



# GTSE1 promotes prostate cancer cell proliferation via the SP1/FOXM1 signaling pathway

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## Abstract

G2 and S phase-expressed-1 (GTSE1) has been implicated in the pathogenesis of several malignant tumors. However, its specific role in prostate cancer (PCa) remains unclear. In this study, RNA-Seq data from patients with PCa and controls were downloaded from the FIREBROWSE database, and it was found that the GTSE1 mRNA level was significantly upregulated in PCa. Moreover, patients with higher GTSE1 mRNA levels had higher Gleason scores ( $P < 0.001$ ), a more advanced pT stage ( $P = 0.011$ ), and a more advanced pN stage ( $P = 0.006$ ) as well as a shorter time to biochemical recurrence ( $P = 0.005$ ). In addition, overexpression of GTSE1 could promote proliferation in LNCaP cells, whereas silencing GTSE1 could inhibit the growth of C4-2 cells in vitro and in vivo. Mechanistically, GTSE1 enhanced the expression of FOXM1 by upregulating the SP1 protein level, a transcription factor of FOXM1, which ultimately promoted PCa cell proliferation. In summary, GTSE1 is a new candidate oncogene in the development and progression of PCa, and it can promote PCa cell proliferation via the SP1/FOXM1 signaling pathway.

## Introduction

Prostate cancer (PCa), the second most common cause of malignant tumors in males worldwide, has shown an increasing incidence in recent years [1, 2]. Although great progress has been made in the screening and comprehensive treatment of PCa, the survival rate of patients is still low due to the limitations of the various treatment methods [3, 4]. Therefore, there is an urgent need to perform intense research and explore novel molecular targets, which could

lead to advances in the development of new diagnostic and therapeutic strategies for PCa.

G2 and S phase-expressed-1 (GTSE1) is a cell cycle-related protein located in microtubules and an essential factor in cell mitosis that is encoded by the 22q13.2–q13.3 gene and expressed specifically during the G2 and S phases of the cell cycle [5]. In mammals, GTSE1 can act as a target gene of p53 [6] or as a negative regulatory factor, translocating from the cytoplasm to reaccumulate in the nucleus to affect the cell cycle by establishing negative feedback regulation with the p53 signaling pathway [7–10]. In cancer, Lin et al. [11] found that GTSE1 activated the AKT pathway in different p53 mutation-dependent ways and promoted the proliferation, migration, and invasion of breast cancer cells. Guo et al. [12] reported that silencing GTSE1 could inhibit the phosphorylation of AKT, BCL-2, and cyclinB1 but promote the phosphorylation of Bax, which ultimately inhibited the growth of hepatocellular carcinoma cells. However, the role of GTSE1 in PCa remains unclear.

In this study, we found that GTSE1 mRNA and protein levels were higher in the PCa group than in the control group and that a high GTSE1 mRNA level was positively correlated with poor outcomes in PCa patients. Further investigation showed that GTSE1 could promote the proliferation of PCa cell via the SP1/FOXM1 signaling

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**Table 1** The information of clinical features from FIREBROWSE database.

Tumor	File name of clinical data	Clinical features	Sample
PRAD	PRAD.clin.merged	<p>“patient.bcr_patient_barcode”</p> <p>“patient.age_at_initial_pathologic_diagnosis”</p> <p>“patient.tumor_samples.tumor_sample.method_of_sample_procurement”</p> <p>“patient.tumor_samples.tumor_sample.other_method_of_sample_procurement”</p> <p>“patient.stage_event.tnm_categories.pathologic_categories.pathologic_n”</p> <p>“patient.stage_event.tnm_categories.pathologic_categories.pathologic_t”</p> <p>“patient.tumor_samples.tumor_sample.disease_details.gleason_score_combined”</p> <p>“patient.tumor_samples.tumor_sample.disease_details.gleason_score_primary”</p> <p>“patient.tumor_samples.tumor_sample.disease_details.gleason_score_secondary”</p> <p>“patient.tumor_samples.tumor_sample.disease_details.psa_result_preop”</p> <p>“patient.days_to_last_followup”</p> <p>“patient.follow_ups.follow_up_days_to_last_followup”</p> <p>“patient.follow_ups.follow_up_2.days_to_last_followup”</p> <p>“patient.days_to_first_biochemical_recurrence”</p> <p>“patient.follow_ups.follow_up_days_to_first_biochemical_recurrence”</p> <p>“patient.follow_ups.follow_up_2.days_to_first_biochemical_recurrence”</p>	499 tumors had clinical data but only 257 tumors with detailed information

PRAD prostate adenocarcinoma.

pathway, suggesting a possible mechanism by which GTSE1 exerts a tumor-promoting effect on PCa.

## Materials and methods

### Analysis of RNA-Seq data and clinical data from FIREBROWSE database

RNA-Seq data for 497 tumor samples and 52 normal samples were downloaded from FIREBROWSE database (<http://www.firebrowse.org>). The RNA-Seq by Expectation Maximization (RSEM) of GTSE1 was analyzed, which extracted from “PRAD.rnaseqv2\_illuminahisec\_rnaseqv2\_unc\_edu\_Level\_3\_RSEM\_genes\_normalized\_data.data”.

