Original Article

Comprehensive analysis of the functional and prognostic value of E2F transcription factors in human prostate cancer through data mining and experimental validation

Decai Wang[1#], Wensen Tang[1#], Pingbao Zhang[2#], Zijian Liu[3], Fang Lyu[1], Yajun Xiao[1], Dong Ni[1], Pu Zhang[1]

[1]Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [2]Department of Urology, Zhongshan Hospital, Fudan University, Shanghai, China; [3]Department of Head and Neck Oncology and Department of Radiation Oncology, Cancer Center and State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, China

Contributions: (I) Conception and design: P Zhang; (II) Administrative support: D Ni; (III) Provision of study of patients: D Wang, W Tang, F Lyu; (IV) Collection and assembly of data: D Wang, W Tang, P Zhang, Z Liu; (V) Data analysis and interpretation: D Wang, W Tang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Dong Ni; Pu Zhang. Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Avenue, Wuhan 430022, China. Email: dni@hust.edu.cn; d202081801@hust.edu.cn.

Background: A growing body of evidence shows that E2F transcription factors play a significant role in the tumorigenesis of prostate cancer. However, their functional and prognostic value has not been fully illustrated. Therefore, we used bioinformatics methods to further analyze the possible roles of E2F transcription factors in the development and progression of prostate cancer.

Methods: We explored the expression levels of E2F transcription factors using data from The Cancer Genome Atlas (TCGA) and Oncomine database in paired and unpaired samples. The clinical correlation and prognostic value of E2F transcription factors were assessed. Using the R package "pROC", we judged the diagnostic value of E2F transcription factors. The online website tool cBioPortal was also employed to find possible gene alterations of E2F transcription factors in samples from TCGA. The R package "clusterprofiler" was used to conduct functional analysis. Moreover, we also used the Tumor Immune Estimation Resource to search for the associations between E2F transcription factors and the infiltration levels of 6 kinds of immune cells. Finally, quantitative real-time polymerase chain reaction (PCR) was conducted to validate the expression levels of E2F transcription factors in human paired prostate tissues.

Results: E2F1/2/3/5 messenger RNA (mRNA) expression levels were higher in prostate cancer tissues than in normal tissues, while E2F4 and E2F6 mRNA expression levels were lower ($P<0.05$). All E2F transcription factors were associated with clinical parameters. Kaplan-Meier analysis revealed that E2F1/4/6/8 were notably associated with the overall survival of patients with prostate cancer ($P<0.05$). Receiver operating characteristic (ROC) curve results showed that except for E2F7, the other E2F transcription factors had diagnostic value for prostate cancer ($P<0.05$). We further found close associations between E2F transcription factors and the infiltration levels of immune cells. The results of quantitative real-time PCR were consistent with those from public databases.

Conclusions: E2F transcription factor family members are differentially expressed in prostate cancer and are significantly related to the prognosis of patients, suggesting that they may be adopted as biomarkers for prognosis prediction and the treatment of prostate cancer.

Keywords: E2F transcription factors; prostate cancer; bioinformatics analysis; prognosis

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Introduction

As one of the most common male malignancies in the United States, prostate cancer (PCa) is also the second leading cause of male-related cancer death (1). In recent years, the rate of PCa in Chinese men has gradually increased, and the age of onset has gradually become younger, which poses a serious threat to the health of Chinese men (2). Studies have shown that about 30% of men over 65 years are diagnosed with PCa, and many patients are diagnosed in the middle and late stages (3). Although the prognosis of most PCa patients is relatively good, the prognosis of PCa patients who relapse or metastasize after treatment is poor. Various biomarkers have been reported to be used for the monitoring of prognosis and predicting the recurrence of PCa, including preoperative prostate-specific antigen (PSA) level, Gleason scores, and lymph node invasion, among others. However, these markers are not cancer-specific and accurate, and it is difficult to make personalized postoperative follow-up plans based on them (4). Ultimately, the optimal window of time to control the disease passes, and recurrence and metastasis of PCa occur, thereby reducing the overall survival (OS) rate of patients. Therefore, the screening of markers related to PCa may contribute to correct clinical decision-making and improve the prognosis of patients with PCa.

