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Original Article

SOX2 control activation of dormant prostate cancer cells in bone metastases by promoting CCNE2 gene expression

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Abstract: Background: Cancer stem cells (CSCs) have a powerful tumor initiation ability, which can promote the early dissemination of single disseminated tumor cells (DTCs), leading to tumor progression. SOX2, a pluripotent inducible transcription factor, is key to maintaining self-renewal and pluripotency of prostate cancer stem cells. However, there is a lack of comprehensive understanding of how SOX2 regulates DTCs dormancy and proliferation in the bone marrow microenvironment. Methods and Results: By constructing a mouse bone metastasis model to simulate the progression of prostate cancer with bone metastasis, the bone tissue immunofluorescence showed that SOX2 expression increased with the progression of prostate cancer in the bone marrow microenvironment. We validated this phenomenon with publicly available single-cell and transcriptome datasets and found that SOX2 is involved in multiple phenotypes associated with prostate cancer dormancy, proliferation, and invasion. Further, CCNE2, a potential target downstream of SOX2, was identified through multiple transcription factor databases and protein interaction networks. Conclusion: The expression of SOX2 affects multiple phenotypes related to dormancy, proliferation and invasion of prostate cancer, and may indirectly activate the dormant prostate cancer cells through the downstream target gene CCNE2, thus affecting the progression and bone metastasis of prostate cancer.

Keywords: Prostate cancer, SOX2, CCNE2, dormancy, bone metastasis

Introduction

Prostate cancer is the second leading cause of cancer-related death in men [1]. At present, androgen deprivation therapy (ADT) is the main treatment for prostate cancer. Although the tumor was well controlled in the early stage, castration resistance still appeared in some patients in the late stage, eventually leading to bone metastasis and recurrence [2]. A large number of studies have shown that most tumor cells that metastasise from the primary site to distant organs through blood are cleared by the immune system or undergo apoptosis, but a small number of residual DTCs colonize in the body and remain in a dormant state. These DTCs that survive immune system attack and drug killing form cancer metastases. These dormant DTCs are generally CSCs with stem cell activity, which are generally considered to be the root cause of resistance to conventional chemoradiotherapy and cause tumor recurrence and metastasis [3, 4]. After CSCs adapt to the microenvironment or receive some kind of stimulation, they will reactivate, divide and proliferate, and eventually become clinical metastatic lesions. However, the interaction between tumor cells and the bone marrow microenvironment is still unclear. Therefore, further understanding of the molecular mechanism of DTCs dormancy in tumor microenvironment will lay a theoretical foundation for elucidating the molecular mechanism of prostate cancer metastasis, which has important scientific significance and clinical guiding value.

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SOX2 is an important pluripotent inducible transcription factor that plays a key role in maintaining self-renewal and pluripotency of embryonic stem cells [5]. Moreover, it is expressed in both benign and malignant prostate tissues, and is also associated with various tumor functions in non-stem cell prostate cancer cells [5-9]. These findings imply a potential link between SOX2 and prostate cancer progression, although whether it is involved in regulating and activating dormant tumor cells has not been clearly defined.

Here, we simulated the process of prostate cancer bone metastasis by constructing an intracardial bone metastasis model in mice, verified the close correlation between SOX2 and prostate cancer progression through multiple transcriptome data and transcription factor databases, and speculated that SOX2 may regulate the cell cycle of prostate cancer cells through the downstream target gene CCNE2, thereby regulating dormant cancer cell activation.

Materials and methods

Cell culture

PC3 (RRID: CVCL_0035) cells were purchased from Prenoxel. The cells were tested for mycoplasma before treatment. As previously mentioned, the cells were cultured in DMEM (Gibco) supplemented with 10% heat-inactivated FBS (Hyclone) and 1% penicillin/streptomycin (Corning). To generate stable transfection cell lines, a lentiviral vector based on GFP-Luciferase-Puromycin (Genechem, GV633) was used, and the lentivirus was packaged into PC3 cells. The GFP expression efficiency was initially measured by fluorescence microscopy 3 days after transfection, and the recipient cell lines were exposed to complete medium supplemented with 10 μg/mL purinomycin (Beyotime) to produce stable cell lines.

Animal studies

Male BALB/c nude mice aged 4 to 6 weeks were purchased from Vitonlihua and raised in SPF animal centres. All mice were exposed to 12 hours of light and 12 hours of darkness, eating and drinking freely. All procedures and experimental protocols involving mice have been approved by the Laboratory Animal Welfare and Ethics Committee of the Army Medical University. Intracardiac injection (I.C.) was used to construct a bone metastasis model. 100 μL cells were injected into the left ventricle of mice using a 26G needle [10]. Bioluminescence signals were evenly distributed throughout the body through BLI imaging within 24 hours, confirming the successful injection. Bone metastasis load was monitored weekly with BLI imaging. The mice were euthanized, and their hindlimbs were collected within 24 hours, at week 1, and at week 5.

Histological analysis and immunofluorescence (IF) staining

After skin and muscle removal, the tibia of mice were fixed with 4% paraformaldehyde, decalcified with EDTA for 4 weeks, and then paraffin embedding was performed to make continuous coronal sections (5 mm thick) of the entire tibia. Permeate with 0.2% Triton X-100 for 5 minutes, block with 5% BSA in PBS for 1 hour, incubate with appropriate primary antibody at 4°C overnight, then incubate with secondary antibody at 4°C in the dark for 1 hour. The antibodies used for IF staining were anti-SOX2 (1:500, abcam, ab97959) and anti-SOX9 (1:500, abcam, ab185230). Staining was performed with the corresponding AlexaFlour secondary antibody of Life Technologies. The images were obtained using the SP5 confocal microscope (Leica Microsystems) and the micrographs were analyzed using the ImageJ, Imaris and Metamorph software packages.

scRNA-seq data processing

Using R software (version 4.1.0) and the Seurat R package (v4.3.0), scRNA-seq data were converted into Seurat objects. Quality control at the cell level was performed by: (1) selecting the top 99% of cells based on unique molecular identifier counts; (2) retaining cells with > 200 genes; (3) excluding cells with mitochondrial gene expression in < 25% of UMI counts. The NormalizeData function with the LogNormalize method was used to standardize gene expression levels for each gene in every cell. Highly variable genes were identified using FindVariableFeatures, which simulated the mean-variance relationship of normalized counts for each gene and determined 2,000 genes per sample. After removing low-quality

cells, the gene expression matrix was normalized to the total UMI count per cell and transformed to a natural log scale. The IntegrateData function was applied using precomputed anchors to integrate the dataset, and the ScaleData function was used to scale the integrated dataset. PCA and Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction were performed based on the first 20 principal components. Clusters were visualized on a 2D map generated using the UMAP method. Cells were clustered using FindNeighbors with dimensions 1-30 and FindClusters with a resolution of 0.5. The SubsetData function was also used to extract subclusters for downstream analysis, and UMAP analysis was executed using the RUNUMAP function.