A total of 257 patients who underwent radical prostatectomy and had complete clinical information were divided into high (128 patients) and low (129 patients) expression groups according to the median RSEM of GTSE1, and the relationships between GTSE1 and age, pT stage, pN stage, PSA level, Gleason score (GS), and biochemical recurrence (BCR) were analyzed. The detail items extracted from FIREBROWSE database are shown in Table 1.

### Clinical specimens

To investigate the protein levels of GTSE1, we obtained 20 PCa tissue samples and 20 benign prostatic hyperplasia tissue samples from the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) to perform immunohistochemistry (IHC). Informed consent was obtained from all patients. All the enrolled tissue samples were approved by the Clinical Ethics Board of the Third Affiliated Hospital of Sun Yat-sen University.

### Immunohistochemistry (IHC)

Briefly, paraffin-embedded samples were cut into 4- $\mu$ m sections, deparaffinized with xylene, and rehydrated using a graded alcohol series. After incubating in 3% H<sub>2</sub>O<sub>2</sub> at 37 °C for 10 min, the sections were boiled in a citrate antigen retrieval solution (pH = 6.0) for 15 min in an electric pressure cooker for antigen retrieval and incubated again in 3% H<sub>2</sub>O<sub>2</sub> at 37 °C for 10 min. After the cell membranes were disrupted by soaking in PBST (0.5% Triton-X) for 15 min, the tissue sections were blocked with goat serum (Telenbiotech, China) in an incubator at 37 °C. After serum removal, the tissue sections were incubated with primary antibodies at 4 °C overnight, followed by addition of an HRP-labeled secondary antibody (DAKO, Denmark) to the sections and incubation for 30 min in an incubator at 37 °C. Then, the sections were developed with

3,3-diaminobenzidine, counterstained with Mayer's hematoxylin, dehydrated, cleared, and sealed. Finally, the samples were observed under a microscope (Olympus Optical, Japan).

A final immunoreactivity score represented the product of the score for the staining intensity grade and that for the percentage of positive cells. The percentage of positive cells was classified as follows: none = 0, <10% = 1, 11–50% = 2, 51–80% = 3 and >80% = 4. The staining intensity was classified as follows: no expression = 0, weak intensity = 1, moderate intensity = 2, and strong intensity = 3.

### Cell culture

LNCaP and C4-2 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). LNCaP and C4-2 cells were cultured in RPMI medium (HyClone, USA) supplemented with 10% fetal bovine serum (Bovogen, Australia). All cells were cultured at 37 °C with 5% CO<sub>2</sub>.

### Construction and transfection of plasmids and lentivirus

Lentiviruses carrying GTSE1 or a GTSE1-knockdown sequence and control lentiviruses were purchased from Obio Technology Corporation (Shanghai, China). According to the virus manual, the lentiviruses overexpressing GTSE1 and corresponding control lentiviruses were separately transfected into LNCaP cells, and the lentiviruses carrying a GTSE1-knockdown sequence and corresponding control lentiviruses were separately transfected into C4-2 cells. The cells were then cultured in medium supplemented with 4 µg/mL (LNCaP) or 8 µg/mL (C4-2) puromycin. After 2 weeks, stable cell lines were selected for experiments. The following knockdown sequences were used: GTSE1-RNAi1, 5'-GCTGTAGGATCTGAAAGCA-3'; GTSE1-RNAi2, 5'-GGGATGTTCTCCCTGACAA-3'.

Plasmids: The shFOXM1 plasmid was constructed from the pSUPER.retro.neo vector, and the pSUPER.retro.neo empty vector was used as the control. The sequence of shFOXM1 was 5'-GATCCCCGCCAAACGAACACAGA CAGTTCAAGAGACGGTTTGCTTGTGTCTGTCTTTTT A-3'. SP1 and its control plasmids were designed and synthesized by Obio Technology Corporation (Shanghai). Cells were transfected with plasmids using Lipofectamine 3000 (Invitrogen, USA) according to the transfection method provided by the manufacturer.

### RNA interference

SP1-siRNA and a corresponding scrambled siRNA were designed and synthesized by Obio Technology Corporation (Shanghai). The sequence of SP1-siRNA was 5'-CCAGGT

GCAAACCAACAGATT-3'. Cells were transfected with siRNA using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions.