The *E2F* gene was discovered by Kovesdi *et al.* (5) when they were studying the interaction between the nuclear extracts of adenovirus-infected cells and the E2 promoter of adenovirus, a new type of gene family that transcriptionally encodes cytokines. As a family of transcription factor proteins, *E2Fs* can regulate cell differentiation, cell cycle, apoptosis, and DNA damage response by affecting downstream gene transcription (6-8). There are 8 members in the *E2F* gene family, namely *E2F1–E2F8*, among which *E2F3* includes *E2F3a* and *E2F3b*. Each *E2F* member has a certain degree of homology, and they constitute a complex transcriptional regulatory network in the cell. According to the molecular structure and transcription characteristics of *E2Fs*, they can be divided into 2 groups: transcriptional activators and transcriptional repressors. Among them, *E2F1/2/3a* are described as transcriptional activators, while *E2F3b* and *E2F4-E2F8* are described as transcriptional repressors. Current studies have found that members of the *E2F* transcription factor family can affect the progression of PCa (9-12). However, there are few reports on the expression of *E2Fs* and their prognostic significance in PCa.

This study comprehensively analyzes the expression and prognostic role of *E2F* transcription factor family members in PCa through public databases and experimental validation, so as to provide a theoretical basis for further research on their role in the diagnosis and treatment of PCa. We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-1532).

Methods

The Cancer Genome Atlas (TCGA)

We downloaded the level 3 HTseq-FPKM RNA sequencing (RNA-seq) data of PRAD from TCGA. The RNA-seq data in FPKM format was converted into TPM format, and log2 transformation was performed to compare the expression among samples. The R package "ggplot2" in R version 3.6.3 (The R Foundation for Statistical Computing, Vienna, Austria) was also employed to draw the boxplots and line plots. The statistical analysis was conducted using the Wilcoxon rank -um test and Wilcoxon signed rank test for unpaired and paired samples, respectively. A P value <0.05 was considered to be statistically significant.

Oncomine analysis

Oncomine is a cancer microarray database and integrated data mining platform designed to promote discovery from genomewide expression analysis (http://www.oncomine.org). Oncomine currently contains 65 gene expression datasets comprising nearly 48 million gene expression measurements for researchers to use. We compared the messenger RNA (mRNA) expression levels of *E2Fs* in various kinds of tumors with those in normal tissues through Oncomine. Statistical analysis was conducted by Student's *t*-test. The threshold of P values and fold change were 0.01 and 2, respectively.

The association of E2Fs with clinical parameters and the prognosis of patients

We also downloaded clinical and survival data from TCGA, consisting of 499 tumor samples to explore the association between the expression of the *E2F* family and clinical parameters, such as tumor stage, age, serum level of prostate-specific antigen (PSA), and the prognosis of patients. The R package "survminer" was used for data visualization while the R package "survival" was used for statistical analysis. The statistical analysis methods were the Kruskal-Wallis test and log-rank test. A P value <0.05 was

considered to be statistically significant.

Diagnostic ability

To judge the potential of *E2Fs* as diagnostic biomarkers between normal and tumor samples, we employed the R package "pROC" to generate receiver operating characteristic (ROC) curves with the data in TPM format from TCGA.

cBioPortal

cBioportal is an online website integrating data from 126 tumor genome studies, which includes large-scale cancer research projects, such as the International Cancer Genome Consortium (ICGC) and TCGA (http://www.cbioportal.org). The Prostate Adenocarcinoma dataset (TCGA, Firehose Legacy) containing data from 499 cases with pathology reports was identified for further analysis of *E2Fs* with cBioportal. We explored the gene alterations of *E2Fs* on the website.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

Through the newly developed website Gene Expression Profiling Interactive Analysis 2 (GEPIA2), which is used for analyzing the RNA-seq data from TCGA database, we found the top 20 similar genes of every gene in the *E2F* family in the PRAD dataset. We conducted GO and KEGG analysis with these genes using the R packages "clusterProfiler" and "org.Hs.eg.db". The threshold of an adjusted P<0.05 and *q* value <0.2 were considered to indicate statistical significance.

Tumor Immune Estimation Resource (TIMER)

TIMER is a powerful web server used to comprehensively explore the molecular characterization of tumor-immune interactions. This tool allows the users to interactively explore the relationships between gene expression and immune infiltrates. We determined the associations between the expression levels of *E2Fs* and immune infiltrates. A P value <0.05 was considered to be statistically significant.

Human specimens and real-time polymerase chain reaction

Human prostate samples were obtained from 23 patients undergoing radical prostatectomy. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and the protocol was approved by the Ethics Committee of Wuhan Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China. Total RNA was extracted from the tissues of 23 frozen prostate specimens using TRIzol reagent (Invitrogen, 15596026) according to the manufacturer's protocol. According to the manufacturer, a SYBR Green One-Step qRT-PCR Kit (Invitrogen, 11736059) was used to measure total RNA (100 ng).