Cell type annotations and differential gene expression analysis

Cell types in the GSE240056 prostate cancer scRNA-seq data were annotated by screening literature [11] and searching the CellMarker 2.0 database [12], revealing that most cells were of epithelial cell type. Differentially expressed genes (DEGs) were identified using the "FindAllMarkers" function (min.pct = 0.25, logfc.threshold = 0.25, $P < 0.05$).

Cell cycle analysis

We utilized the CellCycleScoring function from the Seurat package, which is based on cell cycle phase marker genes described previously [13]. This function provides quantitative scores for G1, G2/M, and S phases for each cell and nucleus based on the expression of cell cycle stage-specific marker genes. Subsequently, bar plots were generated to depict the proportion of cells in each cell cycle phase by group.

Functional enrichment analysis

Gene sets from the Molecular Signatures Database (MSigDB, http://software.broadinstitute.org/gsea/msigdb/index.jsp), including KEGG and Biological Processes gene sets, were downloaded for enrichment score calculation. To identify tumor-specific biological pathways at different stages, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed. These analyses were primarily conducted using the clusterProfiler package (v4.10.1) [14].

CytoTRACE

CytoTRACE offers an innovative approach for measuring cell differentiation potential, significantly enhancing the prediction of cell differentiation at the single-cell level [15]. After computing the gene expression matrix, the CytoTRACE algorithm provides a stemness score for each malignant epithelial cell. A higher CytoTRACE score indicates greater stemness (i.e., lower differentiation) in malignant epithelial cells.

Pseudo-time trajectories analysis

Pseudo-time trajectories of tumor cells at different stages were determined using Monocle v.2 [16]. Initially, the raw counts were converted from a Seurat object to a CellDataSet object using the importCDS function in Monocle. The differentialGeneTest function from the Monocle 2 package was used to select and rank genes (qval < 0.01) that are likely to provide information on cell ordering along the pseudo-time trajectory. Dimensionality reduction and cell ordering were performed using the DDRTree method and the orderCells function. Gene expression changes along pseudo-time were visualized using the plot_genes_in_pseudo_time function.

Data acquisition

Prostate cancer patients of RNA - seq data and clinical information from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/projects/TCGA-PRAD) to download. The data included 497 cancer cases and 52 para-cancer samples. The per million transcript (TPM) data is converted from the gene count data based on the effective length of the gene, and they are normalized by logarithmic scale transformation to ensure normalization. RNA - seq data and scRNA for prostate cancer - seq data are from Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo) to download. The study included three public datasets: GSE240056 (n = 16) [17], GSE175975 (n = 30) [18], and GSE166184 (n = 6) [19]. The selected gene sets in this study (GO, KEGG, GSVA-Hallmark) are available on MsigDB.

Clinical features and survival prognosis

In order to explore the expression of SOX2 and CCNE2 in the TCGA-PRAD dataset, we used the "limma" R package to conduct differential analysis of para-cancer tissue and tumor tissue. We analyzed the clinical features collected in the TCGA-PRAD dataset for clinical correlation with SOX2 expression and CCNE2 expression. Kaplan-Meier curves and Time-dependent receiver operating characteristic (ROC) were used to evaluate the performance of SOX2 and CCNE2 in predicting PFI [20, 21].

Biological function enrichment analysis

We used the "limma" R package to screen differential genes (DEGs) for the transcriptome datasets, respectively, with the criteria $P < 0.05$ and $|log2FC| > 1$. To further explore the potential role of SOX2 and CCNE2, We used the "ClusterProfiler" R package to do kyoto encyclopedia of genes and genomes (KEGG) analysis and gene set enrichment on the transcriptome datasets respectively analysis (GSEA) [22, 23].

Transcription factor database screening and COX regression analysis

The "TFTF" R package is used to obtain the downstream target genes associated with SOX2 by predict_target function in the five databases of hTFtarget, KnockTF, FIMO_JASPAR, TCGA and GTEx. Among them, TCGA is defined in TCGA-PRAD dataset, while GTEx is defined in Prostate organization with $P < 0.05$ as the screening condition to obtain prostate target genes [24].

Using the online website STRING (https://cn.string-db.org/), the protein interaction network diagram of SOX2 was constructed to obtain the proteins associated with SOX2. The survival and survminer R packages were used to conduct multivariate COX regression analysis for these related protein genes to further screen out candidate target genes. Pearson correlation analysis was used to investigate the correlation between SOX2 and candidate target genes.

Statistic analysis

All statistical analyses were performed using R software (v.4.1.0). Student's T-test or Wilcoxon test were used to analyze the differences between the two groups. The Kruskal-Wallis test was used for comparison among multiple groups. Survival curves were described by Kaplan-Meier plots and compared with log-rank tests. $P < 0.05$ was considered statistically significant.

Results

Activation of SOX2 expression may lead to macrometastasis in the advanced stage of the prostate cancer

To comprehend prostate cancer's activity status at various stages of advancement, sc-RNA-seq data (GSE240056) were used to conduct gene heatmaps in the early and late stages of prostate cancer respectively to analyze the expression of genes related to dormancy and proliferation (**Figure 1A**) [25, 26]. We found that proliferation-related genes such as MCM family and CDC family genes showed a tendency to be lower in the early stage and higher in the late stage with tumor progression in this dataset. On the contrary, dormancy related genes such as LIF, Wnt5a, and cell cycle capture related genes such as GADD45a showed a trend of high early and low late. These trends are consistent with advanced prostate cancer progression, reflecting the early dormancy of tumor cells and more active proliferative behavior in later stages.