### Western blotting

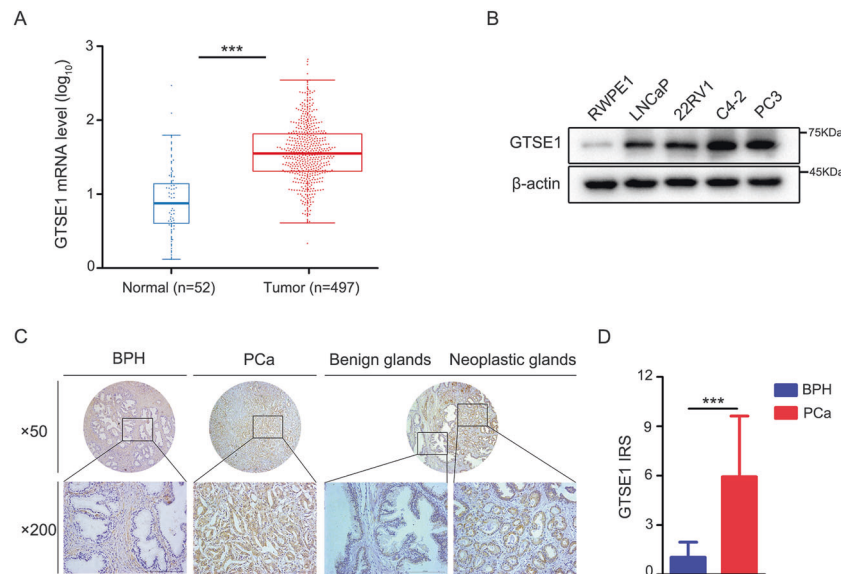
Western blotting was performed following a standard protocol. Cells were collected, lysed with lysis buffer (containing 0.1% protease inhibitor, 1% phosphatase inhibitor, and 1% phenylmethanesulfonyl fluoride) and centrifuged for 10 min at 15,000 × *g* and 4 °C. Subsequently, 20 µg of protein was separated by 10% SDS-PAGE and then transferred to 0.45-µm PVDF membranes (Merck Millipore, USA). After blocking with 4% BSA in TBST for 1 h at 37 °C, the membranes were incubated with a primary antibody at 4 °C overnight and then with an HRP-labeled secondary antibody for 1 h at 37 °C. Finally, the signals of target proteins were detected using ECL (Advansta, USA) and ChemiDoc MP System (Bio-Rad, USA). The primary antibodies included anti-GTSE1 (Proteintech, 21319-1-AP, USA), anti-Ki67 (Abcam, ab16667, USA), anti-β-actin (Santa Cruz Biotechnology, sc-8432, USA), anti-FOXM1 (Abcam, ab207298, USA), anti-FOXO1 (CST, 2880, USA), anti-FOXO3a (Abcam, ab109629, USA), anti-FOXO4 (Abcam, ab128908, USA), anti-CCNB1 (Abcam, ab32053, USA), anti-CCND1 (Abcam, ab16663, USA), anti-HIF-1α (Abcam, ab1, USA), anti-E2F1 (Abcam, ab179445, USA), and anti-SP1 (Abcam, ab124804, USA). Thioestrepton (TargetMol, USA) was used to inhibit FOXM1 expression.

### Cell viability assay

Cell viability was assessed with a Cell Counting Kit-8 (Dojindo, Japan) assay. LNCaP cells at a density of 4 × 10<sup>3</sup> per well or C4-2 cells at a density of 2 × 10<sup>3</sup> per well were seeded in 96-well plates and then incubated in 200 µL of medium in a cell incubator containing 5% CO<sub>2</sub> at 37 °C for one to five days. Then, after the culture medium was replaced with fresh medium containing 10% CCK-8 and incubated for another 1 h at 37 °C, the absorbance of the samples at 450 nm was measured using a microplate reader (Bio-Rad).

### Colony formation assay

LNCaP or C4-2 cells (1 × 10<sup>3</sup> cells per well) were seeded in six-well plates and incubated at 37 °C with 5% CO<sub>2</sub>. After 14 days, the supernatants were removed, and the cells were fixed with 4% paraformaldehyde for 15 min and then stained with a 0.5% crystal violet solution (KeyGEN, China) for 10 min. Finally, the colony number was counted and analyzed after washing the cells with pure water.



**Fig. 1 GTSE1 expression was upregulated in prostate cancer.** **A** The expression level of GTSE1 was elevated in 497 prostate cancer samples compared with 52 normal prostate samples in the FIRE-BROWSE database. **B** The GTSE1 protein levels in five types of prostate cancer cells were confirmed by western blotting.

**C** Representative immunohistochemical staining for GTSE1 in benign prostatic hypertrophy tissue specimens and prostate cancer specimens is shown (scale bar: 200  $\mu$ m; magnification: 50 $\times$  and 200 $\times$ ). **D** The immunoreactivity score for GTSE1 was higher in prostate cancer than in benign prostatic hypertrophy.

## EdU assay

An EdU kit (RiboBio, C10310, China) was used to detect the proliferative ability of stably transfected PCa cells, and the experiment was conducted in strict accordance with the kit instructions. Visible cells were counted under a fluorescence microscope (Olympus Optical).

## Flow cytometry

Cells were fixed in 75% ice-cold ethanol ( $1 \times 10^6$  cells per well) at 4  $^{\circ}$ C overnight. After centrifugation at 200  $\times g$  for 10 min, the supernatant was discarded, and the cells were gently washed with PBS for three times. Then, the cells were incubated with 500  $\mu$ L of propidium iodide (Becton, Dickinson and Company, USA) at 37  $^{\circ}$ C for 15 min in the dark. Finally, the stained cells were evaluated on a flow cytometer (Calibur, BD Bioscience, USA) and analyzed with FlowJo software (Tree Star, San Carlos, USA).

## Mouse xenograft assay

Four- to six-week-old BALB/c nude mice were purchased from the Sun Yat-sen University Laboratory Animal Center and maintained under specific pathogen-free conditions. All experimental animal procedures were approved by the Sun Yat-sen University Animal Care and Use Committee and

performed in accordance with the Sun Yat-sen University Laboratory Animal Center Guidelines.

