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Original Article

LncRNA TYMSOS is a novel prognostic biomarker associated with immune infiltration in prostate cancer

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Abstract: The long noncoding RNA thymidylate synthetase opposite strand (lncRNA TYMSOS) plays an important role in cancers; however, its impact on prostate cancer (PCa) is still unclear. By analyzing the online data, we found that lncRNA TYMSOS was highly expressed in PCa and associated with T stage, Gleason score, age, and primary therapy outcome. The results of the ROC curve showed that lncRNA TYMSOS has a significant diagnostic ability. Furthermore, Kaplan-Meier analyses suggested that lncRNA TYMSOS plays an important role in progression-free survival (PFS). Increased lncRNA TYMSOS expression was an independent risk factor correlated with PFS in PCa patients. GSEA and GSVA indicated that the lncRNA TYMSOS was involved in the cell cycle, neurodegenerative diseases, oxidative phosphorylation, spliceosomes, and adaptive immune system pathways. Additionally, lncRNA TYMSOS expression was also associated with immune cell infiltrates and tumor mutational burden in PCa. Functional experiments were further conducted, and we verified that lncRNA TYMSOS played an oncogenic role in regulating PCa aggressiveness. Specifically, silencing of lncRNA TYMSOS suppressed cell proliferation, division and epithelial-mesenchymal transition (EMT) but promoted cell apoptosis in PCa cells, and conversely, lncRNA TYMSOS overexpression had the opposite effects. In summary, our study revealed that the lncRNA TYMSOS could be a biomarker and therapeutic target in PCa and participate in tumor-immune cell infiltration.

Keywords: lncRNA TYMSOS, prostate cancer, prognostic, immune infiltration

Introduction

Prostate carcinoma (PCa) is the second most common solid tumor in males and the fifth leading cause of cancer mortality worldwide [1]. The number of patients diagnosed with distant stage PCa has increased from 3.9% to 8.2% over the past decade [2]. Most metastatic prostate cancers will develop after receiving antiandrogen or chemotherapy based on androgen deprivation for 18-24 months and only have a mean survival of 13-32 months with a 15% 5-year survival rate [3-5]. There are no satisfactory therapeutic strategies for metastatic castration-resistant prostate cancer (mCRPC) [6]. However, existing molecular markers are not as effective as sensitive detection. Therefore, novel molecular biomarkers for improving the diagnosis and treatment of PCa are needed.

Recently, various treatment combinations have achieved good results in cancer treatment, thereby improving the survival rate of patients [7]. Additionally, the application of immunotherapy in solid tumors has attracted attention. Notably, immune-checkpoint inhibitor (ICI) therapies targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD-1), and programmed cell death-ligand 1 (PD-L1) have been approved to treat most cancers [8]. However, ICI treatment is ineffective in many patients, possibly due to the lack of personalized, targeted treatment biomarkers. A previous study reported that the effectiveness of immunotherapy is related to the tumor microenvironment (TME) [9]. We previously identified a relationship between androgen deprivation therapy and immune regulation [8]. Therefore, a better understanding of the

unique tumor-immune microenvironment of cancer is important.

With the development of RNA sequencing technology, several noncoding RNAs (ncRNAs) have been reported. Long ncRNA (lncRNA) is a ncRNA subclass whose transcripts are more than 200 nt and lack protein coding potential [10]. lncRNAs regulate gene expression at multiple levels, including chromatin remodeling, transcription, translation, protein modification and functional protein induction [11]. Studies have also found that lncRNAs are abnormally expressed in breast, bladder, prostate, and lung cancers and other tumors and regulate the expression of their downstream genes in many pathways to act as oncogenes or tumor suppressors [12-15]. For instance, lncRNA-GATA3-AS1 is involved in immune escape in breast cancer by stabilizing the PD-L1 protein and degrading the GATA binding protein 3 (GATA3) protein [16]. The lncRNA thymidylate synthetase opposite strand (TYMSOS) was first reported as a competitive endogenous RNA (ceRNA) that promotes gastric cancer progression [17]. However, there are no comprehensive studies on the immunomodulatory, molecular biomarker or potential biological functions of lncRNA TYMSOS in PCa.

Epithelial-mesenchymal transition contributes to tumor invasion, metastasis, and drug resistance [18]. It has been reported that epithelial-mesenchymal transition (EMT) is a key factor in malignant tumor invasion and metastasis in prostate cancer [19]. We analyzed the effects of lncRNA TYMSOS on proliferation, apoptosis and epithelial-mesenchymal transition in PCa cell lines.

Therefore, this study evaluated the relationship between lncRNA TYMSOS expression and PCa and analyzed the prognostic role of lncRNA TYMSOS in PCa based on RNA-seq data from The Cancer Genome Atlas (TCGA). We also used gene set variation analysis (GSVA) and gene set enrichment analysis (GSEA) to examine lncRNA TYMSOS biological functions and pathways. Subsequently, we analyzed the relationship of lncRNA TYMSOS conveyance to immune cell infiltration, TME, microsatellite instability (MSI), and tumor mutational burden (TMB). Finally, the role of lncRNA TYMSOS in the regulation of PCa invasiveness was verified by functional experiments. Our results provide insights into the role of lncRNA TYMSOS in the occurrence and development of PCa.

Methods

Data collection and differential expression analysis

The RNA-seq data and clinical information were collected from TCGA (https://genome-cancer.ucsc.edu/), which included 499 PCa samples and 52 cases with matched adjacent tissues. We then converted level 3 HTSeq-FPKM data to transcription number per million (TPM) format for the following analyses. In addition, we also downloaded the TPM format RNA-seq data in the TCGA and Genotype-Tissue Expression (GTEx) databases from the UCSC XENA website (https://xenabrowser.net/datapages/) and used them to analyze the differential expression of lncRNA TYMSOS in PCa and normal tissues [20]. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic performance of lncRNA TYMSOS.

Analysis of prognosis, model construction, and evaluation

Univariate and multivariate Cox regression analyses were performed to compare the influence of low and high lncRNA TYMSOS expression on overall survival (OS), disease-specific survival (DSS), and progression-free survival (PFS) along with other clinical characteristics. Based on the independent prognostic factors collected from multivariate analysis, a nomogram was constructed by the R package rms. Next, we used calibration curves to assess the nomogram-predicted probabilities.

Gene set enrichment analyses

GSEA and GSVA were used to investigate the potential biological lncRNA TYMSOS processes in pancancers using the “clusterprofiler” R package with GSEA [21]. GSVA is commonly applied to estimate the variation in pathway and biological processes in the samples of an expression dataset. The hallmark pathway dataset was downloaded from the updated Molecular Signatures Database [22], and the tumors were scored using GSVA. The correlation between gene expression and pathway score was calculated for each tumor, and a correlation map was created. The R language “GSVA” package was used for GSVA [23].

TME analysis

We used previously published methods [24] to analyze the correlation between the high and low lncRNA TYMSOS expression groups and immune-relevant signature scores, mismatched DNA repair signatures, and stromal-relevant signature pathways. A heatmap drawn using R packages summarizes the association of lncRNA TYMSOS conveyance with immune-relevant, stromal-relevant, and mismatched DNA repair signatures.

Analysis of immune cell infiltration, TMB and MSI

Immune cell infiltration scores for TCGA PCa patients were retrieved from the ImmuCellAI platform (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI/) [25]. The linear correlation analysis for lncRNA TYMSOS expression and immune infiltration score were processed using the downloaded data. Subsequently, PCa samples from TCGA were divided into high- and low-expression groups according to gene medians, and the differential expression of immune cells was plotted. Moreover, we downloaded the mutation data from the GDC TCGA cohort in UCSC XENA, and the MSI data were derived from a previously published article [26]. The correlation between the lncRNA TYMSOS level and TMB and MSI was then analyzed.

Cell culture

Human prostate cancer cell lines (PC-3, DU145 and LNCaP) were obtained from the Cell Bank of the Chinese Academy of Sciences. Briefly, cells were cultured in RPMI-1640 medium (HyClone; Cytiva) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific Corporation), 37°C, 5% CO_2 in a humidified incubator. The medium was changed every 2 days. Cells were used for experiments between passages 2 and 5.

Vector transfection

The overexpression vectors for lncRNA TYMSOS were designed and synthesized by GenePharma (Shanghai, China) according to the sequences of the lncRNA TYMSOS gene from the NCBI database (https://www.ncbi.nlm.nih.gov/). In addition, short harpin RNAs (shRNAs) directed against lncRNA TYMSOS (GenePharma, Shanghai, China) were generated, and the associated sequences were as follows: sh-lncRNA TYMSOS #1: 5'-TAA GAA GAT CTA ATG CAT CCT-3'; sh-lncRNA TYMSOS #2: 5'-AAC ACT TTA TTA TCA CAT CAG-3', and the sh-NC sequence was 5'-CCG GAG AAT TAT CTC TTC ATC TGG GCT CGA GCC CAG ATG AAG AGA TAA TTC TTT TTT G-3'. The above vectors were delivered into the cells by using Lipofectamine 3000 transfection reagent (Thermo Fisher Scientific, USA) in keeping with the manufacturer's protocol.