CSCs have a powerful tumor initiation ability, which is conducive to the early dissemination of single DTC and the maintenance of continuous tumor growth [27, 28]. In order to further explore the proliferation or dormancy of prostate cancer cells and whether it is affected by the stemness of tumor cells themselves, we selected some transcription factors (SOX2 and SOX9) representing stem-like characteristics for analysis, which are believed to play an important role in the proliferation, migration, castration resistance and other progression of prostate cancer [9, 29, 30]. We found that SOX9 expression remained constant but declined as the tumour grew. However, SOX2 continued to increase, contrary to the pattern of dormance-related gene expression. In order to investigate the relationship between the expression of SOX2 and SOX9 and the activation of tumor cell dormancy, we constructed a mouse bone metastasis model using intracar-

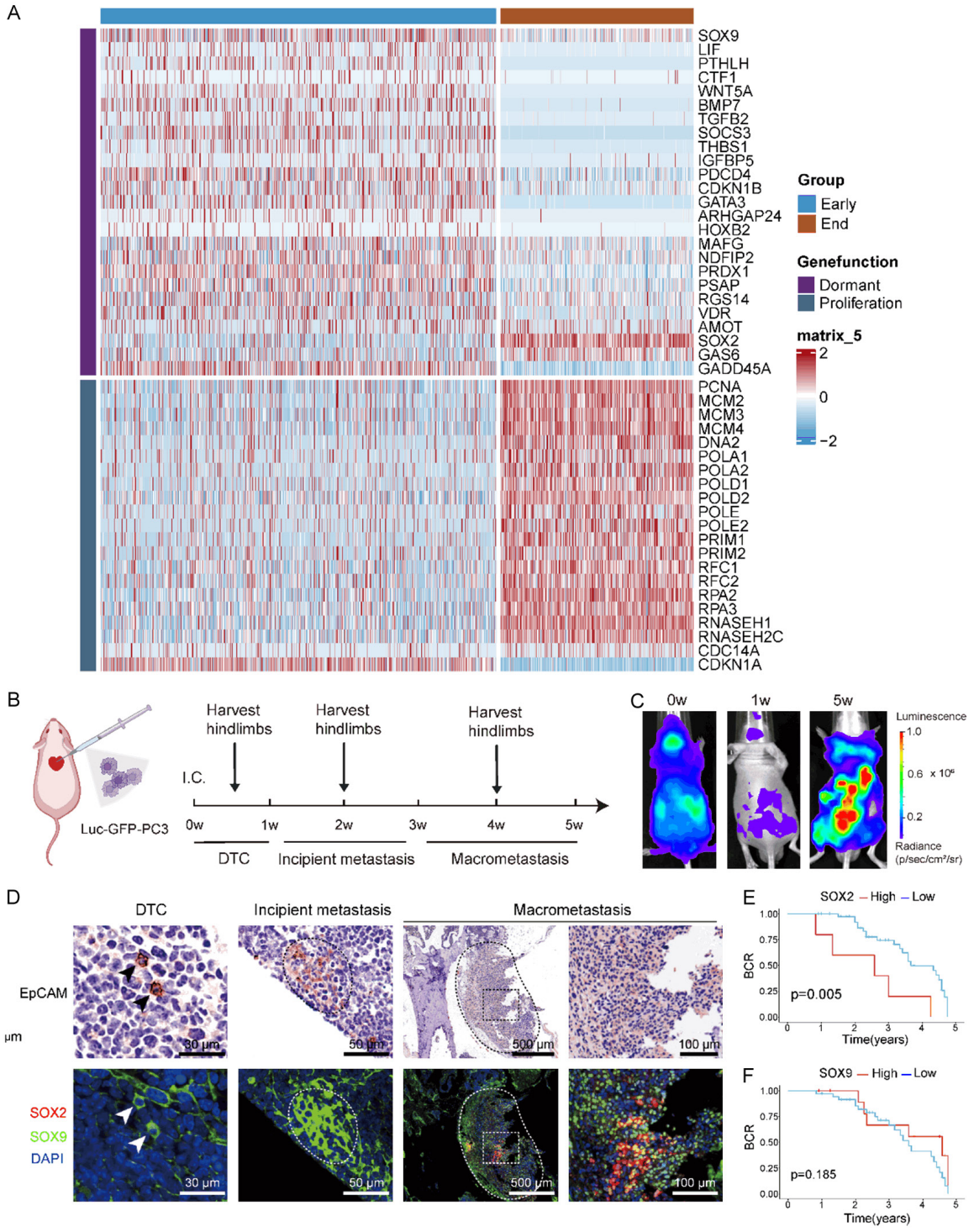


Figure 1. Expression trend of SOX2 in different stages of prostate cancer progression. A. sc-RNA-seq data (GSE240056) was used to draw gene heatmap to show the expression of genes related to dormancy and proliferation in mice at different stages. B. Construction of prostate cancer bone metastasis model and sample collection process. C. Whole-body fluorescence imaging in mice after intracardiac injection at different stages. D. Tibia was dissected at different stages after tumor inoculation for immunohistochemical and immunofluorescence analysis of SOX2 and SOX9 expression. E. 5-year KM curves for SOX2-high and SOX2-low groups in the GSE116918 dataset. F. 5-year KM curves of SOX9-high and SOX9-low groups in the GSE116918 dataset.

dial injection, and collected bone metastasis samples at different stages after inoculation to further verify this phenomenon (**Figure 1B**). On the day of the intracardiac injection, most of the circulating tumor cells had not been colonized and were in the DTC stage. A small number of DTCs colonized and formed micrometastases at about 1 week, and SOX2 expression was hardly observed in bone metastases at this time. At week 5, macrometastasis was observed in nude mice by in vivo imaging, and SOX2 expression was significantly elevated in bone metastases. SOX9 is consistently expressed throughout prostate cancer (**Figure 1C** and **1D**). In addition, we verified the 5-year survival prognosis of prostate cancer patients with SOX2 and SOX9 using the GSE116918 dataset, and found that only SOX2 was associated with the survival prognosis of prostate cancer (**Figure 1E** and **1F**). In summary, since SOX2 is closely related to tumor biochemical recurrence and macrometastasis, we speculate that the dormancy detachment and the formation of macrometastasis in late tumors are related to the expression and activation of SOX2.

