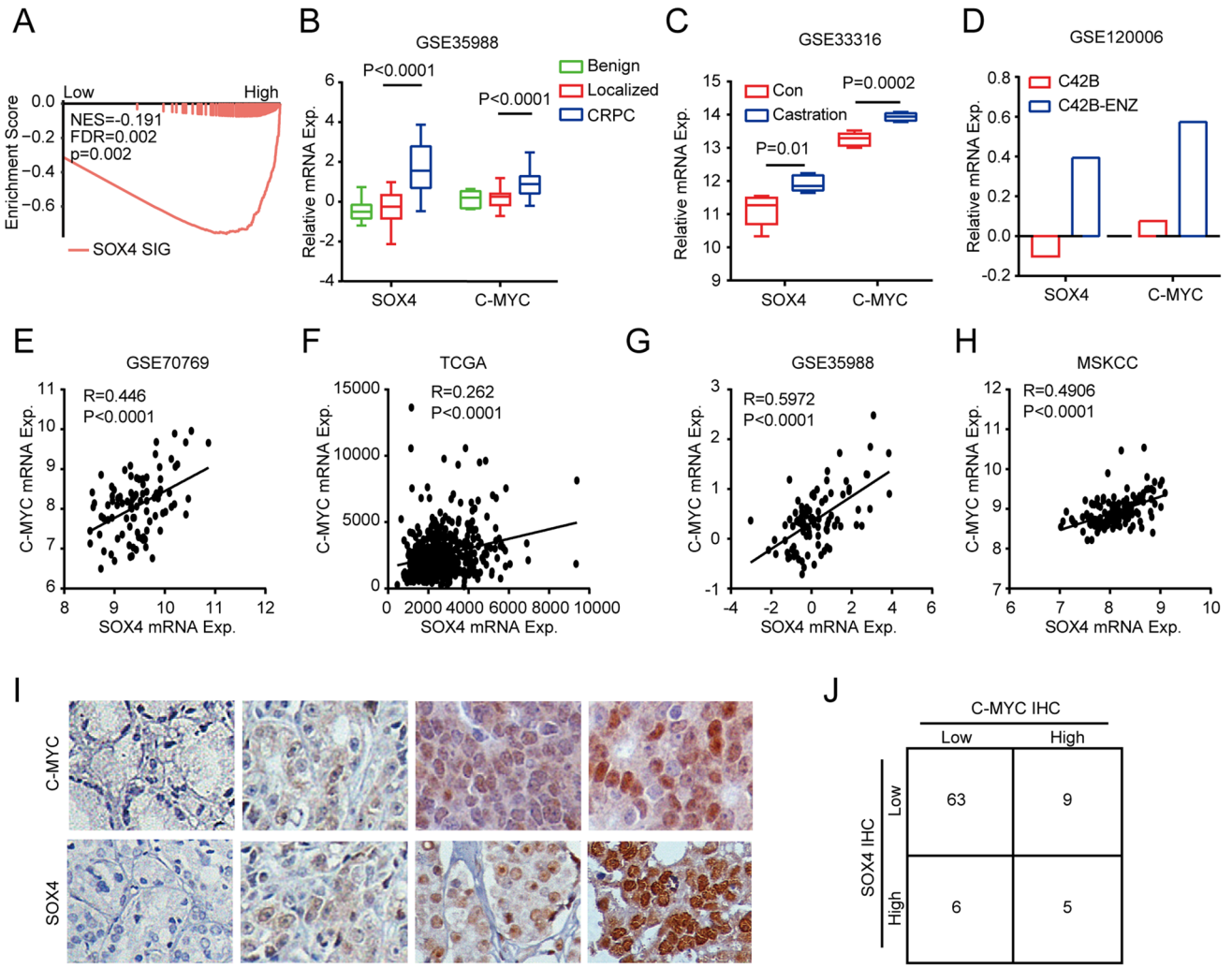

Fig. 2 C-MYC expression is positively correlated with SOX4. **a** Gene set enrichment analysis (GSEA) of SOX4 co-expressed signatures (derived from TCGA) in the TCGA cohort grouped by C-MYC expression. ES = −0.191, $P=0.002$, FDR q = 0.002. **b–d** Expression of C-MYC and SOX4 in GSE35988 (**b**), GSE33316 (**c**), and GSE120006 (**d**). **e–h** Analysis showing correlation of C-MYC and SOX4 expression in GSE70769 (**e**), TCGA (**f**), GSE35988 (**g**), and MSKCC (**h**). **i** Representative images of IHC staining of C-MYC and SOX4 in PCa patients with a different Gleason score from the Qilu cohort. **j** Contingency table for C-MYC and SOX4 expression status from IHC data in the Qilu cohort ($P=0.022$, Chi-square test)

expression was significantly associated with C-MYC expression by IHC (Fig. 2i–j, $P = 0.022$).

SOX4 is a direct target of C-MYC

As shown in Fig. 3a, the mRNA and protein expression levels of C-MYC and SOX4 were shown in human PCa LNCaP and VCaP cell lines. Knockdown of C-MYC suppressed SOX4 expression at both the mRNA and protein levels in VCaP and LNCaP cells (Fig. 3b). Accordingly, in vitro overexpression of C-MYC significantly increased SOX4 expression levels (Fig. 3c). To investigate whether C-MYC regulates SOX4 by direct transcriptional regulation, we referred to a publicly available C-MYC ChIP-seq dataset and identified that C-MYC binds at the SOX4 promoter region (Fig. 3d). By integrating the result from ChIP-seq and predicted binding site using online tools, five regions were selected as potential C-MYC binding sites (Fig. 3e). ChIP assay revealed that the antibody against C-MYC efficiently immunoprecipitated − 1420 bp to − 1226 bp upstream from TSS of SOX4 gene in VCaP cells (Fig. 3f). To confirm the direct transcriptional regulation of C-MYC to SOX4, a