Flow cytometry analysis of cell apoptosis

Cell apoptosis was measured by a cell apoptosis kit. After plasmid transfection (OE-TYMSOS, KD-TYMSOS), PC-3 and DU145 cells were trypsinized from 6-well plates, and apoptotic reagents were added in steps at 37°C for 15 min in the dark. Finally, diluted solution was added, and apoptosis was determined by flow cytometry.

Western blotting

RIPA buffer was used to extract total protein from cultured PC-3 and DU145 cells. Proteins were quantified by BCA, separated by 12% SDS-PAGE, and transferred to PVDF membranes. The membranes were blocked with 5% nonfat dry milk diluted with TBST (Tris-HCl 20 mmol/L, NaCl 150 mmol/L, pH 7.5, 0.1% Tween 20) for 1 h at room temperature and washed three times with TBST. Subsequently, specific primary antibodies (anti-Vimentin, anti-E-cadherin, anti-CDK2, anti-CDK6, anti-CyclinD1, anti-Bax, anti-Bcl-2, and anti-Cleaved Cas-3 (Sigma, USA)) were incubated with the membrane overnight at 4°C. Next, the membranes were washed three times with TBST and incubated with the secondary antibody for 2.5 h at room temperature. ECL luminescence reagent was used for chemiluminescence to observe proteins. The band intensities were quantified using Image-Pro Plus 8.0 (Media Cybernetics, Inc.). GAPDH was used as a normalized loading control.

Statistical analysis

Statistical analyses were performed using R software (version 3.6.3). There was a correlation between lncRNA TYMSOS expression, in vitro experimental data and clinicopathology in patients with PCa after using Wilcoxon rank

sum test, Fisher exact test, Chi-square test, and logistic regression. Kaplan-Meier curves were used to plot survival analyses. ROC curves evaluated the prediction accuracy of lncRNA TYMSOS. Univariate and multivariate analyses were performed to determine the clinical characteristics associated with survival using the Cox proportional hazards regression model. A *p* value of less than 0.05 was considered statistically significant.

Results

Abnormally high expression of lncRNA TYMSOS in PCa

First, the expression of the lncRNA TYMSOS in 33 types of cancers and corresponding normal tissues in the TCGA and GTX datasets was analyzed. The results showed that lncRNA TYMSOS was overexpressed in PCa and 26 other types of tumors (**Figure 1A**). Second, we evaluated the significant differences in lncRNA TYMSOS conveyance between 499 PCa and normal tissues based on the TCGA dataset and found that lncRNA TYMSOS was highly expressed in PCa tissues (**Figure 1B**). Furthermore, we confirmed that the expression levels of lncRNA TYMSOS were significantly upregulated in PCa tissue compared with matched adjacent tissues (**Figure 1C**). In addition, the expression level of lncRNA TYMSOS in normal cases of GTEx combined with TCGA database and PCa samples of TCGA were examined. Our results also showed that the lncRNA TYMSOS was significantly high in PCa tissues (**Figure 1D**). Finally, ROC curves were used to analyze the effectiveness of distinguishing lncRNA TYMSOS expression in PCa from nontumor tissues. The area under the curve (AUC) was 0.621, suggesting that lncRNA TYMSOS may serve as a potential biomarker for PCa tissues (**Figure 1E**).

Correlations between lncRNA TYMSOS expression and clinical characteristics in PCa

The basic pathological data and gene expression data of 499 patients with PCa from the TCGA database were collected. According to the median value of lncRNA TYMSOS expression, the patients were divided into a high expression group (n = 250) and a low expression group (n = 249). Moreover, we evaluated the relationship between the expression level of lncRNA TYMSOS and clinicopathological features and showed that the expression level of lncRNA TYMSOS was associated with T stage (P < 0.001), Gleason score (P < 0.001), age (P = 0.003), and primary therapy outcome (P = 0.008). However, there was no correlation between the expression of the lncRNA TYMSOS and other clinical features (Supplementary Table 1).

Univariate logistic regression analysis demonstrated the relationship between lncRNA TYMSOS expression and poor prognostic clinicopathologic characteristics of patients with PCa (Supplementary Table 2; **Figure 2A-F**). The results suggested that the increased expression of the lncRNA TYMSOS was associated with T stage (T3&T4 vs. T2, OR = 2.202, P < 0.001), Gleason score (8&9&10 vs. 6&7, OR = 2.381, P < 0.001), residual tumor (R1&R2 vs. R0, OR = 1.505, P = 0.039), and primary therapy outcome (CR vs. PD&SD&PR, OR = 0.492, P = 0.003).

Prognostic value of lncRNA TYMSOS expression in PCa

Kapla-Meier curves were used to analyze the relationship between lncRNA TYMSOS level and PFS, DSS, and OS in patients with PCa, which revealed that high lncRNA TYMSOS expression was related to poor PFI, but there was no significant correlation with OS or DSS (**Figure 3A-C**).

To further clarify the risk factors affecting the prognosis of PCa patients, we conducted univariate and multivariate Cox regression analyses of clinicopathological parameters, including age, T stage, N stage, M stage, PSA, Gleason score, primary therapy outcome, and residual tumor status. We found that high expression of the lncRNA TYMSOS was an independent prognostic factor associated with poor PFS (HR: 1.694; CI: 1.052-2.725; P = 0.030), along with Gleason score and primary therapy outcome (all P < 0.05), as shown in Supplementary Table 3. However, lncRNA TYMSOS expression levels showed no association with poor DSS and OS (Supplementary Tables 4, 5). Next, we constructed a nomogram of PFS based on lncRNA TYMSOS and two other independent clinical risk factors (**Figure 3D**). The calibration curve then evaluated the prediction probability of the nomogram, and the C-index of the PFI was 0.78. The calibration plots showed that the predictive

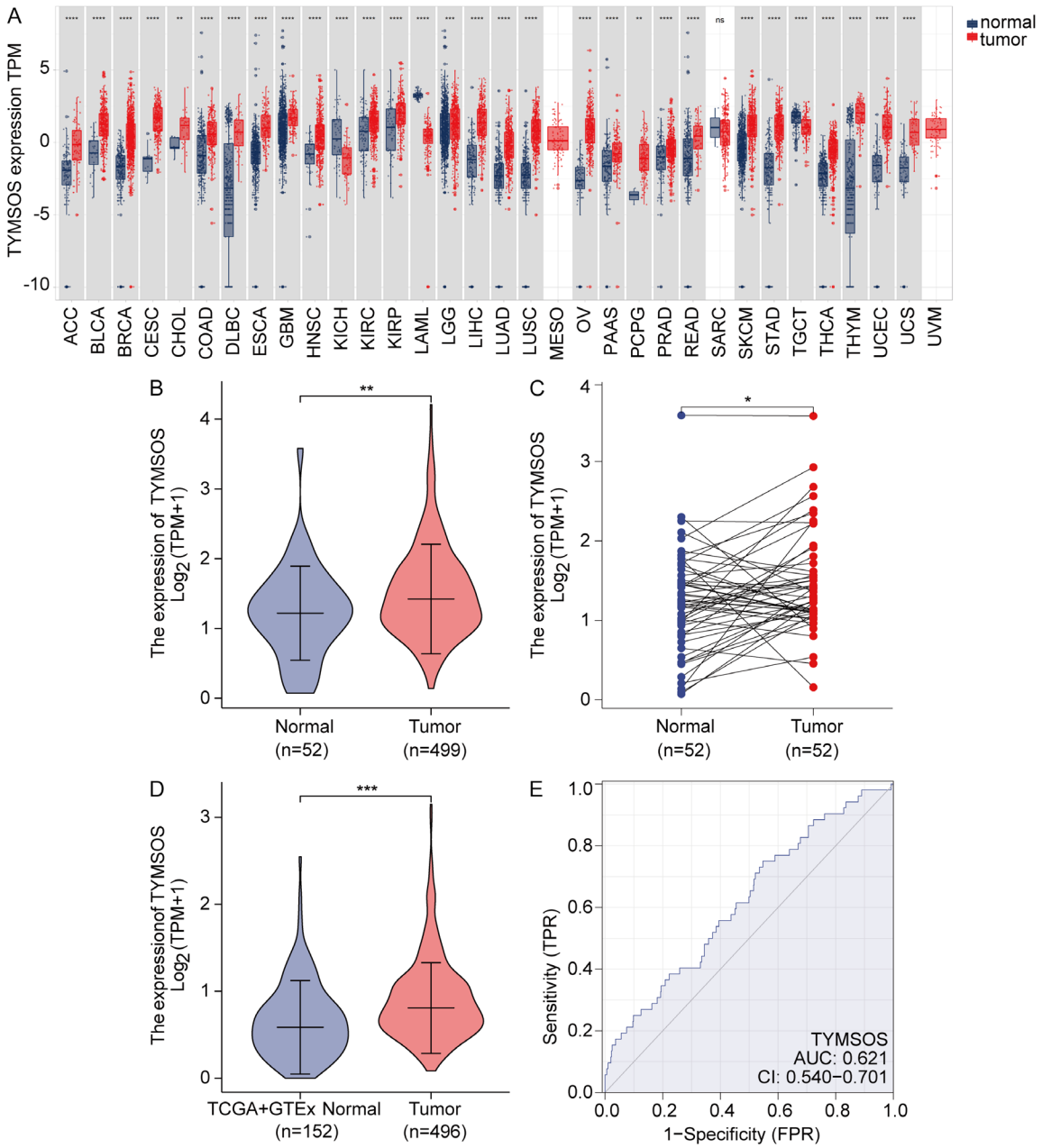

Figure 1. Differential expression of lncRNA TYMSOS in PCa. A. lncRNA TYMSOS expression in pan-cancer. The red and blue boxes represent tumor tissues and normal tissues, respectively. B. lncRNA TYMSOS showed significantly higher expression in cancer tissues than in normal tissues. C. Comparison of lncRNA TYMSOS expression between tumor and pairs non-cancerous adjacent tissues. D. Differential expression of lncRNA TYMSOS in normal prostate tissues of GTEx combined with TCGA and PCa tissues of TCGA. E. ROC curves of lncRNA TYMSOS expression to predict PCa. *P < 0.05, **P < 0.01, ***P < 0.001.

performance of the nomogram was reliable (**Figure 3E**).