SOX2 may be involved in prostate cancer dormancy activation through HEDGEHOG signaling pathways

To investigate the impact of SOX2 changes on prostate cancer phenotype, transcriptomic data of overexpression SOX2 (SOX2-OE) and knockdown SOX2 (SOX2-KD) in prostate cancer cell lines were selected for analysis. We found that SOX2 had significant effects on prostate cancer cell proliferation, cell cycle, invasion, apoptosis, inflammatory response, hypoxia adaptation, androgen signaling response, EMT, angiogenesis and other phenotypes (**Figure 2A**). Further, we use GSEA analysis to find pathways that are significantly up-regulated in SOX2-OE (GSE175975) and down-regulated in SOX2-KD (GSE166184): HEDGEHOG SIGNALING, PATHWAYS IN CANCER, EPITHELIAL MESENCHYMAL TRANSITION, TNFA SIGNALING VIA NFKB (**Figure 2B**). The up-regulation of these core pathways is associated with increased tumor aggressiveness [31-33]. Previous studies have reported that SOX2 plays a crucial role in tumorigenic progression and metastasis of prostate cancer, as well as for prostate cancer stem cells through HEDGEHOG SIGNALING [8, 34]. It also promotes epithelial-mesenchymal transformation (EMT) and stimulates castration resistance by reducing the dependence of prostate cancer cells on androgen receptor signaling [35, 36]. By showing some genes of these core pathways, we found that some genes related to tumor proliferation, invasion, and cell cycle were significantly up-regulated (**Figure 2C**). These results suggest that SOX2 is closely associated with tumor proliferation and invasion, and may be involved in tumor dormancy activation through HEDGEHOG SIGNALING and other pathways.

CCNE2 may be a potential downstream target gene for SOX2 transcription factors

As a key transcription factor regulating stem cell pluripotency and self-renewal, the regulation of SOX2's mRNA and protein occurs precisely at transcriptional, post-transcriptional and post-translational levels, influencing the promoter of a large number of other genes [37, 38]. To find the downstream target genes that SOX2 may act on, we used multiple transcription factor databases such as KnockTF, hTFtarget, JASPAR, TCGA, and GTEx to identify 375 downstream target genes of SOX2 through Wayne analysis (**Figure 3A**). Thirteen target genes were identified by intersecting 375 target genes with four core pathway-related genes (**Figure 3B**). Analysis of the protein interaction network revealed that the genes MAPK8, NFKBIA, EP300, MAX, RB1, CCNE2, and CDK6 have a strong correlation with SOX2, all of which are downstream of SOX2 (**Figure 3C**). Further, univariate COX analysis was conducted on these 13 target genes, and COX regression forest maps showed that CCNE2 and MAX were significantly correlated with survival prognosis, identifying them as two key genes (**Figure 3D**). In the correlation analysis, SOX2 was indicated to be co-expressed with the target gene CCNE2 across multiple prostate cancer data sets, while SOX2 and MAX were only correlated in the TCGA database (**Figure 3E** and **3F**). As a result, we chose CCNE2 as the target gene for our downstream study instead of MAX.

CCNE2 and SOX2 share a similar differentiation fate in prostate cancer progression

To enhance our understanding of how SOX2 and CCNE2 are expressed and function during different stages of prostate cancer progres-