C4-2 cells with stable GTSE1 silencing and control cells were separately resuspended in 200  $\mu$ L of HBSS and subcutaneously injected into the right axillary area of BALB/c nude mice. There were two groups: the control group and the GTSE1-RNAi group (four mice per group). After 1 week, the mice were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg) (Sigma, USA), and the bilateral testicles were removed at the same time. The mice were observed twice per week to monitor their weight and status. Four weeks after surgery, an IVIS system (PerkinElmer, USA) was used for in vivo imaging, and then the mice were euthanized after being deeply anesthetized. Tumor size ( $V = \pi/6 \times \text{length} \times \text{width}^2$ ) and weight were measured, and the corpses were given to the experimental animal center for harmless treatment.

## Gene co-expression and enrichment analyses

To explore the co-expression genes and possible pathways of GTSE1 in PCa, the RNA-Seq data for PCa in The Cancer Genome Atlas (TCGA) database were analyzed in cBioPortal (<http://www.cbioportal.org/>), and the genes highly related to GTSE1 were obtained ( $\rho \geq 0.6$ ,  $P < 0.05$ ). Enrichment analysis was then performed with the identified genes using the “cluster profiler” package of R3.5.1 software.

## RT-qPCR

Total RNA was extracted using TRIzol reagent (Invitrogen) and reverse transcribed with SuperScript™ III Reverse Transcriptase (Invitrogen). Real-time PCR was conducted using SYBR Green qPCR reagent (GenStar, China). GAPDH was used as the internal control, and the primers used for RT-qPCR were as follows: FOXM1 forward 5'-GGAGGAAATGCCACACTTAGCG-3', and reverse 5'-TAGGACTTCTTGGGTCTTGGGGTG-3'; and GAPDH forward 5'-GACTCATGACCACAGTCCATGC-3', and reverse 5'-AGAGGCAGGGATGATGTTCTG-3'.

## Statistical analysis

All the experiments were performed in triplicate, and results were statistically analyzed using R3.5.1 or SPSS22.0. Data are expressed as the mean  $\pm$  standard deviation (SD). An independent *t*-test was used to compare the results of IHC, CCK-8, colony formation, EdU, flow cytometry and animal assays, while the  $\chi^2$  test or Fisher's exact test was used to analyze the relationships between the GTSE1 mRNA level and clinicopathological parameters. Kaplan–Meier curves were employed to determine the correlation between the mRNA level of GTSE1 and BCR. A *P* value less than 0.05 was considered statistically significant for all tests (\**P* < 0.05, \*\**P* < 0.01, and \*\*\**P* < 0.001).

## Results

### GTSE1 mRNA and protein levels are significantly increased in PCa

RNA-seq data from FIREBROWSE database showed that GTSE1 mRNA level was significantly increased in PCa patients (Fig. 1A). Western blotting confirmed that the expression of GTSE1 in PCa cells, including LNCaP, 22RV1, C4-2, and PC3 cells, was significantly higher than that in RWPE1 cells (Fig. 1B). IHC results also showed that the protein level of GTSE1 was significantly upregulated in PCa tissue samples (Fig. 1C, D).

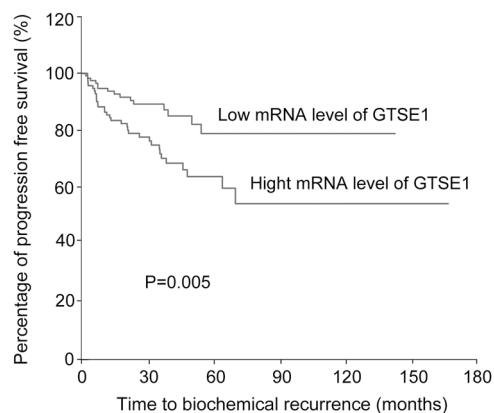
The above results reveal that both the mRNA and protein levels of GTSE1 are significantly increased in PCa.

### High mRNA levels of GTSE1 are positively correlated with poor outcomes

To further confirm the correlations between GTSE1 and the progression and prognosis of PCa, clinical data for 257 PCa patients were extracted from the FIREBROWSE database and analyzed. The results showed that the higher the mRNA level of GTSE1 was, the higher the GS score (*P* < 0.001),