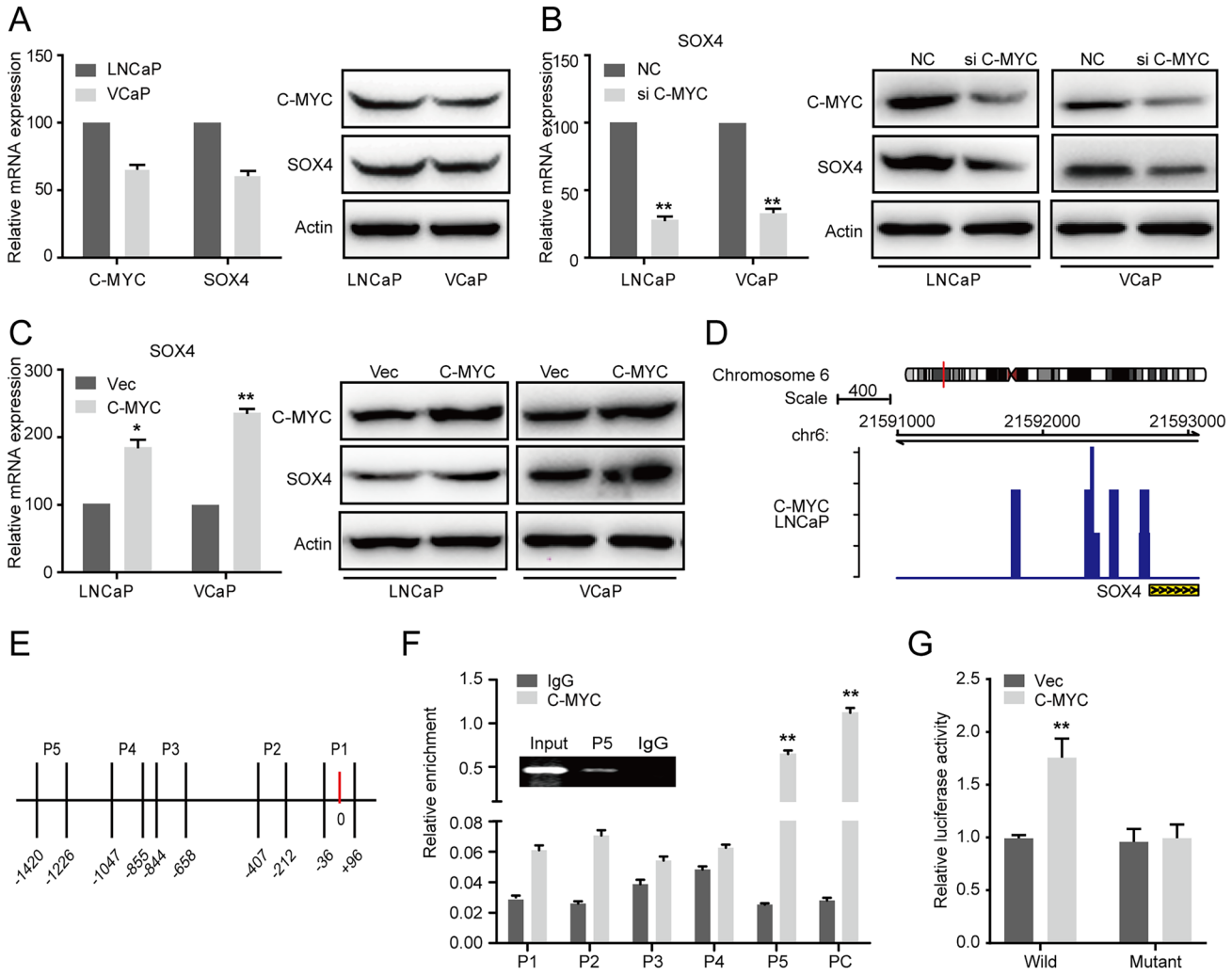

Fig. 3 SOX4 is a direct target of C-MYC in PCa. **a** qRT-PCR (left) and Western blot (right) analyses of C-MYC and SOX4 expression at the mRNA and protein level in LNCaP and VCaP cells. **b** qRT-PCR (left) and Western blot (right) analyses of C-MYC and SOX4 expression at the mRNA and protein level in LNCaP and VCaP cells after transfection with siRNA targeting C-MYC. **c** qRT-PCR (left) and Western blot (right) analyses of C-MYC and SOX4 expression at the mRNA and protein level in LNCaP and VCaP cells after transfection with plasmids expressing C-MYC. **d** C-MYC chromatin binding at the SOX4 promoter region was determined by ChIP-seq experiment (GSM1907205). **e** The schematic binding region (− 1420 to + 96 bp) of the SOX4 gene was shown. **f** ChIP-qPCR analysis of C-MYC recruitment at SOX4 promoters in VCaP cells. Purified rabbit IgG was used as a negative control. Prime of validated C-MYC binding site was used as positive control (PC). **g** A luciferase reporter assay was used to determine whether SOX4 was a direct target of C-MYC in HEK293T cells. 293T cells were transiently transfected with the wild-type construct of pGL3-SOX4 (Wild), mutant construct of pGL3-SOX4 (Mutant), empty plasmid (Vec), or C-MYC expression plasmid (C-MYC) as indicated. $^{*}P < 0.05$, $^{**}P < 0.01$

luciferase reporter assay was performed on 293T cells transfected with wild-type or mutant pGL3-SOX4 promoter vectors. As shown in Fig. 3g, C-MYC increased the promoter activity in cells transfected with wild-type vectors but not in cells with mutant vectors.

Physical interaction of C-MYC and SOX4 in PCa cell lines

Considering the significant association of the two transcriptional factors C-MYC and SOX4, we asked whether they share similar transcriptional programs. According to online tool Cistrome (http://cistrome.org), we obtained the putative target genes of both C-MYC and SOX4. Vennen plots showed an overlap of their target genes (Fig. 4a). In addition, bioinformatics analysis revealed that both C-MYC and SOX4 had the strongest ChIP-seq read intensity at the TSS region (Fig. 4b). Notably, Co-IP analysis demonstrated C-MYC interacted with SOX4 in VCaP (Fig. 4c, upper) and reciprocal Co-IP further confirmed the existence of C-MYC in protein immune-precipitated by anti-SOX4 antibody (Fig. 4c, bottom). More importantly, siRNA knockdown of SOX4 in VCaP cells significantly decreased the binding of C-MYC to SOX4 (Fig. 4d). This result suggests that SOX4 expression is essential for C-MYC binding to SOX4 at the protein levels.