GSEA and GSVA analysis

We performed GSEA and GSVA to clarify the molecular mechanism of lncRNA TYMSOS in PCa regulation. We used GSEA to identify the pathways associated with lncRNA TYMSOS regulation in PCa and found that lncRNA TYMSOS affected several important gene ontology factors, including the regulation of chromatin organization, molecule metabolism, protein modification, and cell cycle regulation (**Figure 4A**). In

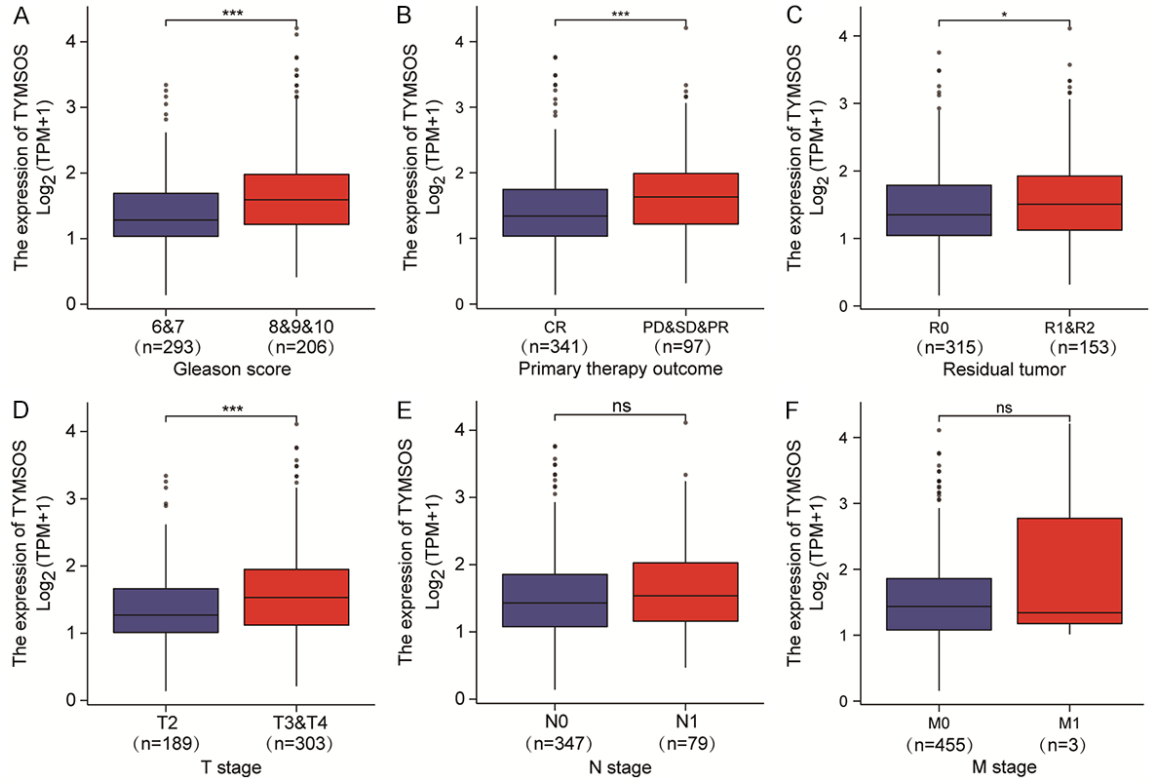

Figure 2. Associations between lncRNA TYMSOS expression and Gleason score (A), Primary therapy outcome (B), Residual tumor (C), TNM stage (D-F) in PCa. *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significance.

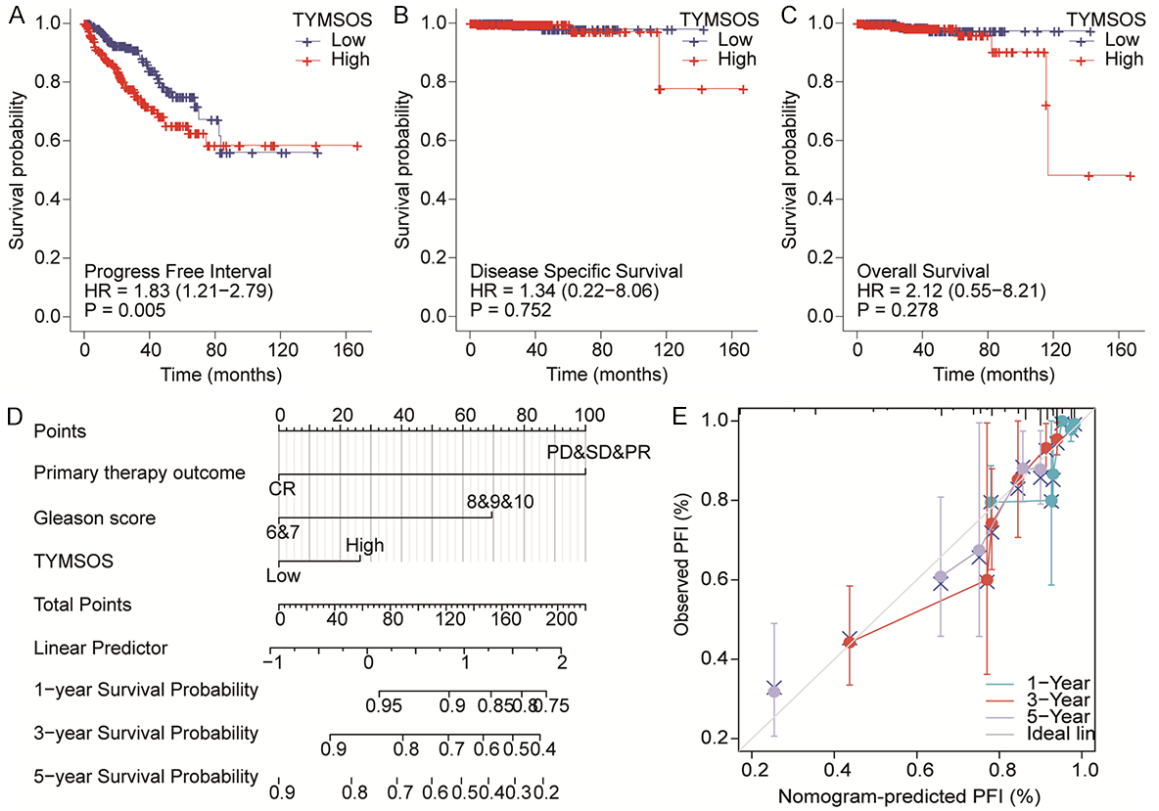

Figure 3. Survival analysis of lncRNA TYMSOS in PCa patients. A. The Kaplan-Meier curves of PFS. B. The Kaplan-Meier curves of DSS. C. The Kaplan-Meier curves of OS. D. Nomogram predicting the probability of PFS in PCa patients. E. Calibration plot of the nomogram for predicting the probability of PFS at 1, 3, and 5 years. OS, overall survival; DSS, disease-specific survival; PFS, progression-free survival.