**Table 2** Association of GTSE1 mRNA expression with clinicopathological features in PCa.

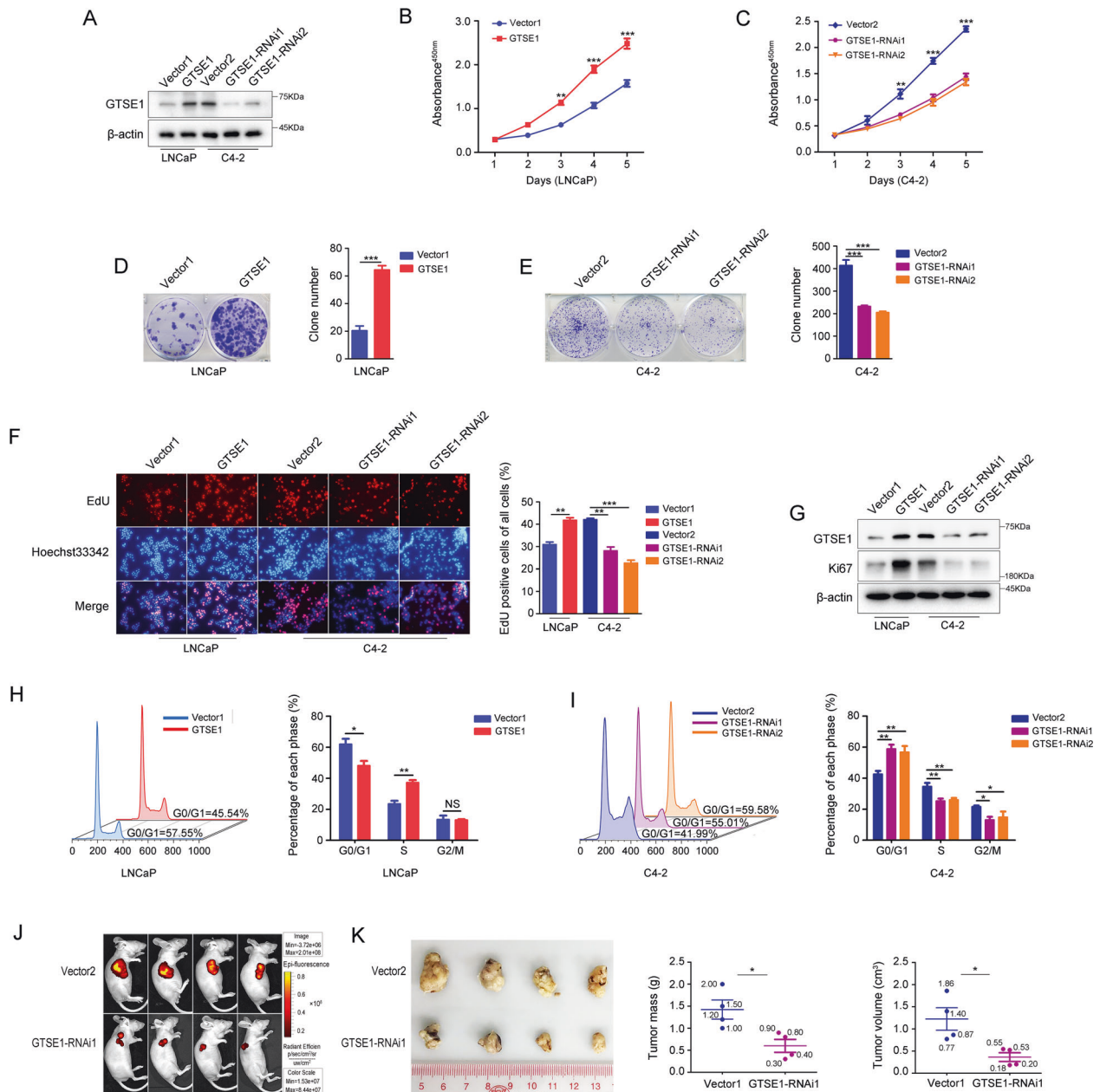
Characteristics	Total no. (%)	GTSE1 mRNA expression		<i>P</i>
		Low no. (%)	High no. (%)	
All cases	257 (100.0)	129 (50.2)	128 (49.8)	–
Age				0.062
<65		90 (54.5)	75 (45.5)	
≥65		39 (42.4)	53 (57.6)	
PSA (ng/mL)				0.098
<10		87 (52.4)	79 (47.6)	
10–20		29 (54.7)	24 (45.3)	
>20		13 (34.2)	25 (65.8)	
Gleason score				<0.001
6		16 (84.2)	3 (15.8)	
7 (3 + 4)		50 (56.8)	38 (43.2)	
7 (4 + 3)		34 (61.8)	21 (38.2)	
8–10		29 (30.5)	66 (69.5)	
Pathological T stage				0.011
T2		57 (62.0)	35 (38.0)	
T3		70 (44.6)	87 (55.4)	
T4		2 (25.0)	6 (75.0)	
Pathological N stage				0.006
N0		113 (54.3)	95 (45.7)	
N1		16 (32.7)	33 (67.3)	



**Fig. 2** The mRNA level of GTSE1 correlates with biochemical recurrence in prostate cancer. The progression-free survival of 257 patients with prostate cancer extracted from the FIREBROWSE database was compared between low and high GTSE1 mRNA expression groups using the Kaplan–Meier method.

pT stage (*P* = 0.011), and pN stage (*P* = 0.006) (Table 2), and the shorter the time to BCR (*P* = 0.005) (Fig. 2).

These results imply that a high mRNA level of GTSE1 is significantly correlated with a poor prognosis.