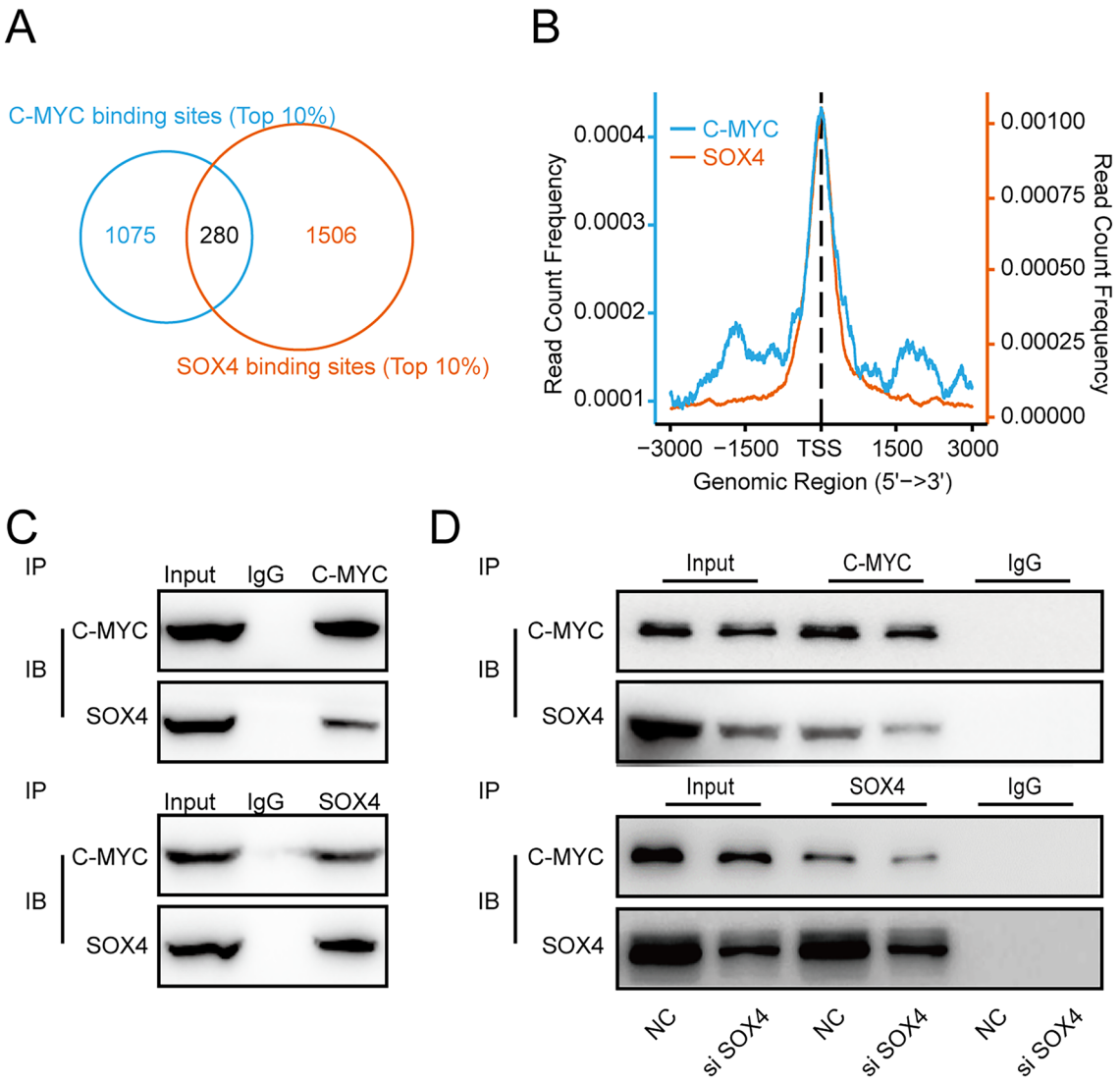

Fig. 4 Interaction of C-MYC and SOX4 in PCa cells. **a** The overlap of C-MYC and SOX4 binding sites. The top 10% putative target genes were used. **b** Average C-MYC and SOX4 ChIP-seq intensity around promoter region. **c** Co-immunoprecipitation assays of C-MYC and SOX4 in VCaP cells. **d** Co-immunoprecipitation assays of C-MYC and SOX4 in VCaP cells after transfection with siRNA targeting SOX4

Synergism effect of C-MYC and SOX4 on oncogenic function

Having shown that there was a significant correlation of expression and function of C-MYC and SOX4, we asked whether they acted synergistically for oncogenic functions. As shown in Fig. 5a, b, siRNA knockdown of C-MYC significantly inhibited the migration and invasive capacity of VCaP cells. In contrast, SOX4 re-expression was able to rescue these phenotypes significantly after C-MYC knockdown. Similar results were obtained by colony formation assays. C-MYC inhibition impaired clonogenic growth, whereas SOX4 re-expression rescued the colony formation ability (Fig. 5c). Further analysis by Western blot showed that C-MYC inhibition significantly increased the expression of the epithelial marker (E-cadherin) but decreased the mesenchymal markers (Vimentin) levels. More importantly, these changes were partially reversed by SOX4 overexpression (Fig. 5d). Collectively, these data suggest that SOX4 is a major factor that associates with the effect of C-MYC in PCa cells.

C-MYC+/SOX4+ defined a subset of PCa patients with poor prognosis

To further reveal the clinicopathological significance of concurrent expression of C-MYC and SOX4, we performed in silico analyses. PCa patients were grouped by median C-MYC and SOX4 expression. As shown in Fig. 6a–c, patients with concurrent C-MYC and SOX4 expression (C-MYC+/SOX4+) was significantly increased in CRPC patients, whereas patients with both C-MYC and SOX4 low expression (C-MYC−/SOX4−) were present in a higher percentage in localized PCa patients. We next performed a cross-platform analysis to stratify CRPC patients from localized PCa patients. In GSE35988 and GSE70769 PCa datasets, C-MYC+/SOX4+ patients tightly clustered apart from other patients and were congruent with CRPC