Statistical analysis

The values of different groups are represented by the mean ± SD (standard deviation). A paired, two-sided Student's *t*-test was used to compare differences between two groups. Statistical significance was analyzed by SPSS 22.0 software. P<0.05 was considered statistically significant.

Results

The mRNA expression levels of E2Fs between normal and tumor tissues

We extracted the mRNA expression levels of *E2Fs* from the data downloaded from TCGA and the Oncomine database. For the unpaired tissues from TCGA, *E2F1*, *E2F2*, *E2F3*, and *E2F5* were up-regulated in the tumor tissues while *E2F4* and *E2F6* were down-regulated. Moreover, no significant difference was observed in the expression levels of *E2F7* and *E2F8* between normal and tumor tissues (*Figure 1A*). For paired tissues in TCGA, higher expression levels of *E2F1/2/3/5* were also observed in the tumor tissues, while higher expression levels of *E2F4* and *E2F6* were found in normal tissues. As for *E2F7* and *E2F8*, there was also no significant difference between normal and tumor samples (*Figure 1B*). The results from Oncomine were consistent with those from TCGA (*Figure 1C*).

The association between E2Fs and the clinical parameters of patients with prostate cancer

Using the clinical data from TCGA, we explored the relationships between E2Fs and clinical parameters. Except for those of *E2F4* and *E2F6*, we found that in terms of tumor stage, the expression levels of *E2Fs* increased with the progression of tumors and that a statistical significance was observed in various stages of patients with PCa (*Figure 2A*).