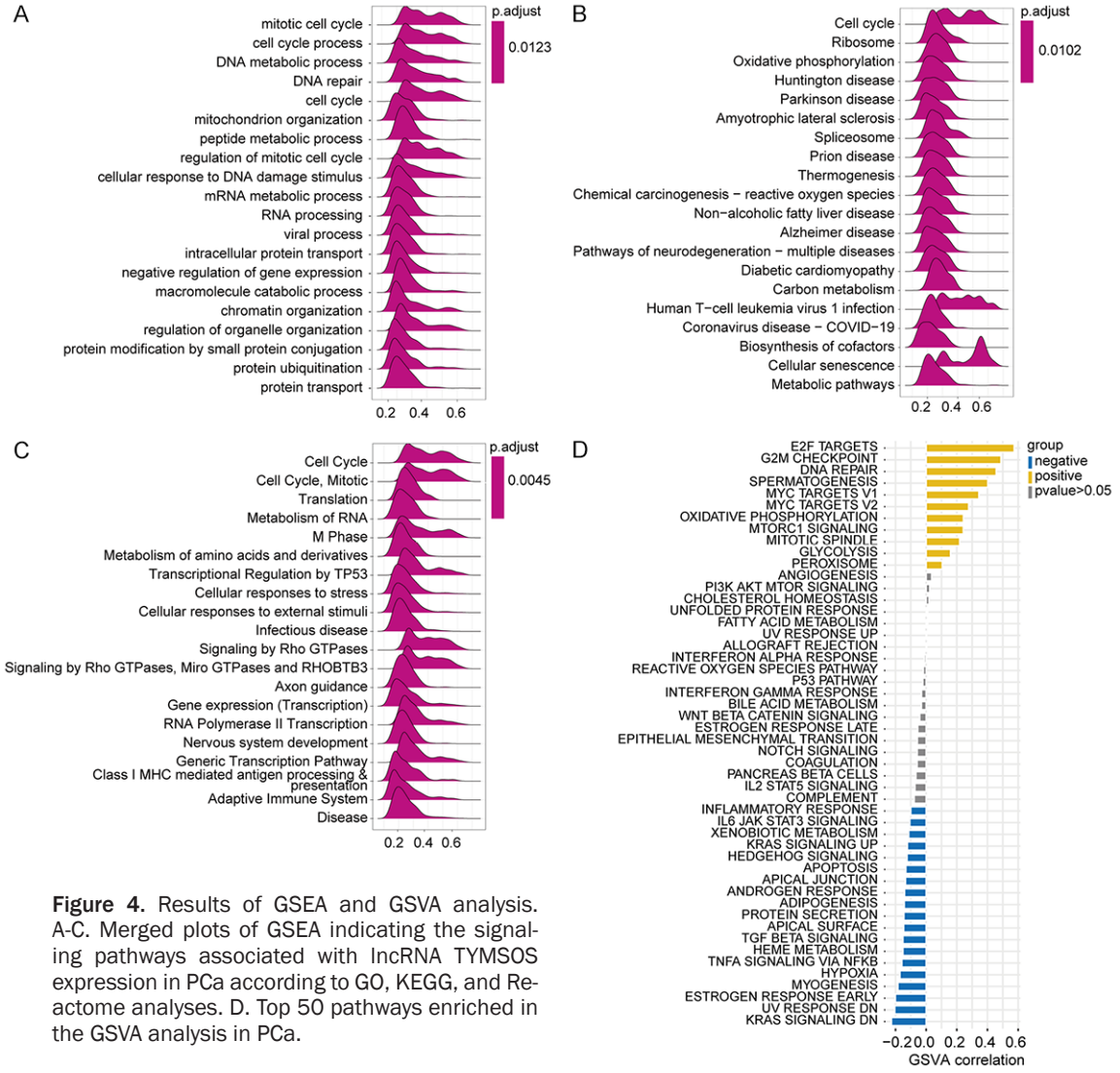

Figure 4. Results of GSEA and GSVA analysis. A-C. Merged plots of GSEA indicating the signaling pathways associated with lncRNA TYMSOS expression in PCa according to GO, KEGG, and Reactome analyses. D. Top 50 pathways enriched in the GSVA analysis in PCa.

PCa, the GSEA results of KEGG analysis indicated that lncRNA TYMSOS was involved in various pathways, including the cell cycle, neurodegenerative diseases, oxidative phosphorylation, spliceosome, viral infection, and metabolic pathways (**Figure 4B**). GSEA of the reactome in PCa showed that lncRNA TYMSOS was related to the adaptive immune system (**Figure 4C**). GSVA showed that lncRNA TYMSOS was associated with cancer-promoting pathways, such as mTOR and MYC target signaling (**Figure 4D**). These results indicated that the lncRNA TYMSOS plays an important role in PCa development and immune regulation.

Correlation analysis of lncRNA TYMSOS expression and the tumor microenvironment

We calculated the TME scores and various TME signatures based on the differential expression of the lncRNA TYMSOS in PCa. Our results showed the TME signatures between samples with high and low lncRNA TYMSOS expression, including genes involved in mismatch repair, nucleotide excision repair, DNA damage response, DNA replication, and base excision repair (**Figure 5A**). The expression of the lncRNA TYMSOS and TME-related biological processes in 33 tumors is presented in **Figure 5B**.

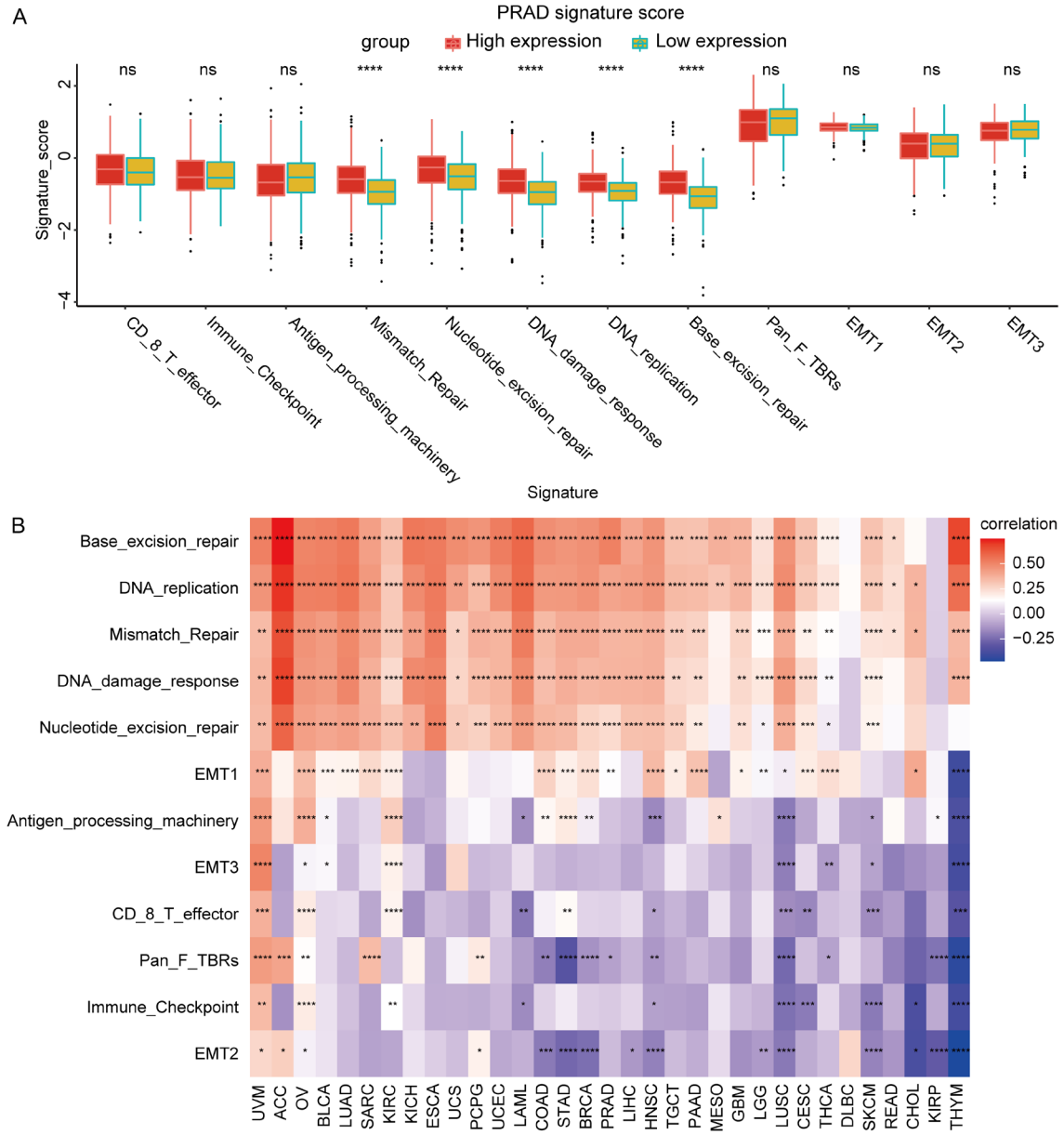

Figure 5. Correlation analysis between lncRNA TYMSOS and tumor microenvironment. A. Correlation between lncRNA TYMSOS expression and TME-related biological processes in PCa. B. Correlation between lncRNA TYMSOS expression and TME-related biological processes in pan-cancer.

Excluding DLBC and KIRP in 33 tumors, lncRNA TYMSOS expression was positively linked with DNA damage repair pathways.

Correlation between lncRNA TYMSOS expression and immune cell infiltration

Subsequently, we evaluated the correlation between lncRNA TYMSOS expression and immune cell infiltration in PCa tissues using the ImmuCellAI database. LncRNA TYMSOS expression was positively correlated with B, CD8 naïve, DC, monocyte, and Th1 and macrophage cells but negatively correlated with $CD4^+$ T, NKT, Tex and Th17 cells in PCa (**Figure 6A-I**).