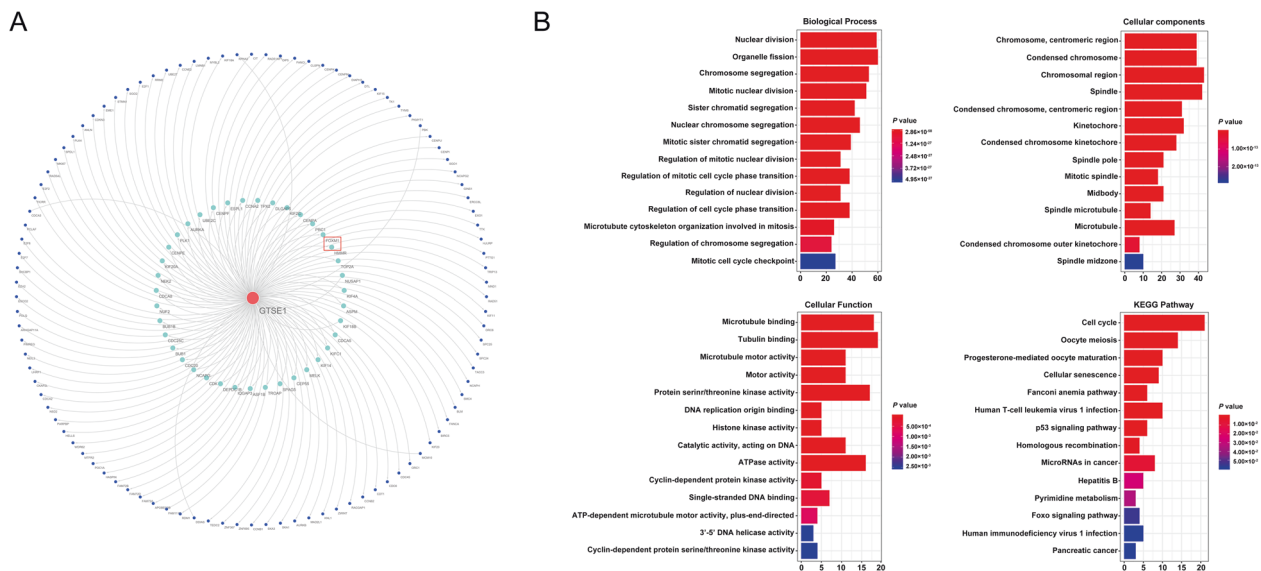
**Fig. 3** GTSE1 promotes prostate cancer cell proliferation *in vitro* and *in vivo*. **A** Efficiency of GTSE1 overexpression and knockdown in LNCaP and C4-2 cells, respectively. **B, C** Cell viability measured by a Cell Counting Kit-8 assay. Colony formation by LNCaP (**D**) and C4-2 cells (**E**). The left side contains representative images, and the right side contains quantized results. **F** EdU staining of LNCaP and

C4-2 cells. **G** Positive regulation of Ki67 by GTSE1 in LNCaP and C4-2 cells. Cell cycle distributions of LNCaP (**H**) and C4-2 cells (**I**) evaluated by flow cytometry. The left images are raw data from the flow cytometric analysis, and the right side shows the quantitative analysis results. **J, K**, Inhibition of the tumorigenicity of C4-2 cells *in vivo* resulting from GTSE1 knockdown.

**GTSE1 facilitates PCa cell growth in vitro and in vivo**

To investigate whether GTSE1 affects PCa cell proliferation, we initially overexpressed GTSE1 in LNCaP cells and knocked down GTSE1 in C4-2 cells (Fig. 3A). The results showed that overexpression of GTSE1 could significantly increase the cell viability, colony formation and proliferation of LNCaP cells, while the same features were

significantly decreased in C4-2 cells with GTSE1 knock-down (Fig. 3B–F). In addition, the expression of Ki67 in LNCaP cells was upregulated after overexpression of GTSE1, whereas that in C4-2 cells was downregulated after silencing GTSE1 (Fig. 3G). Flow cytometry analyses indicated that overexpression of GTSE1 could significantly promote the G1/S transition in LNCaP cells (Fig. 3H), while inhibition of GTSE1 could delay the transition in C4-2 cells



**Fig. 4 Gene co-expression and enrichment analyses.** **A** Genes highly correlated with GTSE1 in prostate cancer in the TCGA database. **B** Top 14 GO pathways for the biological process, cellular component, and cellular function categories and KEGG-enriched gene pathways.

(Fig. 3I). Moreover, a xenograft assay showed that the weight ( $1.43 \pm 0.22$  g vs  $0.60 \pm 0.15$  g,  $P = 0.020$ ) and volume ( $1.22 \pm 0.25$  cm<sup>3</sup> vs  $0.36 \pm 0.01$  cm<sup>3</sup>,  $P = 0.019$ ) of C4-2 cell xenografts were significantly decreased after GTSE1 knockdown (Fig. 3J, K).

In summary, GTSE1 can promote the growth of PCa cells in vitro and in vivo.

### GTSE1 promotes PCa cell proliferation via the SP1/FOXM1 signaling pathway

Co-expression and enrichment analyses showed that a total of 141 genes were highly associated with GTSE1 in PCa (Fig. 4A), and some classic signaling pathways, such as the p53 and FOXO pathways, showed gene enrichment, suggesting that GTSE1 might play a role through these pathways (Fig. 4B).