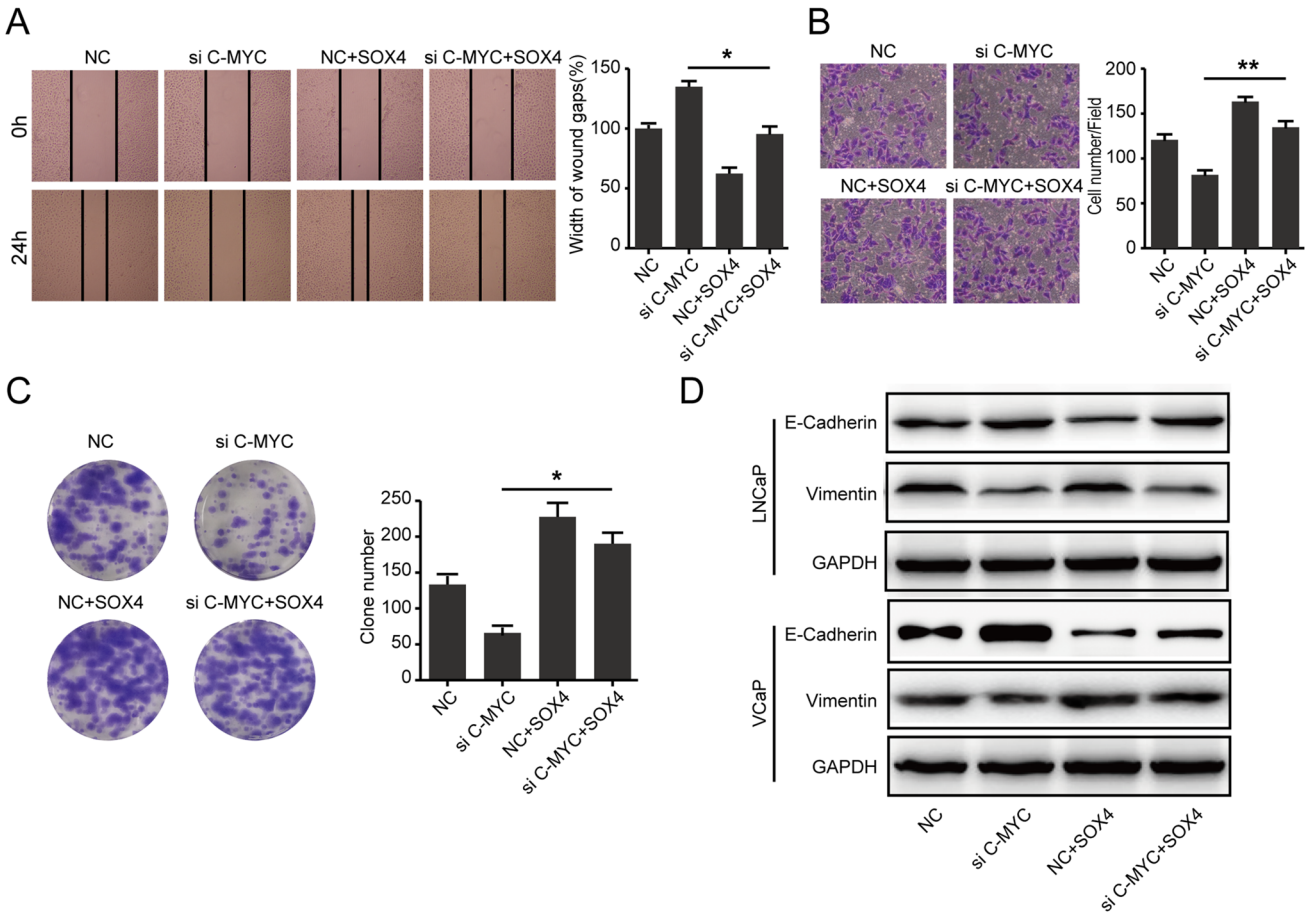

Fig. 5 Synergism effect of C-MYC and SOX4 on oncogenic function. **a**–**c** Effect of SOX4 in oncogenic function of C-MYC. VCaP cell were transiently transfected with control siRNA (NC), C-MYC siRNA (si C-MYC), empty plasmid (NC), or SOX4 expression plasmid (SOX4) as indicated. VCaP cells were subjected to wound healing assay (**a**), transwell assay (**b**), colony formation assay (**c**), and Western blot (**d**). Western blot analysis of E-cadherin and Vimentin were shown as indicated. *$P < 0.05$