Correlation of lncRNA TYMSOS expression with MSI and TMB

TMB is recommended as a biomarker for immune checkpoint inhibitors in mCRPC [27]. The higher the TMB is, the more antigens can be recognized by T cells, and the greater the effect of immunotherapy. **Figure 7A** shows a

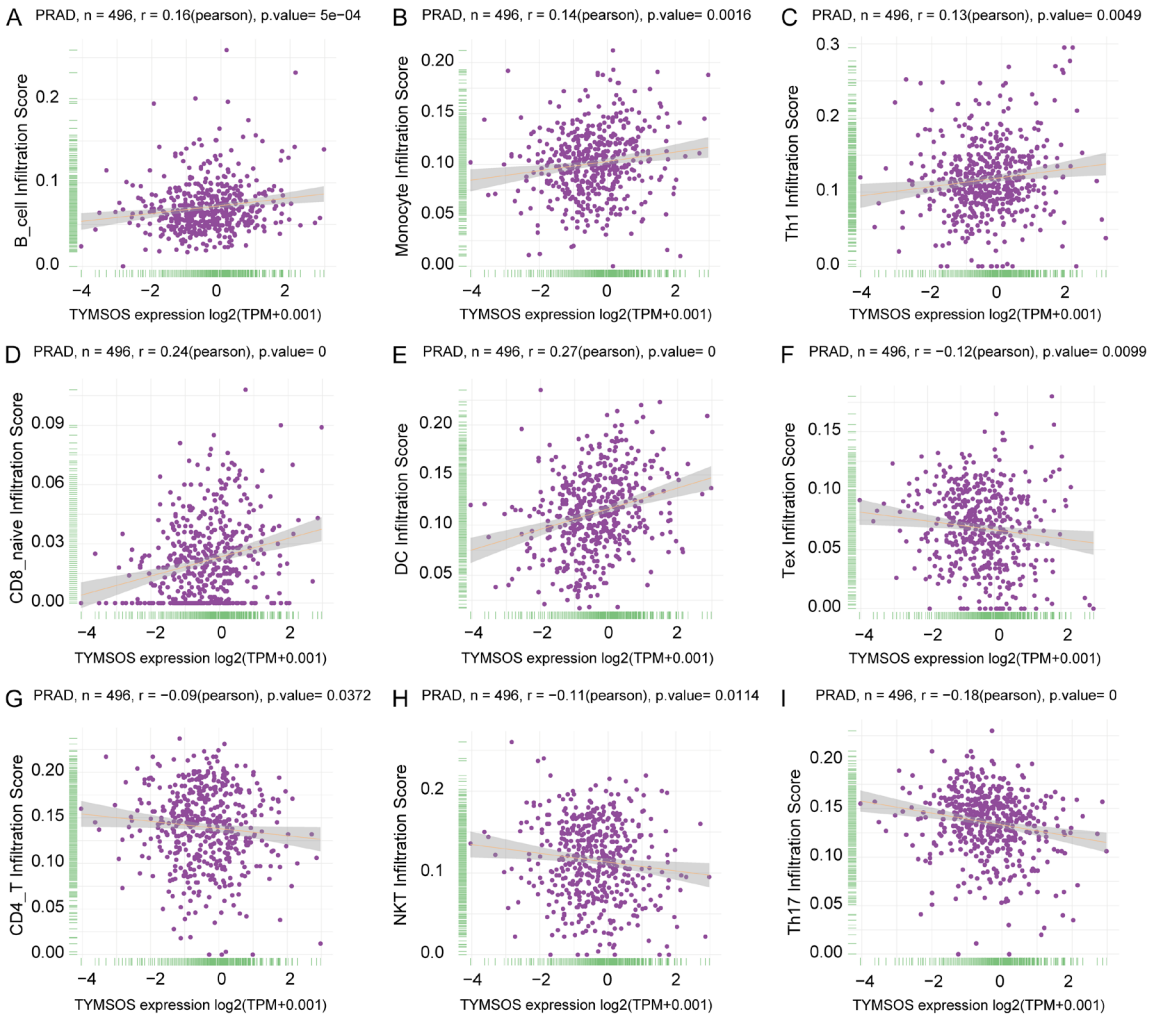

Figure 6. Immune cells infiltration analysis. A-E. Infiltration levels of immune cells correlate positively with lncRNA TYMSOS expression in PCa. F-I. Infiltration levels of immune cells correlate negatively with lncRNA TYMSOS expression in PCa.

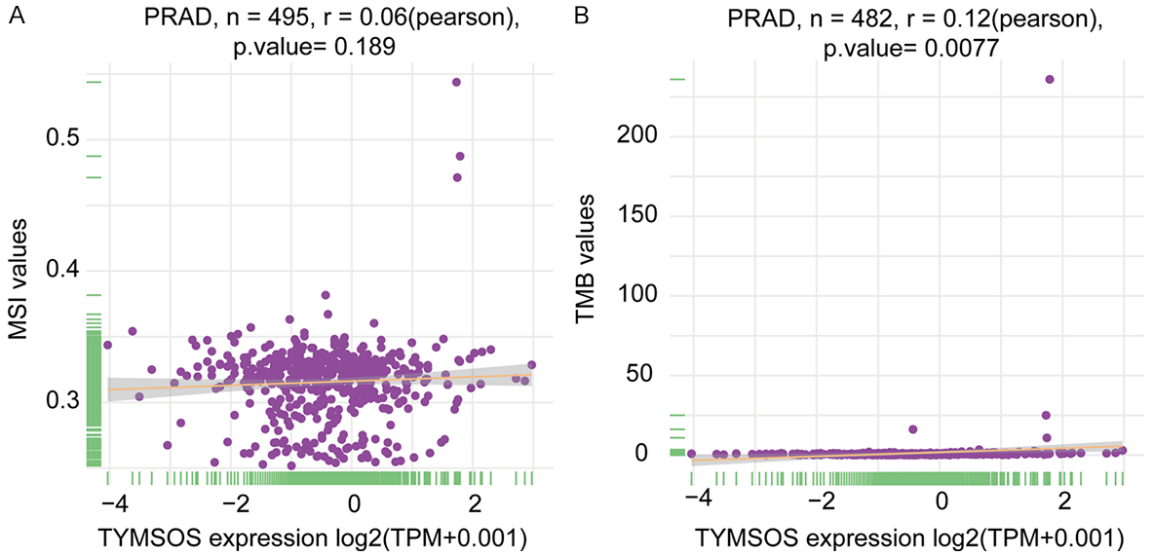

Figure 7. Correlation between TMB, MSI and lncRNA TYMSOS expression in PCa. A. The correlation between lncRNA TYMSOS expression and the MSI. B. The correlation between lncRNA TYMSOS expression and the TMB.

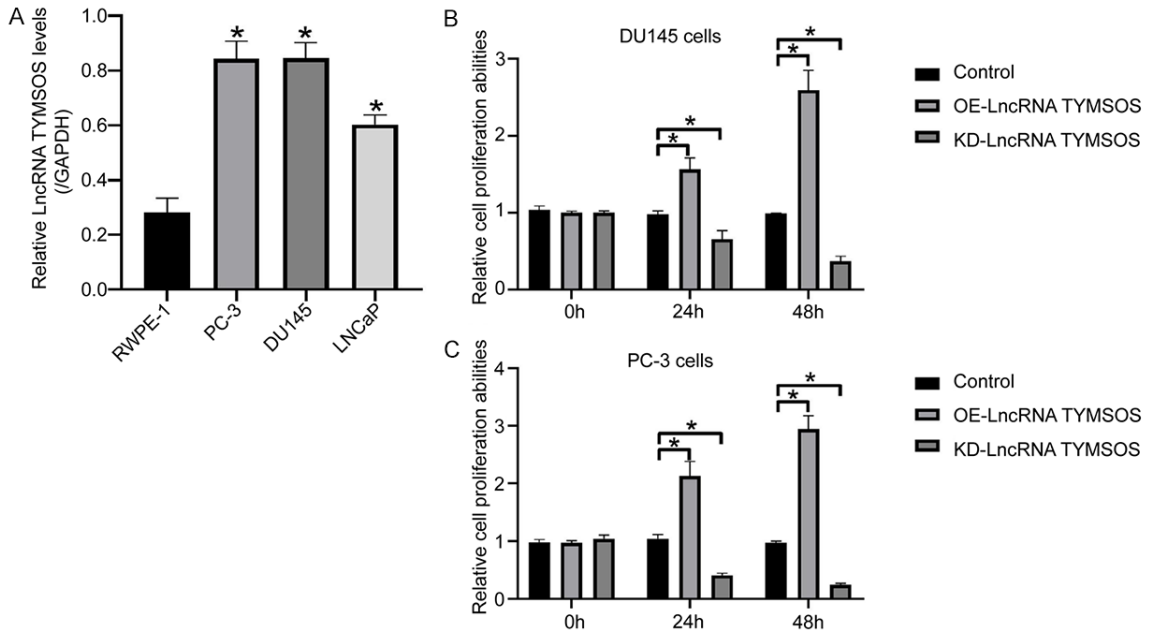

Figure 8. Real-Time qPCR analysis was performed to detect the expression levels of lncRNA TYMSOS in the RWPE-1, PC-3, DU145 and LNCaP cells, respectively (A). lncRNA TYMSOS was respectively overexpressed and downregulated, and MTT assay was employed to examine the cell proliferation abilities in the (B) DU145 cells and (C) PC-3 cells. Each experiment was repeated at least for 3 times, and *P < 0.05.

significant positive correlation between lncRNA TYMSOS conveyance and TMB in PCa. In addition, tumor immunotherapy largely depends on the MSI status, which in turn is frequently affected by functional defects in the match repair system. Previous studies have reported a relationship between expression and mismatch repair; therefore, we analyzed the relationship between the lncRNA TYMSOS and MSI in PCa. However, the lncRNA TYMSOS had no significant association with MSI (**Figure 7B**).