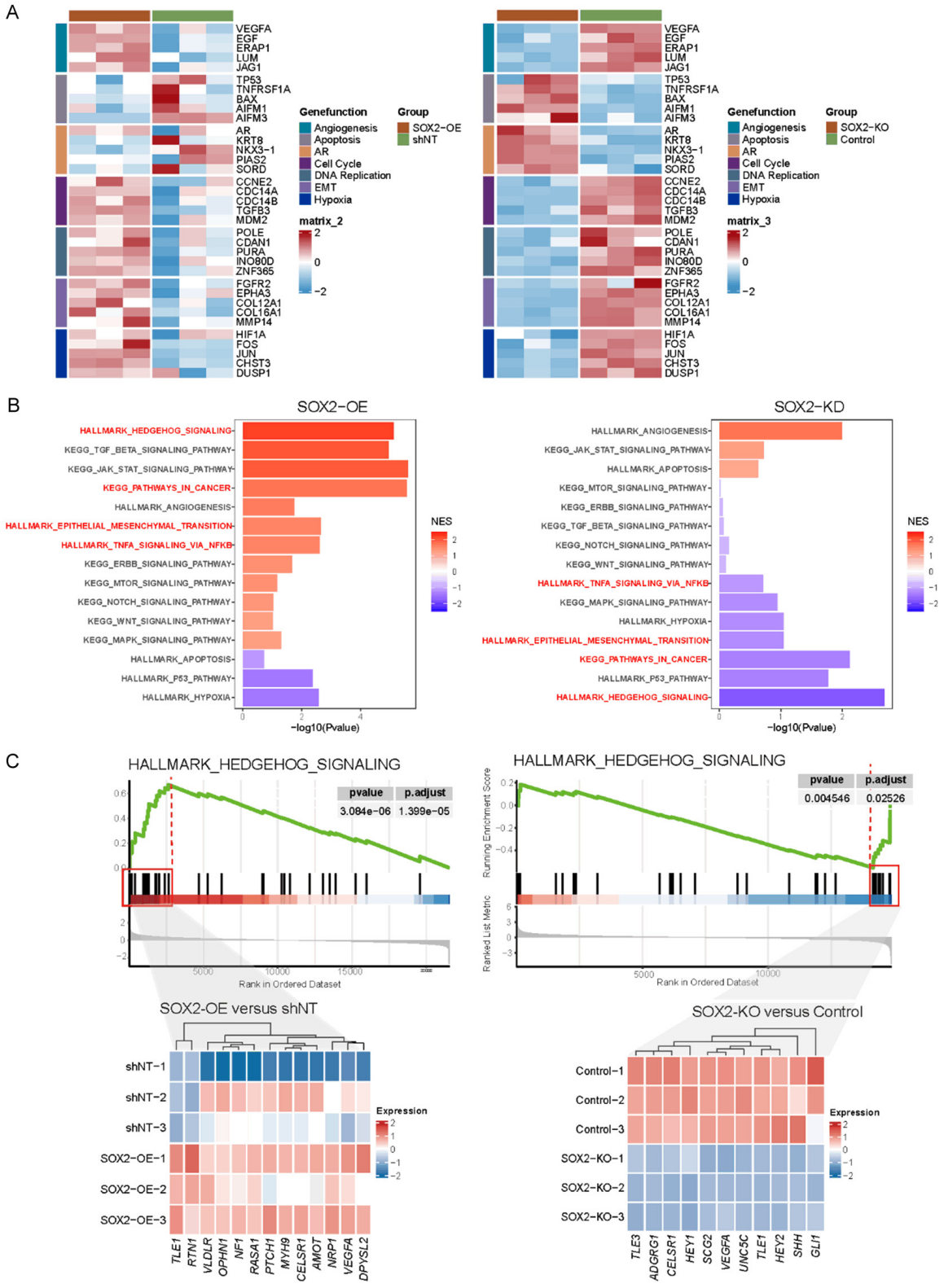
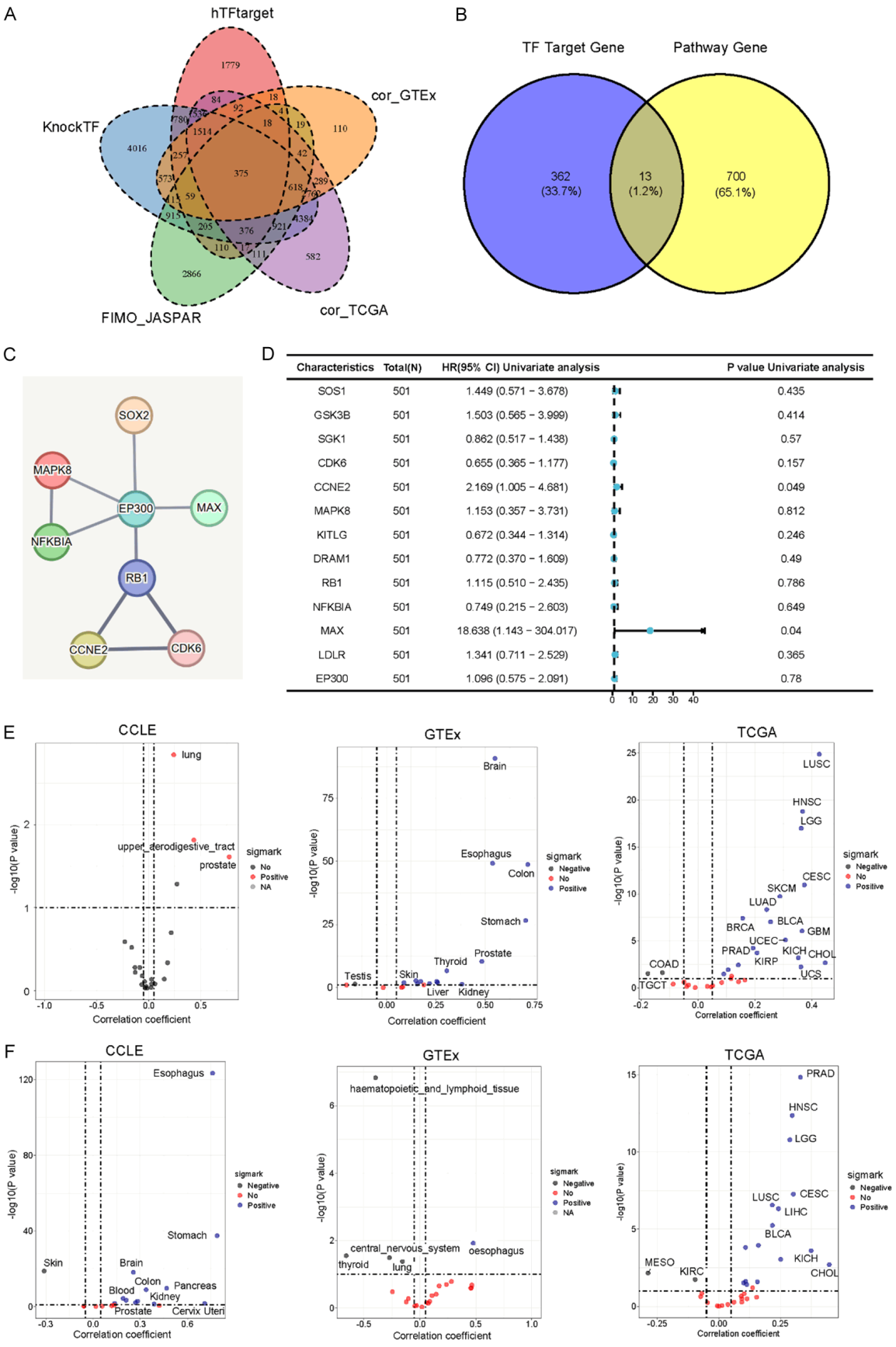