To confirm the results, we subsequently examined FOXO1, FOXO3a, and FOXO4, which have been reported to play roles in PCa [13–15], as well as FOXM1, a gene highly correlated with GTSE1 according to the co-expression analysis, in cells (Fig. 4A). The results showed that GTSE1 overexpression significantly upregulated FOXM1 expression in LNCaP cells, and GTSE1 knockdown downregulated FOXM1 expression in C4-2 cells (Fig. 5A). Moreover, two downstream factors of FOXM1, CCNB1, [16] and CCND1 [17], were also positively regulated by GTSE1 (Fig. 5B). In addition, the pro-proliferation effect of GTSE1 on LNCaP cells could be significantly reversed when FOXM1 expression was knocked down by shFOXM1 or the specific inhibitor thiothrepton [18] (Fig. 5C–F), which showed that

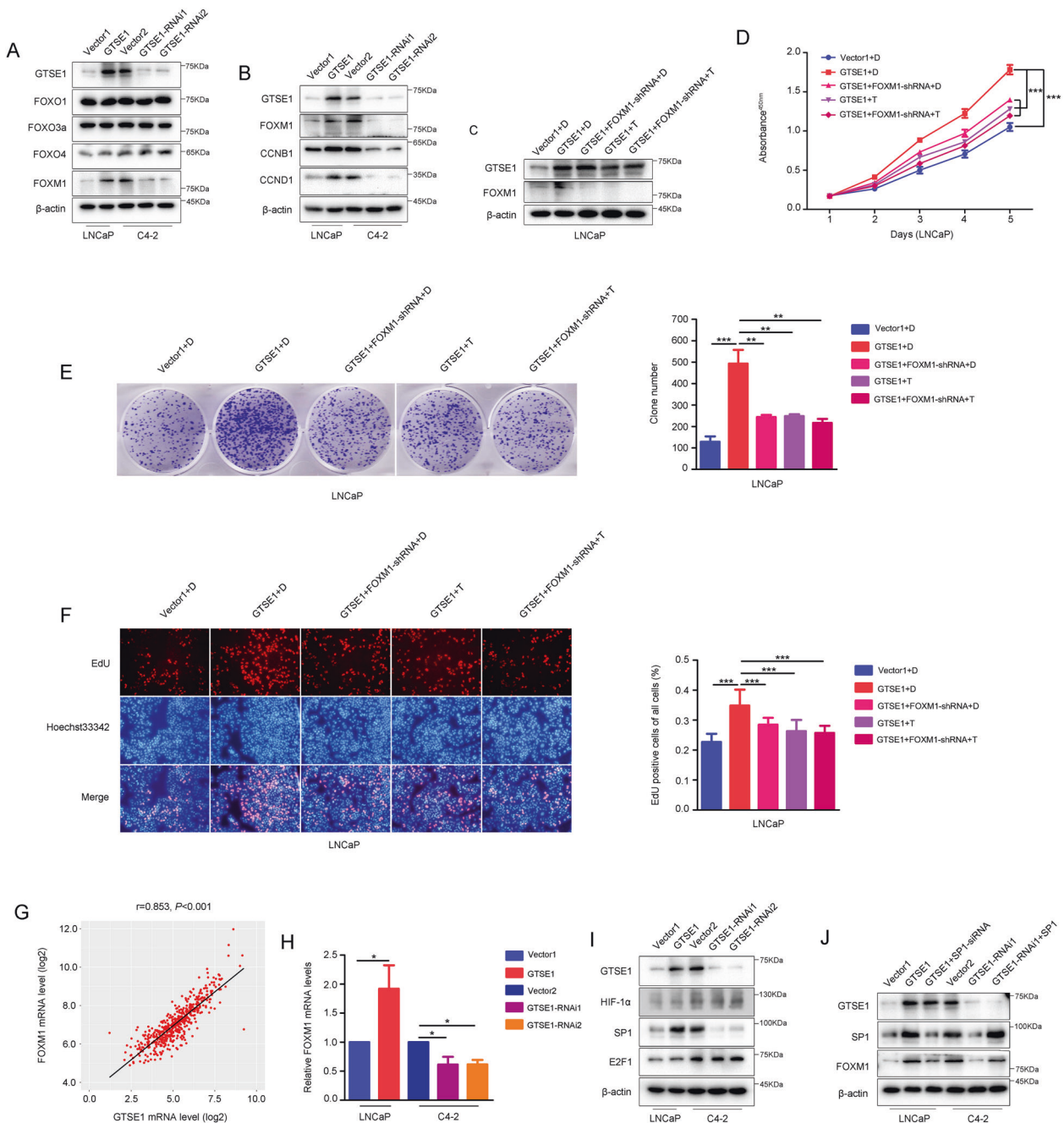
GTSE1 exerted a cancer-promoting function through FOXM1.

Subsequently, correlation analysis and qPCR both showed a high correlation between GTSE1 and FOXM1 at the mRNA level (Fig. 5G, H), suggesting that the effect of GTSE1 on FOXM1 occurs at the transcriptional level. Thus, we further investigated the relationship between GTSE1 and transcription factors of FOXM1, including HIF-1 $\alpha$  [19], SP1 [20], and E2F1 [21]. Western blotting confirmed that GTSE1 overexpression could significantly promote the expression of SP1 in LNCaP cells, and GTSE1 knockdown inhibited SP1 expression in C4-2 cells (Fig. 5I). Furthermore, inhibition of SP1 significantly reversed the promotion of FOXM1 by GTSE1, and SP1 overexpression upregulated FOXM1 expression in the context of GTSE1 silencing (Fig. 5J).

Taken together, these results indicate that GTSE1 promotes PCa cell proliferation via the SP1/FOXM1 signaling pathway (Fig. 6).