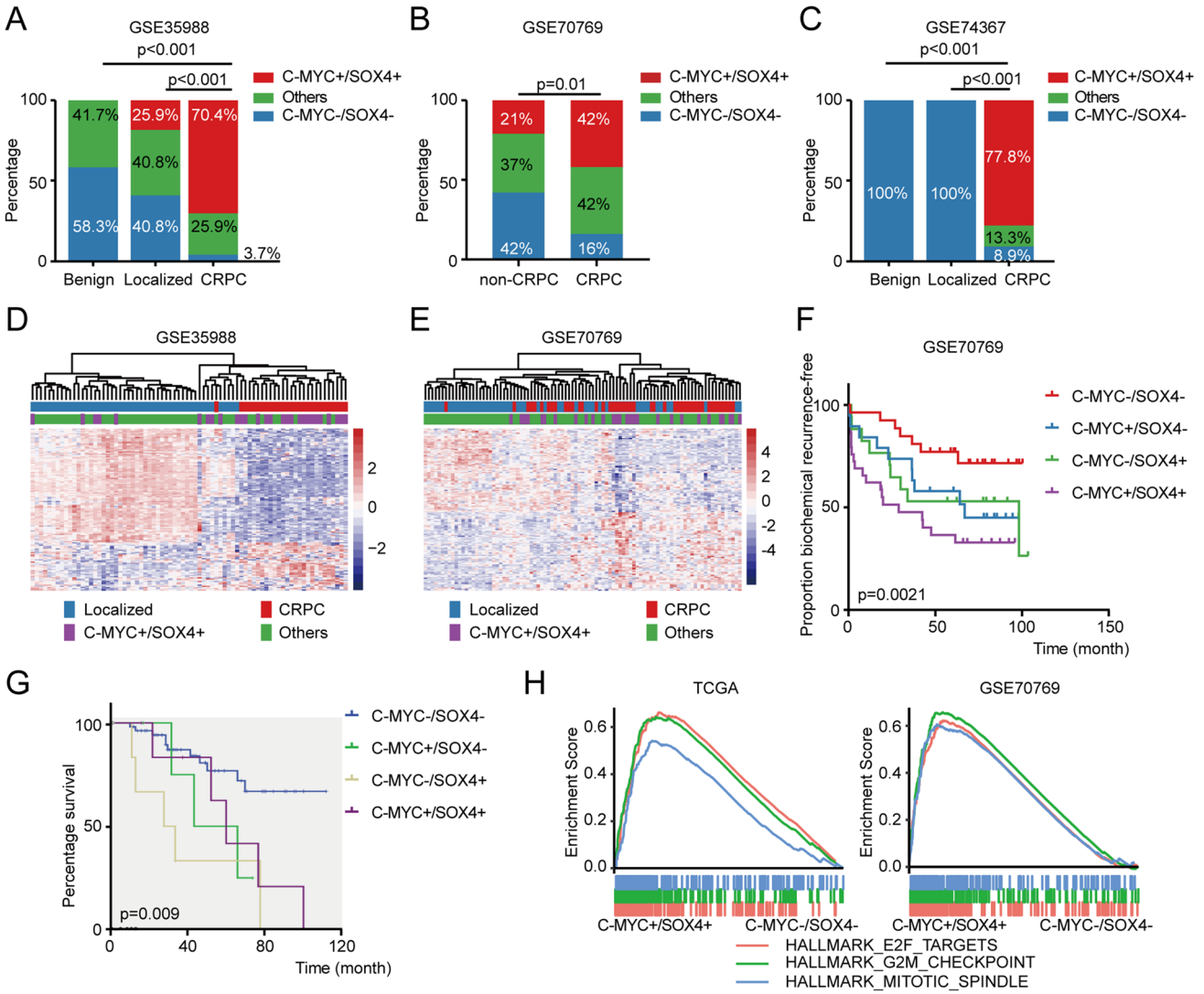

Fig. 6 C-MYC+/SOX4+subgroup with unfavorable outcome in PCa cases. **a**–**c** The percentage of C-MYC and SOX4 expression distributed in benign prostate tissue, localized and CRPC PCa tissues in GSE35988 (**a**), GSE70769 (**b**), and GSE74367 (**c**). **d**–**e** Unsupervised clustering analysis of GSE35988 (**d**) and GSE70769 (**e**) datasets based on differentially expressed genes (DEGs) of localized and castration-resistant PCa. The statuses of patients are shown in the annotation column. Patients' samples were categorized according to C-MYC and SOX4 expression or recurrent state. Blue: patients with localized PCa (localized); Red: Patients with CRPC (CRPC); Purple: patients with concurrent high C-MYC and SOX4 expression (C-MYC+/SOX4+); Green: other patients (others). **f**–**g** Kaplan–Meier survival analysis demonstrates the correlation between the C-MYC+/SOX4+subgroup and overall survival in GSE70769 (**f**) ($P=0.0021$, Log-rank test) and Qilu PCa cohort (**g**) ($P=0.009$, Log-rank test). **h** GSEA of statistically significant overlapping gene signatures in TCGA (left panel) and GSE70769 (right panel) datasets. The E2F-activated signature is red, G2M checkpoint-related signature is green, and signatures containing genes important for mitotic spindle assembly are blue ($P<0.05$ and FDR < 0.2)

subgroup (Fig. 6d, e). Notably, C-MYC+/SOX4+ patients had a worse overall survival rate than the others, including C-MYC−/SOX4− patients (Fig. 6f). This feature was also identified in the Qilu cohort according to IHC results (Fig. 6g). GSEA revealed that DEG in patients with concurrent C-MYC+/SOX4+ expression in TCGA and GSE70769 dataset enriched for statistically significant overlapping gene signature involved in E2F activation, G2M checkpoint, and mitotic spindle assembly (Fig. 6h). These data suggest that C-MYC+/SOX4+ characterizes a subset of aggressive PCa with poor prognoses.