The biological functions of lncRNA TYMSOS in regulating the aggressiveness of PCa cells

To further validate the detailed biological functions of lncRNA TYMSOS in regulating cancer progression in PCa, we initially detected the expression levels of lncRNA TYMSOS in PCa cells and, as expected, found that lncRNA TYMSOS was especially highly expressed in PCa cell lines (PC-3, DU145 and LNCaP) compared with normal RWPE-1 cells (**Figure 8A**). Then, the MTT assay results showed that overexpression of lncRNA TYMSOS promoted cell proliferation abilities in a time-dependent manner, whereas lncRNA TYMSOS ablation had the opposite effects (**Figure 8B**, **8C**). Consistently, our Annexin V-FITC/PI double staining assay results confirmed that silencing of lncRNA TYMSOS promoted apoptotic cell death in both DU145 and PC-3 cells (**Figure 9A-C**). Then, Western blot analysis was performed, and our data validated that lncRNA TYMSOS downregulated E-cadherin and upregulated vimentin to promote epithelial-mesenchymal transition (EMT) in PCa cells (**Figure 10A-C**). Additionally, we noticed that lncRNA TYMSOS upregulated CDK2, CDK6 and Cyclin D1 to facilitate cell cycle and division (**Figure 11A-C**), and silencing of lncRNA TYMSOS upregulated Bax and cleaved Caspase-3 and suppressed Bcl-2 to promote cell apoptosis in PCa cells (**Figure 12A-C**). The above data supported the notion that targeting the oncogenic lncRNA TYMSOS was effective in restraining malignant phenotypes in PCa cells.

Discussion

LncRNAs are involved in the occurrence, development, and prognosis of tumors [12, 13, 15,

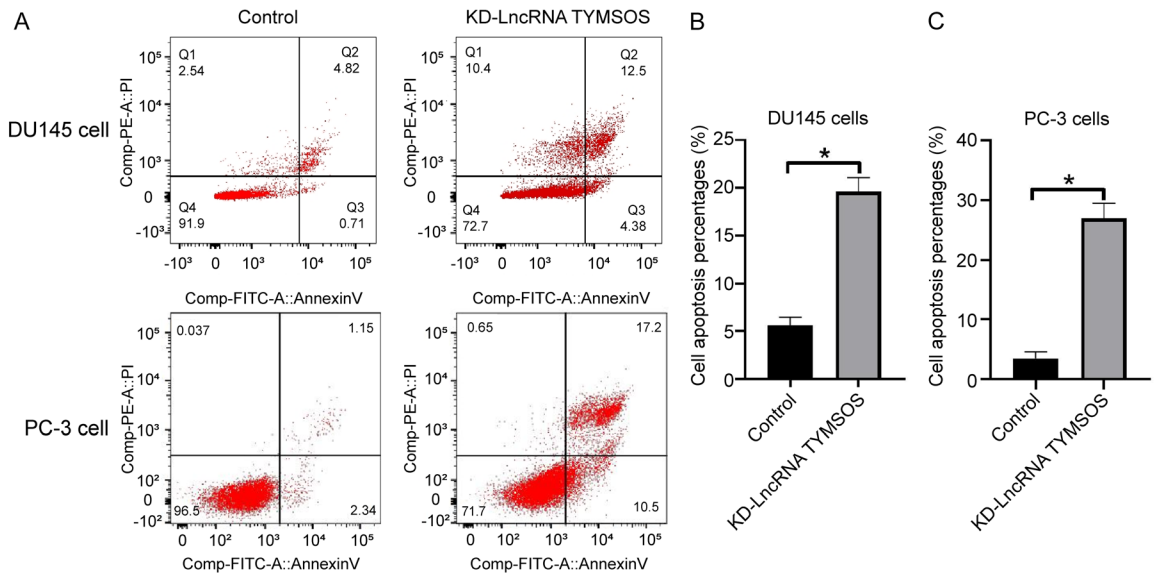

Figure 9. The Annexin V-FTIC/PI double staining assay was performed to detect (A-C) cell apoptosis ratio in the DU145 and PC-3 cells. Each experiment was repeated at least for 3 times, and *P < 0.05.

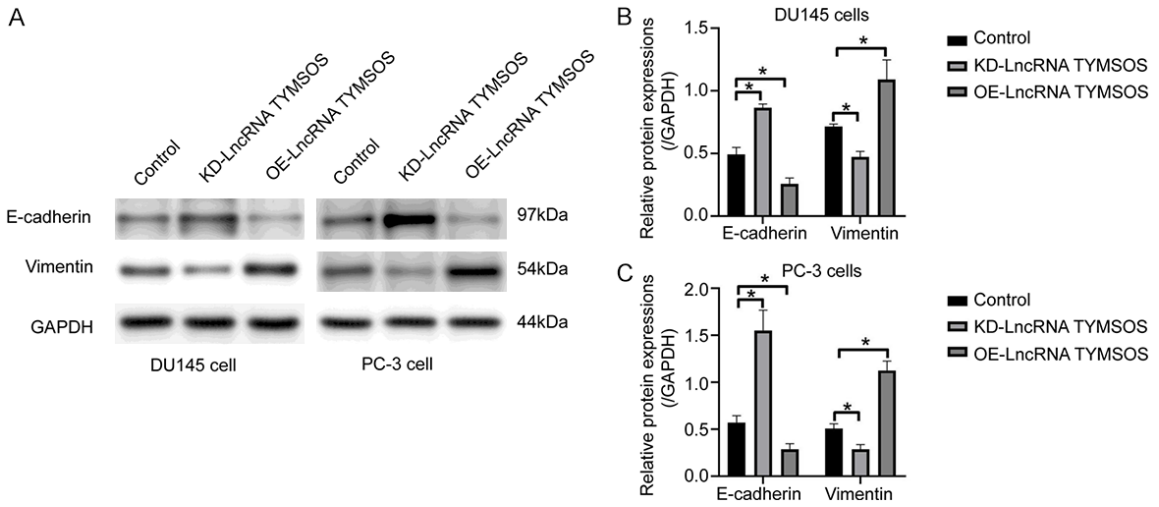

Figure 10. The expression levels of the EMT-associated biomarkers (E-cadherin and Vimentin) were detected by performing the (A-C) Western Blot analysis. Each experiment was repeated at least for 3 times, and *P < 0.05.

28] and have been linked to complex biological processes such as immune cell development and function and immune disorders [28]. Recently, the emergence of immunotherapy has improved the treatment outcomes of various cancers; however, some types of cancers are relatively insensitive to immunotherapy, including PCa [29]. It has also been reported that the prostate microenvironment defends against tumor-infiltrating cells, resulting in a lack of response to immune checkpoint inhibitors and immunotherapy [5]. Therefore, a better understanding of the tumor-immune microenvironment (TIME) is critical for sensitizing prostate cancer to immunotherapy approaches.

Studies have shown that lncRNAs play a key role in promoting immune cell infiltration and malignant progression of PCa. For instance, Chen et al. [30] found that lncRNA KCNQ1OT1 sponges miR-15a to promote immune evasion and progression of PCa by upregulating PD-L1. LINC00184, as a ceRNA, regulates the T-cell-mediated immune response and docetaxel

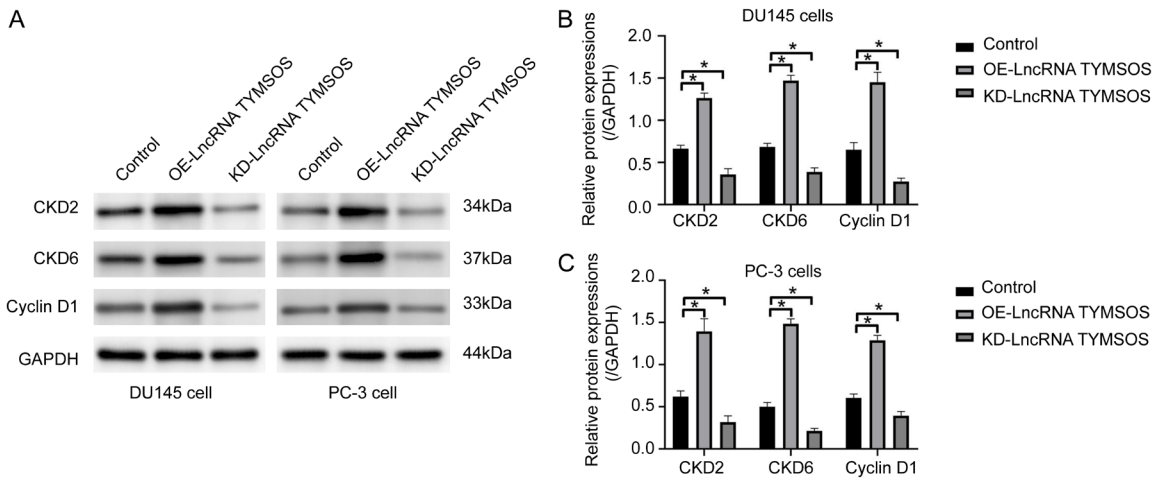

Figure 11. The expression levels of cell cycle-associated proteins (CDK2, CDK6 and Cyclin D1) were detected by using the (A-C) Western Blot analysis. Each experiment was repeated at least for 3 times, and *P < 0.05.