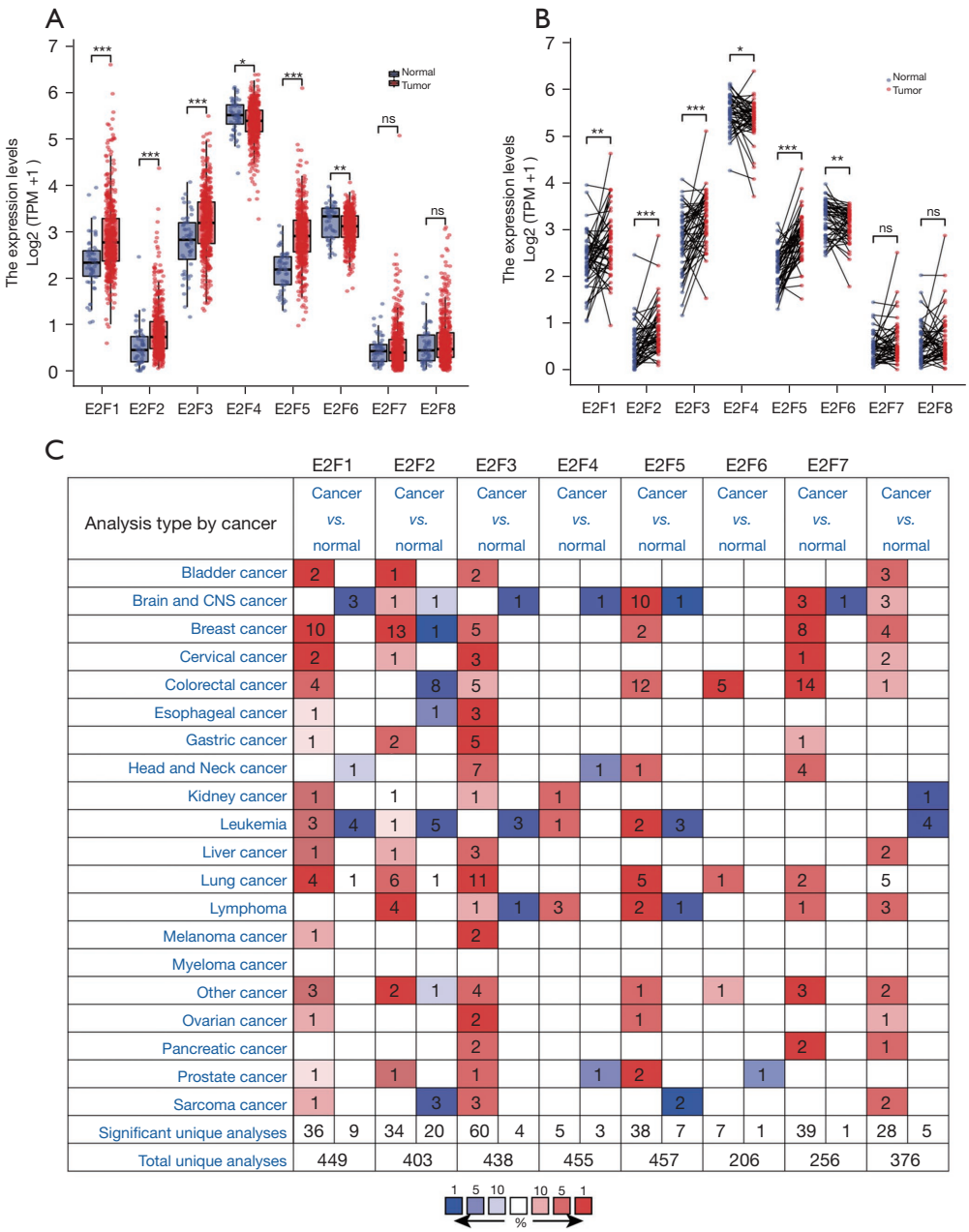


Figure 1 The expression levels of E2Fs in prostate cancer and normal tissues. (A) The expression levels of E2Fs in unpaired tissues; (B) the expression levels of E2Fs in paired tissues; (C) the transcription levels of E2F factors in different types of cancers. *P<0.05; **P<0.01; ***P<0.001. TPM, Transcripts Per Million; ns, not statistically significant.

In terms of N stage, we found that with the development of tumors, the expression levels of *E2Fs* increased, except for those of *E2F4* and *E2F6* (*Figure 2B*). We also found that the mRNA expression levels of *E2F1/2/7* were higher in patients over 60 years than in those under 60 years (*Figure 2C*). For patients with PSA >4, the expression levels of *E2F1-E2F5* and *E2F7-E2F8* were higher compared to those with PSA <4 (*Figure 2D*).

The prognostic value of E2Fs in patients with PCa

We further investigated the value of *E2Fs* in the OS of

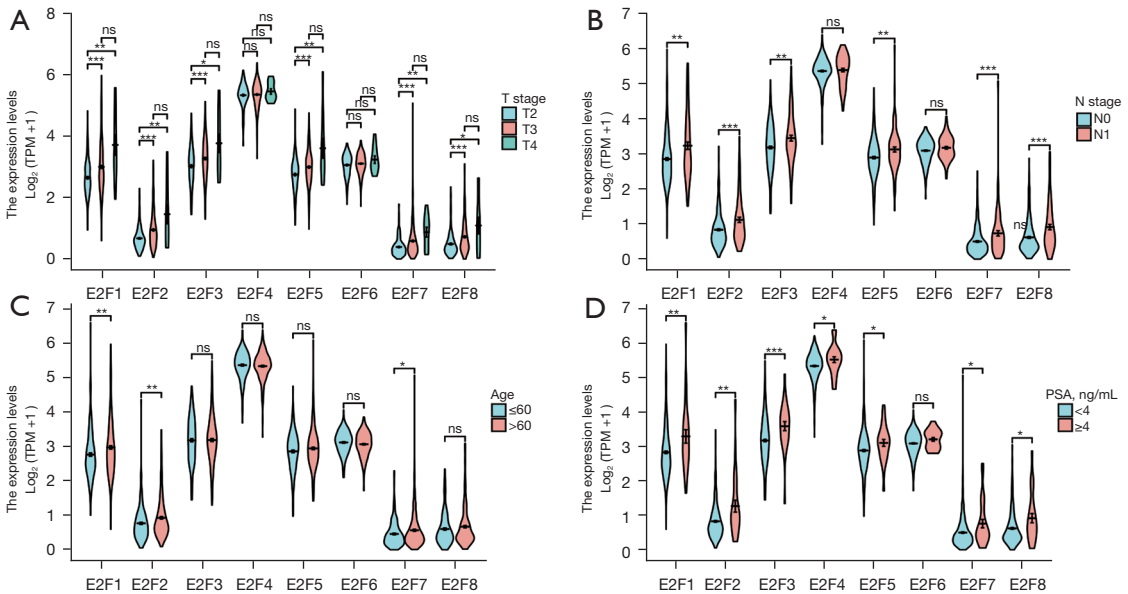


Figure 2 Correlation of E2F expression with tumor stage (A,B) age (C) and Prostate specific antigen (D) patients with PCa. PSA, prostate specific antigen; *P<0.05; **P<0.01; ***P<0.001. TPM, Transcripts Per Million; ns: not statistically significant.

patients with PCa using Kaplan-Meier analysis. The survival data were downloaded from TCGA and consisted of 499 patients. The Kaplan-Meier curves revealed that *E2F1/4/6/8* were markedly associated with the OS of patients with PCa, while others were not (P<0.05; *Figure 3*).