Figure 2. SOX2-related signaling pathways in prostate cancer. A. Effects of SOX2 changes on multiple phenotypes of prostate cancer. B. GSEA enrichment analysis of pathway changes after SOX2 changes. C. Some core pathways, such as HEDGEHOG SIGNALING HALLMAR pathway, were used for GSEA analysis and core gene display.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KnockTF
cor_GTEx
FIMO_JASPAR
cor_TCGA
B
TF Target Gene
Pathway Gene
362 (33.7%)
13 (1.2%)
700 (65.1%)
C
SOX2
MAPK8
EP300
MAX
NFKBIA
RB1
CCNE2
CDK6
D
Characteristics Total(N) HR(95% CI) Univariate analysis P value Univariate analysis
SOS1 501 1.449 (0.571 - 3.678) 0.435
GSK3B 501 1.503 (0.565 - 3.999) 0.414
SGK1 501 0.862 (0.517 - 1.438) 0.57
CDK6 501 0.655 (0.365 - 1.177) 0.157
CCNE2 501 2.169 (1.005 - 4.681) 0.049
MAPK8 501 1.153 (0.357 - 3.731) 0.812
KITLG 501 0.672 (0.344 - 1.314) 0.246
DRAM1 501 0.772 (0.370 - 1.609) 0.49
RB1 501 1.115 (0.510 - 2.435) 0.786
NFKBIA 501 0.749 (0.215 - 2.603) 0.649
MAX 501 18.638 (1.143 - 304.017) 0.04
LDLR 501 1.341 (0.711 - 2.529) 0.365
EP300 501 1.096 (0.575 - 2.091) 0.78
0 10 20 30 40
E
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lung
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Liver
Kidney
LUSC
HNSC
LGG
CESC
SKCM
LUAD
BRCA
BLCA
GBM
UCEC
PRAD
KIRP
KICH
CHOL
COAD
TGCT
UCS
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Esophagus
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HNSC
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CESC
LIHC
BLCA
KICH
CHOL
MESO
KIRC

Figure 3. Screening of downstream target genes of SOX2 transcription factors. A. Venn diagram showing the downstream genes of transcription factors. B. Venn diagram illustrating the target genes of transcription factors and core pathway genes. C. COX forest plot demonstrating the intersection of target genes and core pathway genes. D. Protein interaction network analysis of 13 overlapping genes. E. Correlation analysis of SOX2 and CCNE2 among cell lines of CCLE, GTEx tissues and cancer species of TCGA. F. Correlation analysis between SOX2 and MAX across CCLE cell lines, GTEx tissues and TCGA cancer types.

sion, we used sc-RNA data (GSE240056) to perform GO and KEGG enrichment analysis for different stages of prostate cancer. As the tumor progresses, pathways associated with proliferation, such as the cell cycle and DNA replication, are significantly enriched during the transition and end phases (**Figure 4A** and **4B**). Given that CCNE2-encoded Cyclin plays an important role in promoting cell cycle G1/S transition [39], we hypothesized that it may similarly influence prostate cancer. We demonstrated the cell cycle of prostate cancer in mice at various stages through CellCycleScoring (**Figure 4C**) to observe the proliferation potential of cancer cells at different stages. Given that cells with high proliferative capacity shorten the G1 phase in order to enter the S phase for DNA replication more quickly, advanced prostate cancer cells have a stronger proliferation potential [40]. Correspondingly, CCNE2 expression was low in the early stage and increased with tumor progression (**Figure 4D**), which is consistent with our speculation. Further, we observed the expression of SOX2 and CCNE2 in prostate mouse models at different stages by quasi-time series analysis, and found that they had a similar elevated trajectory at the end of the tumor (**Figure 4E** and **4F**). Interestingly, according to cytotrace scoring of epithelial cells' dryness and differentiation potential, we found that advanced epithelial cells had the highest score and the strongest dryness and differentiation potential, which is contrary to normal malignant epithelial differentiation (**Figure 4G**). This may imply that dormant tumor epithelial cells activate and awaken the malignant potential of cell differentiation with the rise of SOX2 during tumor progression.

CCNE2 increase due to high SOX2 levels and predicts a poor prostate cancer prognosis

To clarify whether CCNE2 is affected by the regulation of SOX2, we conducted difference analysis and GSEA analysis on SOx2-OE and SOX2-KD data sets respectively. Our findings revealed that CCNE2 was highly expressed in the SOX2-OE datasets, whereas it showed the opposite trend in the SOX2-KD datasets. This indicated that the expression of CCNE2 was positively correlated with the expression of SOX2. In addition, in GSEA analysis, we found that CCNE2-related cell cycle pathways had similar changes in expression to CCNE2. All these suggest that changes in SOX2 expression do affect downstream CCNE2 expression (**Figure 5A**). In addition, we verified the relationship between CCNE2 and the clinical features and prognosis of prostate cancer in the TCGA-PRAD dataset. We found that high expression of CCNE2 predicted higher TNM staging and worse survival outcomes (**Figure 5B-F**). Together, these results suggest that the high expression of SOX2 in advanced prostate cancer may lead to the elevation of CCNE2, which promotes the cell cycle transition to G1/S, and indirectly leads to the activation of dormant cancer cells.

Discussion

Prostate cancer is the second most common cancer in men, and most prostate cancer recurrences are initially just elevated PSA or biochemical recurrence (BCR) without significant lower urinary tract symptoms and bone pain symptoms. Although early ADT can effectively control tumor progression, drug resistance and castration resistance in the late stage tend to lead to relapse, which is usually accompanied by bone metastasis and even leads to death [26]. It is worth mentioning that evidence of DTCs has been found in the bone marrow of patients with clinically localized prostate cancer, suggesting that the control of prostate cancer does not mean the complete elimination of cancer cells, rather, some cancer cells may have entered a dormant state [26]. Typically, these dormant cancer cells are dynamically controlled in the ecological niche, and only in the appropriate environment can the tumor cells undergo selective dormant activation. The bone marrow microenvironment is a favorable environment for dormancy reactivation, in which the increase of osteolytic factors such as