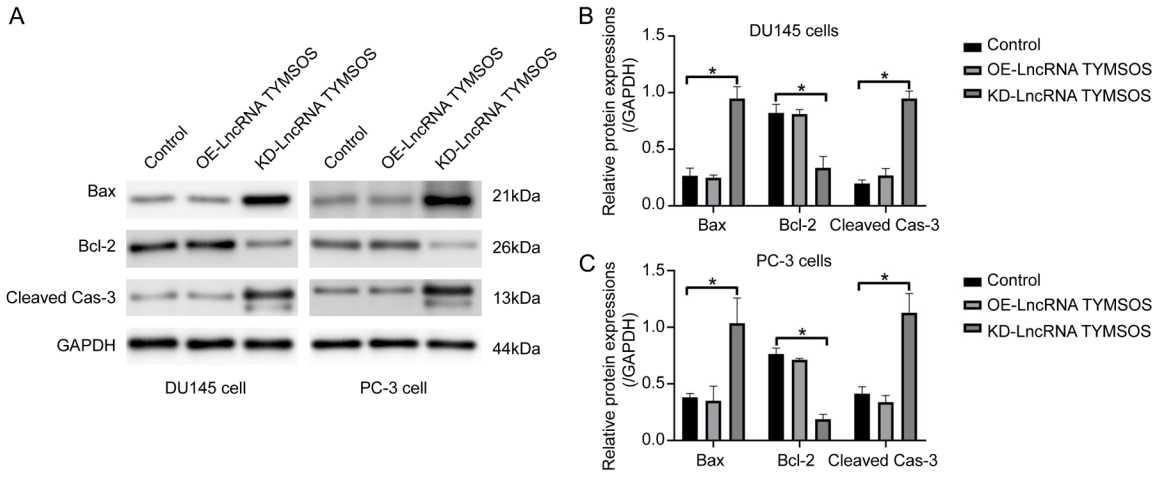

Figure 12. The expression levels of the apoptosis-associated genes (Bax, Bcl-2 and cleaved Caspase-3) were determined by using the (A-C) Western Blot analysis. Each experiment was repeated at least for 3 times, and *P < 0.05.

resistance in PCa [31]. LncRNA HOXD-AS1 promotes the proliferation and castration resistance of PCa by combining WDR5/MLL1 [32]. In addition, through bioinformatics analysis techniques, several immune-related lncRNA prognostic signatures for PCa have been identified [33, 34]. As a novel lncRNA, lncRNA TYMSOS was only reported in gastric and lung cancer [17, 35] and no study has reported the correlation between lncRNA TYMSOS expression and PCa.

Our study showed that the lncRNA TYMSOS gene was highly expressed in PCa compared to normal tissues, indicating that lncRNA TYMSOS plays an important role in tumorigenesis and development. Kaplan-Meyer survival analysis indicated that the overexpression of lncRNA TYMSOS was related to poor PFS in PCa. According to the results of multivariate analysis, lncRNA TYMSOS was an independent factor affecting the PFS of PCa patients. Moreover, we constructed a nomogram combining lncRNA TYMSOS expression and clinical parameters. Calibration plots indicated that the nomogram accurately predicted the 1-, 3- and 5-year PFS of PCa patients. In addition, the results of GSEA and GSVA analysis revealed that cell cycle, neurodegenerative diseases, oxidative phosphorylation, spliceosomes, viral infection, metabolic pathway, and adaptive immune systems pathways were enriched in

PCa patients with high expression of the lncRNA TYMSOS. Additionally, lncRNA TYMSOS expression was significantly correlated with immune cell infiltration and TMB. Based on the above results, we could consider lncRNA TYMSOS as a potential prognostic marker and therapeutic target for PCa.

GSVA and GSEA indicated that lncRNA TYMSOS can potentially impact PCa etiology or pathogenesis via the cell cycle, neurodegenerative diseases, oxidative phosphorylation, spliceosomes, viral infection, metabolic pathways, and adaptive immune systems. Several studies have reported that the TME is involved in tumorigenesis, progression, and metastasis. Meanwhile, we found a positive correlation between lncRNA TYMSOS expression and DNA damage repair-related pathways in PCa. A dedicated DNA damage response (DDR) is essential for maintaining genomic integrity. Studies have shown that PCa often undergoes mutations affecting DDR in germinal and somatic tissues [36]. Single-nucleotide polymorphisms of different DDR genes are associated with an increased risk of PCa [37].

Activating the immune response to treat cancer has become the cornerstone of modern oncology therapy. The composition of resident immune cell types in the TME varies, including cytotoxic T cells, helper T cells, dendritic cells (DCs), tumor-associated macrophages, and related inflammatory pathways [24]. It has been reported that changes in the number of $CD4^+$ T cells and macrophages in the TME of gastric, lung, and breast cancers and melanomas are correlated with clinical results [38, 39]. Accordingly, we evaluated the relationship between lncRNA TYMSOS expression and the infiltration of 24 immune-related cells in PCa. Recently, studies have found that lncRNAs are involved in immune regulation in tumors, such as lncRNA FENDRR as a tumor suppressor in tumor-immune interactions in non-small cell lung cancer. Silencing lncRNA NKILA can reduce T-cell apoptosis and enhance their killing ability, while lncRNA SNHG1 can promote STAT3 and PD-L1 to mediate immune escape in renal cell cancer tissue [40-42]. PCa is known as an immunologically “cold” tumor, and its immune microenvironment is complex [43]. Currently, the therapeutic effect of vaccines (sipuleucel-T) and immune-checkpoint inhibitors has improved the survival of metastatic prostate cancer, yet the efficacy remains far from satisfying; therefore, it is necessary to identify novel targets for immunotherapy [44, 45]. Chronic inflammation has been linked to the development of prostate cancer; the inflammation of the prostate is primarily regulated by T and B cells and macrophages [46]. In PCa, our data showed that lncRNA TYMSOS expression was positively correlated with B, CD8 naive, DC, monocyte, and Th1 cells and macrophages and negatively correlated with $CD4^+$ T, NKT, Tex, and Th17 cells. GSEA also indicated a relationship between the lncRNA TYMSOS and the adaptive immune regulation system. Simultaneously, Fong et al. [47] found that $CD3^+$, $CD4^+FOXP3^-$, and $CD8^+$ T cells infiltrate the periphery of metastatic prostate cancer. Our findings, along with previous results, suggest that lncRNA TYMSOS might be a new target in PCa immunotherapy.

Additionally, previous studies have found that TMB and MSI are specific, sensitive biomarkers for immune checkpoint inhibitors [38, 48, 49]. Patients with high TMB or MSI are more likely to derive long-term survival benefit from immunotherapy [17]. Notably, we found that the lncRNA TYMSOS was positively related to TMB in PCa, while its expression did not correlate with MSI status. MSI is an important biomarker of immune checkpoint inhibitors and is commonly caused by functional defects in the DNA mismatch repair system [49]. Enrichment analysis revealed that the lncRNA TYMSOS was positively correlated with DNA damage repair-related pathways in PCa. This finding may explain the low efficacy of ICI drugs in PCa treatment.

Aberrant expression of lncRNAs has often been reported to be associated with cancer development and metastasis. Compared with mRNA, lncRNA expression has obvious tissue specificity [50]. However, the role of lncRNA TYMSOS in prostate cancer has not been clearly studied. In this study, we performed bioinformatics analysis and found that the expression of the lncRNA TYMSOS was upregulated in PCa cells. Further in vitro studies consistently demonstrated that the lncRNA TYMSOS was highly expressed in PCa cell lines. Loss-of-function studies showed that silencing of lncRNA TYMSOS inhibited the proliferation, division and migration of PCa cells. lncRNAs have been shown to have diagnostic and therapeutic potential in various cancers, including prostate cancer, because lncRNA TYMSOS is highly

expressed in PCa tissues [51] and has tumor-promoting effects in PCa cells. It is possible to use locked nucleic acids (LNAs) to specifically inhibit the expression of lncRNA TYMSOS in PCa patients.

New biomarkers for early diagnosis and prognosis are important to improve the diagnosis of metastatic or recurrent PCa. Recent studies have demonstrated the important role of lncRNAs in tumorigenesis. In particular, lncRNAs are associated with epithelial-mesenchymal transition. We demonstrated that lncRNA TYMSOS silencing inhibits EMT. More importantly, lncRNA has a tissue-specific expression pattern compared with mRNA [52], so lncRNA TYMSOS may have the potential to become a new biomarker to distinguish patients from healthy individuals. However, analysis of large samples is required to evaluate the utility of lncRNA TYMSOS in clinical prediction.

Although we used bioinformatics techniques and in vitro experiments to investigate the expression and prognostic significance of lncRNA TYMSOS and its relationship to immune cell infiltration, there are several limitations in this study. First, the sample size of our healthy control group differed from that of patients with PCa; hence, additional research is warranted for better sample presentation. Second, there was a lack of in vivo and in vitro experiments to confirm our results. Third, we did not determine whether MSI affects the efficacy of ICIs in treating prostate cancer. Fourth, we did not determine the specific mechanism of lncRNA TYMSOS in tumorigenesis. Therefore, as we are currently beginning to understand the molecular mechanisms of lncRNA TYMSOS, there is a great scope and need for future studies in this area.