The diagnostic value of E2Fs in patients with PCa

The diagnostic value of *E2Fs* was investigated using the R package "pROC". The results showed that *E2F1* [area under the curve (AUC) =0.718], *E2F2* (AUC =0.710), *E2F3* (AUC =0.676), *E2F4* (AUC =0.603), *E2F5* (AUC =0.861), *E2F6* (AUC =0.612), and *E2F8* (AUC =0.550) were able to efficiently distinguish PCa tissues from normal prostate tissues (*Figure 4*), while *E2F7* (AUC =0.492) lacked this ability (*Figure 4A*). However, receiver operating characteristic curve results showed that the AUC of PSA is only 0.659 (*Figure 4B*).

The identification of gene alterations, coexpression, and neighbor gene network analysis

We analyzed the alterations and coexpression of *E2Fs* by using the cBioPortal online tool for PCa (TCGA, Firehose Legacy). We found that in all 491 samples with mRNA data, the *E2Fs* were altered in 174 samples (35%). *E2F5* was the most frequently altered gene, which was altered in about 15% of patients. The alteration types of these genes included truncating mutation, missense mutation, deep deletion, amplification, mRNA high, and mRNA low. The frequency of gene alterations are presented in *Figure 5A*. The correlations among *E2Fs* were also calculated through analyzing their mRNA expression in cBioPortal. Spearman's correlation coefficient and Pearson's correlation coefficient were both implemented to explore their relationships. The results showed the following positive and significant correlations between the *E2Fs*: *E2F1* with *E2F2*, *E2F7*, and *E2F8*; *E2F2* with *E2F7* and *E2F8*; *E2F3* with *E2F5*, *E2F7*, and *E2F8*; *E2F5* with *E2F8*; and *E2F7* with *E2F8* (*Figure 5B*). Subsequently, a gene-gene interaction network was constructed by using the online analysis tool GeneMANIA (*Figure 5C*). Using this tool, we also explored the functions of *E2Fs*. The functions of *E2Fs* were transcription initiation from RNA polymerase II promoter, core promoter binding, initiation, G1/S transition of mitotic cell cycle, DNA integrity checkpoint, DNA-templated transcription, signal transduction by *p53* class mediator, and regulatory region DNA binding. Moreover, we found 25 genes which were highly associated with *E2Fs* in physical interactions, colocalization, shared protein domains, pathway, prediction, and genetic interactions.