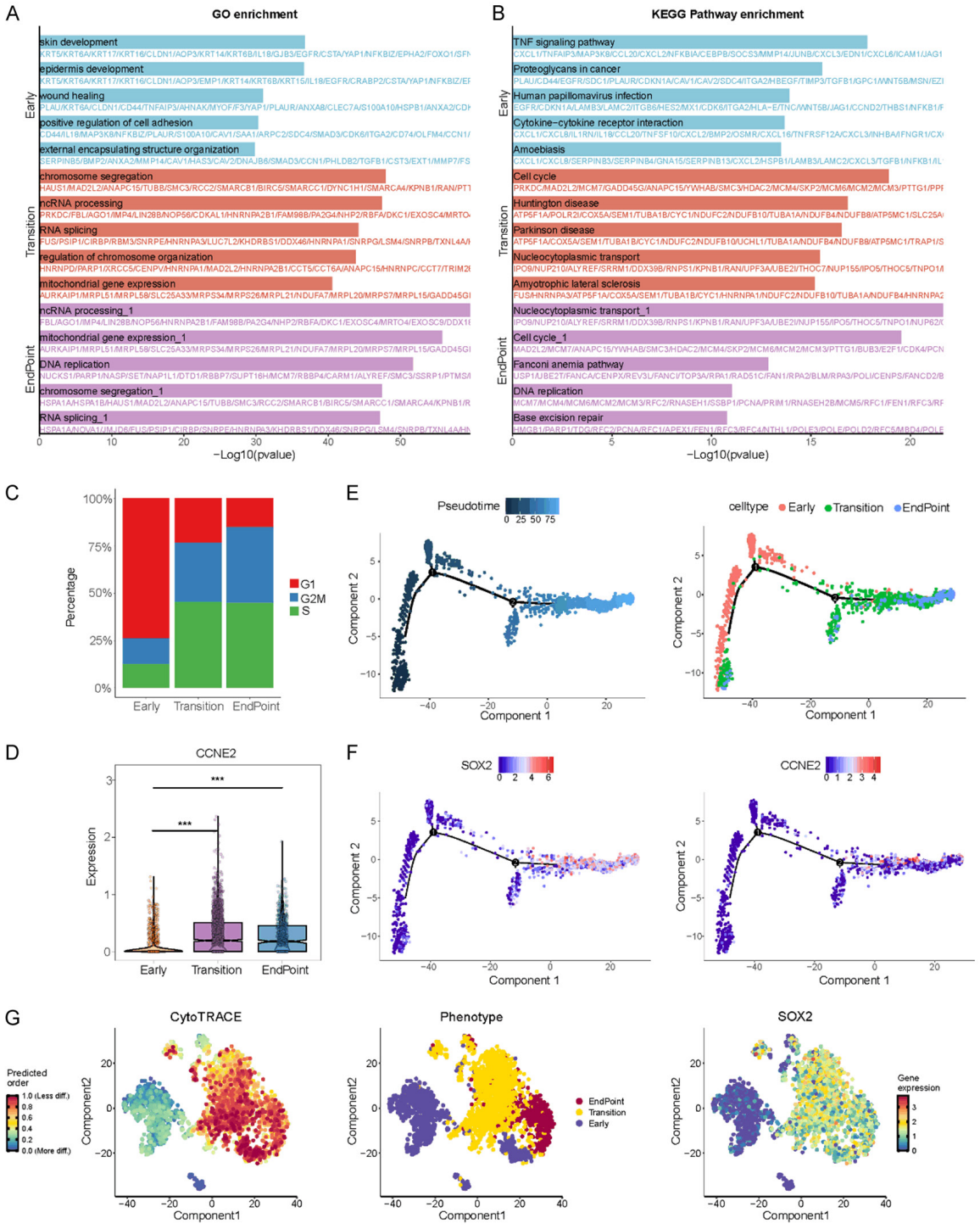

Figure 4. Expression and function of SOX2 and CCNE2 in the progression of prostate cancer. A. GO enrichment analysis at different stages of prostate cancer progression. B. Enrichment analysis of KEGG pathway in different stages of prostate cancer progression. C. Distribution of cells in the cell cycle stage at different stages of prostate cancer progression. D. Expression levels of CCNE2 in prostate cancer at different stages. E. Monocle2 pseudo-time series shows the dynamic changes of epithelial cell progression in prostate cancer. F. Pseudochronogram reveals changes in the expression of SOX2 and CCNE2 during the progression of prostate cancer epithelial cells. G. CytoTRACE reveals the differentiation and malignant potential of prostate cancer epithelial cells at different stages.