Discussion

PCa is the second most commonly diagnosed malignancy in men globally [24]. Oncogenic activation of C-MYC is one of the most frequent cellular events in human malignancies,

especially in PCa [3, 9, 10]. C-MYC is a master regulatory gene that reprograms cells to proliferate or undergo apoptosis through the induction or repression of transcription [3, 4]. It is well established that C-MYC is frequently amplified in advanced stages of PCa, but rarely in localized PCa [25]. By contrast, the prevalence of C-MYC overexpression in PCa has been presented in various reports, ranging from 32 to 81.6% [7, 9, 10, 26]. This broad range might be explained by the heterogeneity of PCa tissue, different ethnic groups, the lack of specific antibodies, and different standards of interpretation.

Although PSA screening test has been widely used to screen PCa patients in western countries, the PCa patients in our cohort were screened based on non-PSA tests but all had symptoms of lower tract urinary obstruction, therefore representing a select subgroup of clinically recognized PCas. Indeed, a significantly higher percentage of high-grade PCa cases (GS > 8) were included in the current study. This differed from most western patients who were found to have PCa due to PSA screening [27]. Our clinicopathological data may be prone to reflect more about the clinical phenotype and the natural history of PCa.

The SOX4 protein is highly overexpressed in many types of human cancers, including PCa [14–17]. Previously, we and others have suggested SOX4 overexpression was correlated with high Gleason scores, high Ki67 index, tumor progression, and metastasis of PCa [14, 19, 28]. Several groups have suggested that SOX4 could be induced by hypoxia and hypoxia-inducible factor 1α (HIF1α) [29], angiogenesis [30], tumor necrosis factor α [31], TGFβ1, and Wnt signaling [32]. Most recently, Bilir et al. reported that SOX4 expression was induced by PTEN loss as a result of the activation of PI3K-AKT-mTOR signaling, suggesting a positive feedback loop between SOX4 and PI3K-AKT-mTOR activity [23]. Additionally, we found that ERG may directly regulate the expression of SOX4 in PCa [22]. SOX4 was also identified as a direct target of miR-30a and miR-132/212 [33]. For the first time, we showed in our study that SOX4 is a direct target of C-MYC and could be activated by C-MYC. It should be mentioned that previous studies on similar topics did not include the Asian population [20, 21, 23, 34]. Although our data demonstrate a link between C-MYC and SOX4 expression, it also is important to emphasize that there are likely multiple mechanisms that affect SOX4 levels, and C-MYC activation may represent one of those pathways.

Furthermore, we showed that C-MYC may cooperate with SOX4 to promote PCa progression. Firstly, Co-IP revealed that a complex could be formed in the PCa cell. Secondly, our data suggest that C-MYC and SOX4 collaborate to influence the migration and proliferation ability of PCa cells. Thirdly, clinical data showed a significant association between SOX4 overexpression and C-MYC overexpression in our cohort. Most importantly, the subset of PCa patients with overexpression of these two genes had the worst cancer-related survival. Collectively, these data suggest that C-MYC and SOX4 are interacting elements, thereby acting as hubs in gene regulatory networks of PCa progression.

To conclude, SOX4 is a direct target of C-MYC. C-MYC overexpression may contribute to PCa progression by activating and cooperating with SOX4. Our findings highlight an important role for the C-MYC/SOX4 axis in the development and progression of PCa.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study was authorized by the Institutional Review Board at the School of Medicine of Shandong University. The procedures performed in the present study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all individual participants included in the study.

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