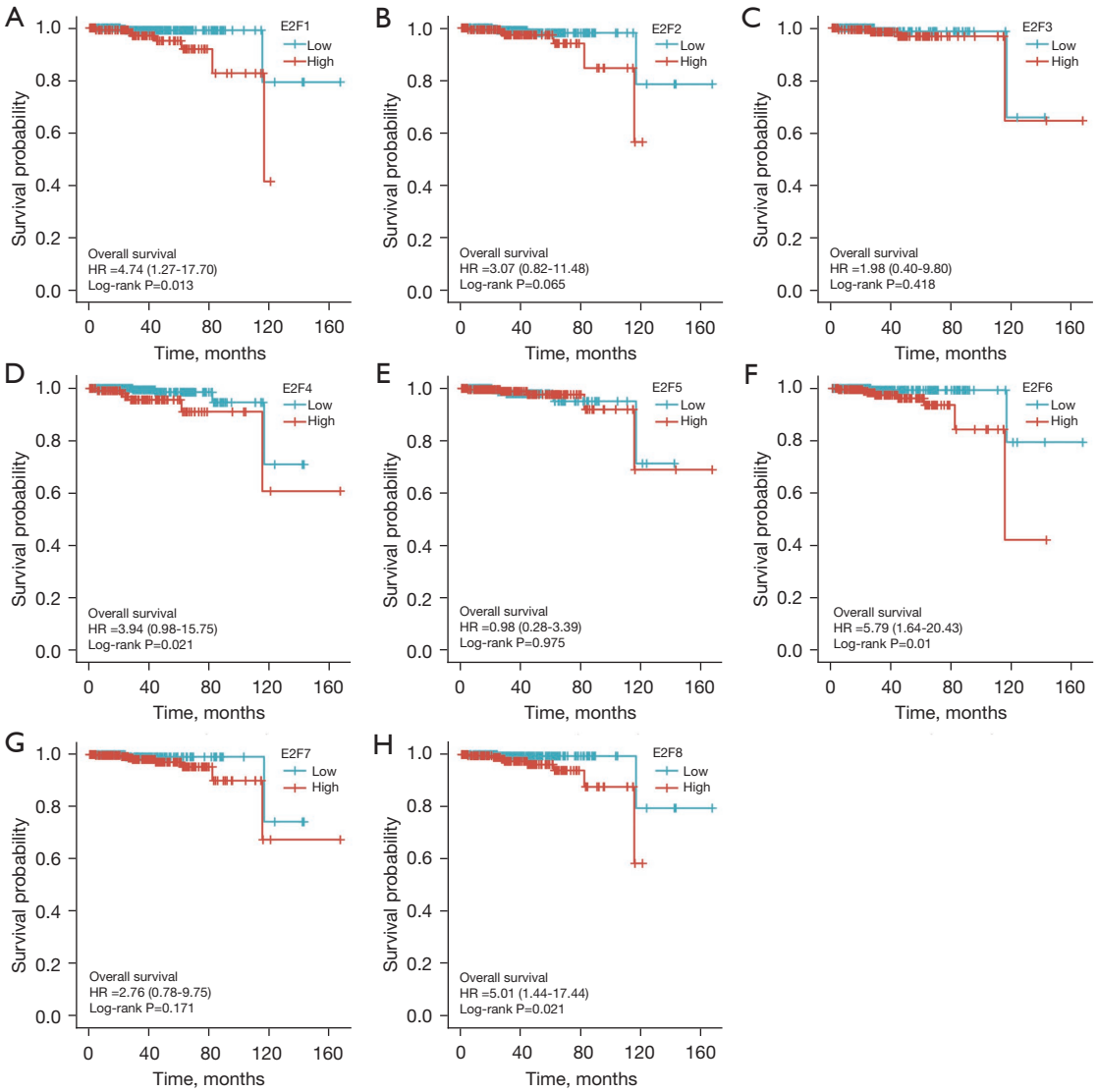


Figure 3 The prognostic value of E2F transcription factors in the patients with PCa. The Kaplan-Meier curves revealed that E2F1/4/6/8 (A,D,F,H) were markedly associated with the OS of patients with PCa, while E2F2/3/5/7 (B,C,E,G) were not (P<0.05). OS, overall survival; HR, hazard ratio.

Functional analysis of E2Fs and their related genes

Using the R package "clusterProfiler", we used GO and KEGG analysis to explore the possible mechanism underlying the PCa with *E2Fs* and their related genes (*Figure 6*). The results demonstrated that in terms of biological process, these genes were mostly enriched in G0 to G1 transition, negative regulation of mitotic cell cycle, and mitotic DNA damage checkpoint. In terms of cellular component, they were mostly enriched in RNA polymerase II general transcription initiation factor activity, nuclear transcription factor complex, and lateral element. In terms of molecular function, they were mostly enriched in DNA-binding transcription activator activity, RNA polymerase II-specific, transcription corepressor activity, and general transcription initiation factor activity. KEGG analysis showed that cellular senescence, cell cycle, transforming growth factor beta (TGF-β) signaling pathway, Epstein-Barr virus infection, PCa, and microRNAs in cancer were enriched.

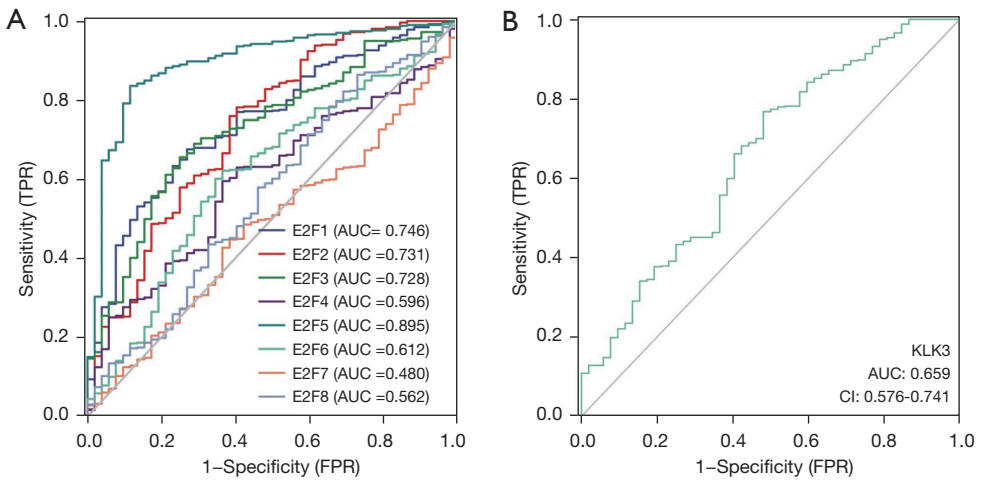


Figure 4 The ROC curves of E2F transcription factors (A) and PSA (B) in PCa. PSA, prostate specific antigen; KLK3, kallikrein related peptidase 3, it is the gene name of PSA.