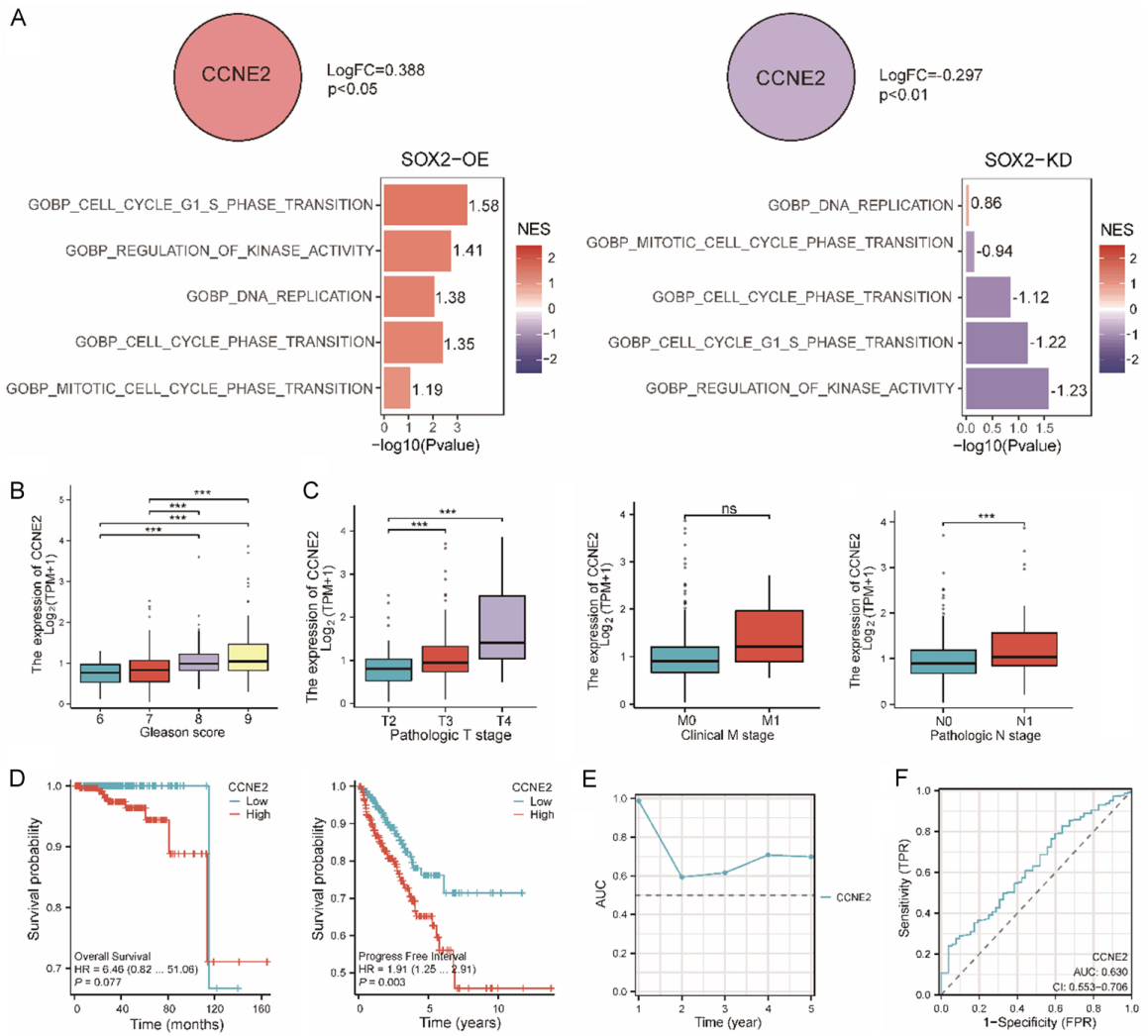

Figure 5. Relationship between CCNE2 expression and clinical features of prostate cancer. A. Changes in CCNE2 and the corresponding path when SOX2 changes. B. Relationship between CCNE2 and Gleason score. C. Relationship between CCNE2 and TMN staging. D. The relationship between CCNE2 and OS and PFI in prostate cancer. E. Trends in 1-5-year survival and progression-free survival predicted by CCNE2 in prostate cancer patients. F. The time of CCNE2 in prostate cancer at years 1, 2, 3, 4 and 5 depends on ROC and ROC at year 5.

nuclear factor κB (NF-κB) ligand receptor activator (RANKL) and parathyroid hormone-related protein (PTHRP) will lead to dormancy activation, and eventually lead to tumor recurrence and metastasis [3].

The stemness of CSCs to self-renew gives these cells the ability to coordinate a range of carcinogenic processes, including metastasis and treatment resistance, and researchers have taken a keen interest in them. CSCs share many similarities with dormant tumor cells [41]. First, CSCs are better adapted to a variety of extreme environments in vivo with strong autonomic adaptability and autonomic invasion. In addition, CSCs can remain dormant for a long time and are insensitive to most of the physicochemical factors that kill tumor cells. The proportion of CSCs in primary tumors is less than 10%, while in DTCs, the proportion of CSCs is as high as 72% [42]. For CSCs, the abnormal expression of pluripotent inducible transcription regulators synergistically promotes the further proliferation and deterioration of cancer cells with carcinogenic signaling pathways. Therefore, it is a promising direction to explore the tumor dormancy mechanism by starting with pluripotent inducible transcriptional regulators. Dormant DTCs increase the post-translational modification of histone H3 and increase

the activity of transcription factors such as SOX2, SOX9, NANOG and NR2F1 [26]. In particular, SOX2, as an important pluripotent inducing transcription factor, is worth further exploration for its clinical value in the progression of prostate cancer.

In order to understand the role of these pluripotent inducible transcription factors at different stages, we attempted to explore this process by constructing a mouse model of bone metastasis and bioanalysis. In this study, we found that SOX2 increases with the progression of prostate cancer and speculated that it may promote the cell cycle shift to G1/S through downstream CCNE2, which could activate dormant cancer cells. In addition, we found some signaling pathways that are closely related to SOX2, Such as HEDGEHOG SIGNALING, PATHWAYS IN CANCER, EPITHELIAL MESENCHYMAL TRANSITION, TNFA SIGNALING VIA NFKB. In particular, SOX2-HEDGEHOG SIGNALING, similar to previous studies, shows that the SOX2-HH axis is critical for the maintenance, survival, and development of prostate cancer [8]. Identifying these pathways and associated genetic alterations in the future could help us understand the underlying molecular mechanisms driving tumor dormancy activation, which could help guide the selection of targeted therapies or treatment strategies.

Due to limited space and experimental conditions, we did not further explore the relationship between SOX2 and prostate cancer. Both SOX2 and SOX9 have been found to play an important role in prostate cancer. Clinically advanced prostate tumors are often accompanied by the overexpression of SOX2 and SOX9 and predict the more aggressive and neurological features of prostate cancer [30]. Interestingly, Joan Massague used LCC to study the expression of SOX2 and SOX9 in breast cancer and lung adenocarcinoma, and found that the expression trend of the two was consistent in lung cancer, while the opposite trend was similar to that of prostate cancer in breast cancer [9]. Since prostate cancer and breast cancer are both hormone-dependent cancers, we hypothesized that it may be due to the influence of hormones that lead to the formation of a unique expression pattern of SOX2 and SOX9, which affects the potential of CSCss and slow-cycling state of tumor cells. In addition, there are still some problems to be solved: there is a lack of sufficient molecular and animal experiments to further verify the specific relationship between SOX2 and CCNE2; There are many factors affecting dormancy activation in the tumor microenvironment. Whether crosstalk between these factors will interfere with the relationship between SOX2 and CCNE2 needs further investigation. In the method and use of data analysis, the existing database is limited, lack of certain reliability, universality and credibility. More importantly, after clarifying these relationships, whether a key method can be found to effectively control the SOX2-CCNE2 clue, so as to achieve a targeted strategy to maintain tumor cell dormancy.

Conclusion

In conclusion, this study suggests that SOX2 may increase the risk of prostate cancer progression and metastasis by modulating downstream CCNE2 expression and lead to poorer survival outcomes in prostate cancer. These genes could serve as potential therapeutic targets to prevent progression and metastasis in prostate cancer patients.

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Disclosure of conflict of interest

None.

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