Conclusions

In conclusion, we performed a comprehensive analysis to reveal that the high expression of the lncRNA TYMSOS was significantly associated with the progression, poor PFS, and immune cell infiltration of PCa for the first time. Moreover, silencing of the lncRNA TYMSOS inhibited cell proliferation, division and epithelial-mesenchymal transition while promoting apoptosis of PCa cells. This study suggested that the lncRNA TYMSOS could be a novel prognostic biomarker and therapeutic target for PCa patients. However, further analysis of the mechanism is warranted to provide a reliable basis for accurate, personalized immunotherapy for PCa.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Correlation between lncRNA TYMSOS expression and clinicopathological characteristics in prostate cancer

Characteristic	Low expression of lncRNA TYMSOS	High expression of lncRNA TYMSOS	p	Test
n	249	250		
T stage, n (%)			< 0.001	Chisq.test
T2	116 (23.6%)	73 (14.8%)		
T3	123 (25%)	169 (34.3%)		
T4	4 (0.8%)	7 (1.4%)		
N stage, n (%)			0.169	Chisq.test
N0	173 (40.6%)	174 (40.8%)		
N1	32 (7.5%)	47 (11%)		
M stage, n (%)			0.619	Fisher.test
M0	224 (48.9%)	231 (50.4%)		
M1	2 (0.4%)	1 (0.2%)		
Age, n (%)			0.003	Chisq.test
≤ 60	129 (25.9%)	95 (19%)		
> 60	120 (24%)	155 (31.1%)		
Race, n (%)			0.781	Chisq.test
Asian	5 (1%)	7 (1.4%)		
Black or African American	30 (6.2%)	27 (5.6%)		
White	207 (42.8%)	208 (43%)		
Primary therapy outcome, n (%)			0.008	Chisq.test
PD	13 (3%)	15 (3.4%)		
SD	12 (2.7%)	17 (3.9%)		
PR	11 (2.5%)	29 (6.6%)		
CR	186 (42.5%)	155 (35.4%)		
Residual tumor, n (%)			0.099	Fisher.test
R0	170 (36.3%)	145 (31%)		
R1	64 (13.7%)	84 (17.9%)		
R2	3 (0.6%)	2 (0.4%)		
Zone of origin, n (%)			0.980	Fisher.test
Central Zone	2 (0.7%)	2 (0.7%)		
Overlapping/Multiple Zones	54 (19.6%)	72 (26.2%)		
Peripheral Zone	56 (20.4%)	81 (29.5%)		
Transition Zone	3 (1.1%)	5 (1.8%)		
PSA (ng/ml), n (%)			0.621	Chisq.test
< 4	213 (48.2%)	202 (45.7%)		
≥ 4	12 (2.7%)	15 (3.4%)		
Gleason score, n (%)			< 0.001	Chisq.test
6	33 (6.6%)	13 (2.6%)		
7	139 (27.9%)	108 (21.6%)		
8	33 (6.6%)	31 (6.2%)		
9	43 (8.6%)	95 (19%)		
10	1 (0.2%)	3 (0.6%)		
Age, median (IQR)	60 (55, 66)	63 (57, 66)	0.002	Wilcoxon

PSA, prostate specific antigen; CR, complete response; PD, progressive disease; SD, stable disease; PR, partial response.

Supplementary Table 2. lncRNA TYMSOS expression associated with clinicopathologic characteristics (logistic regression)

Characteristics	Total (N)	Odds Ratio (OR)	*P* value
T stage (T3&T4 vs. T2)	492	2.202 (1.523-3.201)	< 0.001
N stage (N1 vs. N0)	426	1.460 (0.893-2.414)	0.135
M stage (M1 vs. M0)	458	0.485 (0.022-5.096)	0.556
Primary therapy outcome (CR vs. PD&SD&PR)	438	0.492 (0.307-0.778)	0.003
PSA (ng/ml) (< 4 vs. ≥ 4)	442	0.759 (0.340-1.657)	0.489
Gleason score (8&9&10 vs. 6&7)	499	2.381 (1.656-3.443)	< 0.001
Residual tumor (R1&R2 vs. R0)	468	1.505 (1.021-2.224)	0.039

CR, complete response; PD, progressive disease; SD, stable disease; PR, partial response; PSA, prostate specific antigen.

Supplementary Table 3. Univariate and multivariate analyses of progression-free survival in prostate cancer patients

Characteristics	Total (n)	HR (95% CI), Univariate analysis	*P* value Univariate analysis	HR (95% CI), multivariate analysis	*P* value multivariate analysis
lncRNA TYMSOS (low vs. high)	499	1.833 (1.207-2.786)	0.005	1.694 (1.052-2.725)	0.030
Age (≤ 60 vs. > 60)	499	1.302 (0.863-1.963)	0.208		
T stage (T2 vs. T3&T4)	492	3.785 (2.140-6.693)	< 0.001	1.479 (0.716-3.055)	0.290
N stage (N0 vs. N1)	426	1.946 (1.202-3.150)	0.007	0.790 (0.454-1.374)	0.403
M stage (M0 vs. M1)	458	3.566 (0.494-25.753)	0.208		
PSA (ng/mL) (< 4 vs. ≥ 4)	442	4.196 (2.095-8.405)	< 0.001	1.608 (0.721-3.586)	0.246
Primary therapy outcome (CR vs. PD&SD&PR)	438	6.627 (4.337-10.126)	< 0.001	3.752 (2.169-6.489)	< 0.001
Residual tumor (R0 vs. R1&R2)	468	2.365 (1.566-3.570)	< 0.001	1.048 (0.618-1.778)	0.862
Gleason score (6&7 vs. 8&9&10)	499	4.675 (2.957-7.391)	< 0.001	2.846 (1.585-5.112)	< 0.001
Zone of origin (peripheral zone vs. Central Zone & Overlapping/Multiple Zones & Transition Zone)	275	1.170 (0.726-1.887)	0.519		
Race (Asian & Black or African American vs. White)	484	1.332 (0.739-2.401)	0.339		

CR, complete response; PD, progressive disease; SD, stable disease; PR, partial response; PSA, prostate specific antigen.

Supplementary Table 4. Univariate and multivariate analyses of disease-specific survival survival in prostate cancer patients

Characteristics	Total (n)	HR (95% CI), Univariate analysis	*P* value Univariate analysis	HR (95% CI), multivariate analysis	*P* value multivariate analysis
lncRNA TYMSOS (low vs. high)	497	1.336 (0.221-8.057)	0.752		
Age (≤ 60 vs. > 60)	497	0.241 (0.027-2.162)	0.204		
T stage (T2 vs. T3&T4)	490	519428284.120 (0.000-Inf)	0.999		
N stage (N0 vs. N1)	424	8.116 (0.736-89.560)	0.087	21.628 (1.216-384.801)	0.036
M stage (M0 vs. M1)	456	192.878 (11.629-3198.978)	< 0.001	0.000 (0.000-Inf)	0.999
PSA (ng/mL) (< 4 vs. ≥ 4)	440	32.707 (5.137-208.243)	< 0.001	44.162 (3.143-620.609)	0.005
Primary therapy outcome (CR vs. PD&SD&PR)	437	3868557293.902 (0.000-Inf)	0.999		
Residual tumor (R0 vs. R1&R2)	466	5.865 (0.609-56.523)	0.126		
Gleason score (6&7 vs. 8&9&10)	497	892211776.881 (0.000-Inf)	0.999		
Zone of origin (peripheral zone vs. Central Zone & Overlapping/Multiple Zones & Transition Zone)	273	1.072 (0.151-7.619)	0.945		
Race (Asian & Black or African American vs. White)	482	349191972.846 (0.000-Inf)	0.999		

CR, complete response; PD, progressive disease; SD, stable disease; PR, partial response; PSA, prostate specific antigen.

Supplementary Table 5. Univariate and multivariate analyses of overall survival in prostate cancer patients

Characteristics	Total (n)	HR (95% CI), Univariate analysis	*P* value Univariate analysis	HR (95% CI), multivariate analysis	*P* value multivariate analysis
lncRNA TYMSOS (low vs. high)	499	2.116 (0.546-8.207)	0.278		
Age (≤ 60 vs. > 60)	499	1.577 (0.440-5.648)	0.484		
T stage (T2 vs. T3&T4)	492	3.294 (0.612-17.727)	0.165		
N stage (N0 vs. N1)	426	3.516 (0.778-15.896)	0.102		
M stage (M0 vs. M1)	458	59.383 (6.520-540.817)	< 0.001	22.298 (1.956-254.173)	0.012
PSA (ng/mL) (< 4 vs. ≥ 4)	442	10.479 (2.471-44.437	0.001	4.396 (0.838-23.056)	0.080
Primary therapy outcome (CR vs. PD&SD&PR)	438	4.396 (0.838-23.056)	0.007	3.667 (0.544-24.732)	0.182
Residual tumor (R0 vs. R1&R2)	468	2.598 (0.696-9.694)	0.155		
Gleason score (6&7 vs. 8&9&10)	499	6.664 (1.373-32.340)	0.019	2.009 (0.276-14.644)	0.491
Zone of origin (peripheral zone Vs. Central Zone & Overlapping/Multiple Zones & Transition Zone)	275	1.313 (0.353-4.893)	0.684		
Race (Asian & Black or African American vs. White)	484	1.616 (0.308-8.472)	0.570		

CR, complete response; PD, progressive disease; SD, stable disease; PR, partial response; PSA, prostate specific antigen.