The associations between E2Fs and immune cell infiltration

We used TIMER to estimate the relationships between the expression levels of *E2Fs* and immune cell infiltration in patients with PCa (*Figure 7*). We found that the expression level of *E2F1* was negatively associated with the infiltration levels of B cells, $CD8^+$ T cells, and neutrophils, and positively associated with $CD4^+$ T cells, T regulatory cells, and macrophages. *E2F2* was negatively associated with B cells, but positively associated with the other 5 kinds of immune cells. *E2F3* was positively associated with all 6 kinds of immune cells. In regard to *E2F4*, there existed a negative association between the expression levels of *E2F4* and infiltration levels of $CD8^+$ T cells and T regulatory cells, while the expression levels of *E2F4* were positively associated with the other 4 kinds of cells. *E2F5* and *E2F6* were negatively associated with B cells and $CD8^+$ T cells, while they were positively associated with the other 4 kinds of cells. *E2F7* and *E2F8* were both negatively associated with $CD8^+$ T cells and positively associated with the other 5 kinds of cells.

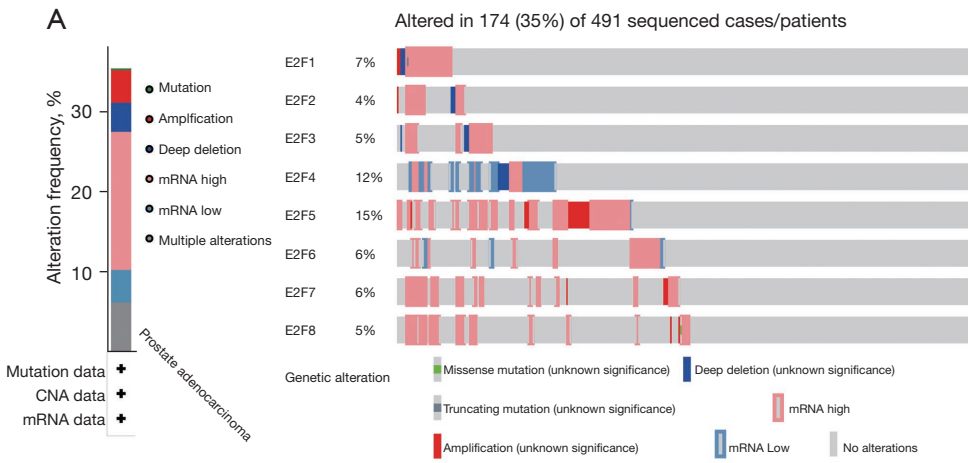
Experimental validation of human prostate cancer tissue

In order to verify the above results, 23 paired cancer and adjacent tissues collected from patients with PCa were selected for real-time polymerase chain reaction (RT-PCR) detection to examine the expression of *E2Fs* in PCa. It was found that in cancer tissues, the expression levels of *E2F1-E2F3* and *E2F5* were higher than those of adjacent tissues ($P<0.05$), while the expression levels of *E2F4* and *E2F6* were lower than those of adjacent tissues (*Figure 8*). There was no statistically significant difference in the expression of *E2F7* and *E2F8* between cancer and adjacent normal tissues. The above results are consistent with the results from both TCGA database and Oncomine database.

Discussion

As important transcription factors, members of the *E2F* transcription factor protein family play a key role in regulating downstream gene transcription (13-17). Therefore, the abnormal expression of *E2Fs* in some tumors may play a dominant role in promoting or suppressing cancer by affecting a variety of downstream genes (18). The function of *E2F* activators in the tumorigenesis and prognosis of several kinds of cancers has been clearly demonstrated (19,20), but further analysis of their roles in PCa has not been elaborated. Our study investigated the expression of *E2Fs* and their clinical, diagnostic, prognostic, functional, and immunological value in patients with PCa. Our findings may help improve the treatment of patients with PCa.