## Discussion

In this study, we found that GTSE1 mRNA and protein levels were upregulated in PCa, and the mRNA level was negatively associated with patient prognosis. Moreover, ectopic expression of GTSE1 could promote proliferation in LNCaP cells, while GTSE1 knockdown impaired proliferation in C4-2 cells in vitro and in vivo. Mechanistically, GTSE1 could upregulate SP1 expression and then promote the proliferation of PCa cells via transcriptional activation of FOXM1.



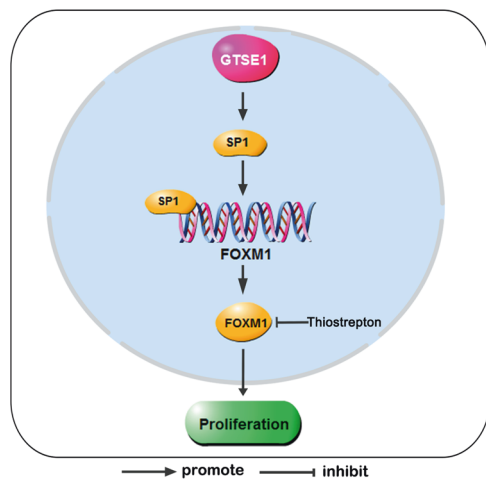
**Fig. 5** GTSE1 promotes prostate cancer proliferation via the SP1/FOXM1 signaling pathway. **A** GTSE1, FOXO1, FOXO3a, FOXO4, and FOXM1 were detected by western blotting. **B** CCNB1 and CCND1 could be positively regulated by GTSE1. **C** The efficiency of shFOXM1 and thiothrepton was measured. **D** Cell viability was measured by a Cell Counting Kit-8 assay. **E** Colony formation by LNCaP cells treated with or without shFOXM1 or Thiothrepton was evaluated. The left side contains representative pictures, and the right side shows the quantized results. **F** LNCaP cells treated with or

without shFOXM1 or Thiothrepton (left image) were evaluated with an EdU assay. Proliferation rates are also shown (right image). **G** Based on the FIREBROWSE database, GTSE1 was positively correlated with FOXM1 at the mRNA level. **H** qPCR confirmed that GTSE1 positively regulates the transcription of FOXM1. **I** GTSE1, HIF-1 $\alpha$ , SP1 and E2F1 were detected by western blotting. **J** SP1 was identified as a critical factor participating in the regulatory effect of GTSE1 on FOXM1.

Forkhead box (FOX) proteins, a superfamily of evolutionarily conserved transcription factors including a total of 19 subfamilies from A to S, have a highly conserved DNA-binding domain containing 80–100 amino acids called the

FOX domain [22]. As a member of the FOX family, FOXM1 is only expressed in proliferative cells and is closely related to the progression of the cell cycle [23]. In addition, several previous studies have confirmed that





**Fig. 6** GTSE1 promotes the proliferation of prostate cancer cell via SP1/FOXM1 signaling pathway. GTSE1 upregulated the expression of SP1 and enhanced the transcription of FOXM1, which ultimately promoted the proliferation of prostate cancer cell. However, the pro-proliferation effect of GTSE1 on prostate cancer could be significantly reversed by thiostrepton, a specific inhibitor of FOXM1.

FOXM1 plays a central role in the development of various types of tumors [24–26], including PCa. Ketola et al. [27] reported that inhibition of FOXM1 activity could significantly reduce the cell growth and stemness of enzalutamide-resistant PCa cells in vitro and in vivo, and Li et al. [17] found that Natura-Alpha inhibited the growth of AR-dependent and AR-independent PCa cells in vitro and in vivo by inhibiting the activity of the FOXM1 protein. These studies emphasize the important functions of FOXM1 in PCa occurrence and development. In the present study, we found that GTSE1 could significantly upregulate the expression of FOXM1 in PCa cells, while silencing FOXM1 with shRNA or inhibiting it with thiostrepton could significantly reverse the tumor-promoting effect of GTSE1. Thus, FOXM1 plays a key role in the proto-oncogene function of GTSE1 in PCa.

To understand the contribution of GTSE1 to PCa progression, it would be of great interest to further clarify the specific mechanism by which GTSE1 regulates FOXM1. We first found that there was a positive correlation between GTSE1 and FOXM1 at the transcriptional level based on bioinformatic analysis and qPCR. Thus, we subsequently focused on the transcription factors of FOXM1. SP1, E2F1, and HIF-1 $\alpha$  have been reported to bind to the FOXM1 promoter and activate its transcription in many cancers [28–30]. Therefore, we hypothesized that GTSE1 may regulate FOXM1 transcription via these factors. Subsequent investigation showed that SP1 but not E2F1 or HIF-1 $\alpha$  could be positively regulated by GTSE1, and SP1 was essential for GTSE1 to regulate FOXM1. These results reveal the specific mechanisms by which GTSE1 promotes the proliferation of PCa cells.

In summary, GTSE1 can promote PCa cell proliferation via the SP1/FOXM1 signaling pathway, which facilitates tumorigenesis and progression, suggesting that GTSE1 has potential value as a prognostic marker and therapeutic target in PCa.

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**Author contributions** XW designed the study. WL and WZ conducted experiments and participated in writing the manuscript. XL and YH were responsible for the statistical analysis. YW, QL, and ML collected the clinical specimens and clinical data. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China ensured that all sample information was kept confidential.

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