The roles of each member of the *E2F* family in tumorigenesis and the development of tumors have been reported, among which *E2F1* is the most explored member (21-27). Previous research found that *E2F1* can play various roles in different cancers (28). It has recently been reported that safranal inhibits cell cycle re-entry of quiescent PCa cells by deregulating the transcriptional activity of



B

	E2F1	E2F2	E2F3	E2F4	E2F5	E2F6	E2F7	E2F8
E2F1	1	0.75	0.13	0.08	0.12	−0.06	0.42	0.44
E2F2	0.75	1	0.35	−0.01	0.27	−0.06	0.63	0.64
E2F3	0.13	0.35	1	−0.23	0.52	0.03	0.44	0.49
E2F4	0.08	−0.01	−0.23	1	−0.36	0.19	−0.11	−0.17
E2F5	0.12	0.27	0.52	−0.36	1	−0.19	0.39	0.41
E2F6	−0.06	−0.06	0.19	0.19	−0.19	1	−0.12	−0.08
E2F7	0.42	0.63	−0.11	−0.11	0.39	−0.12	1	0.67
E2F8	0.44	0.64	−0.17	−0.17	0.41	−0.08	0.67	1

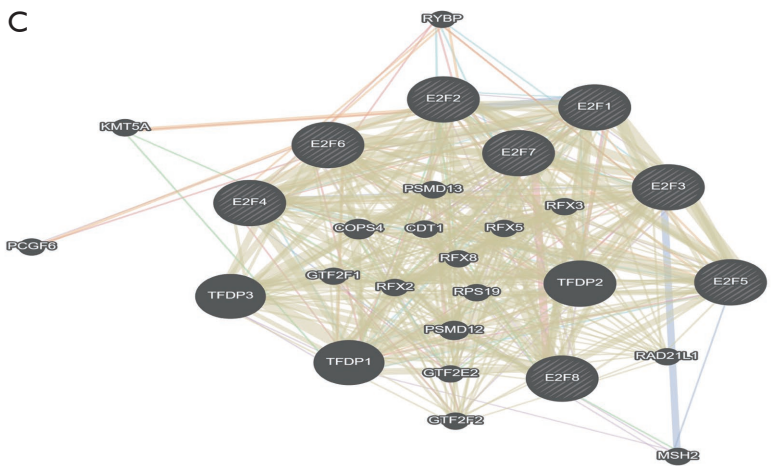


Figure 5 Genetic alteration, correlation analysis and neighbor gene network of E2F transcription factors in patients with PCa. (A) Summary of alterations of E2F transcription factors; (B) correlation heat map of E2F transcription factors; (C) neighbor gene network of E2F transcription factors.

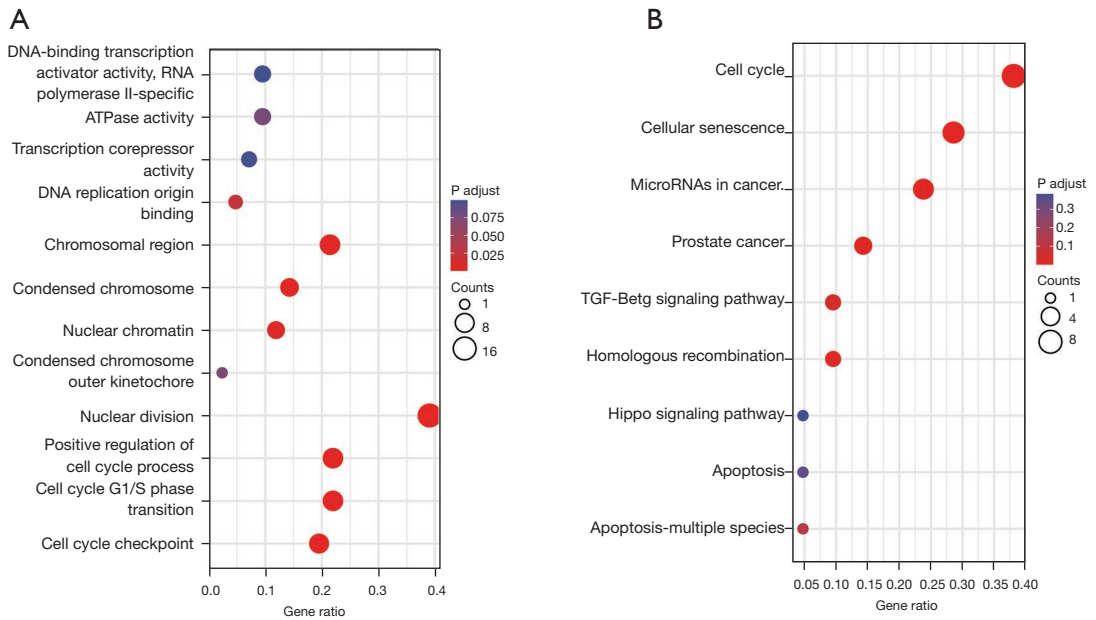
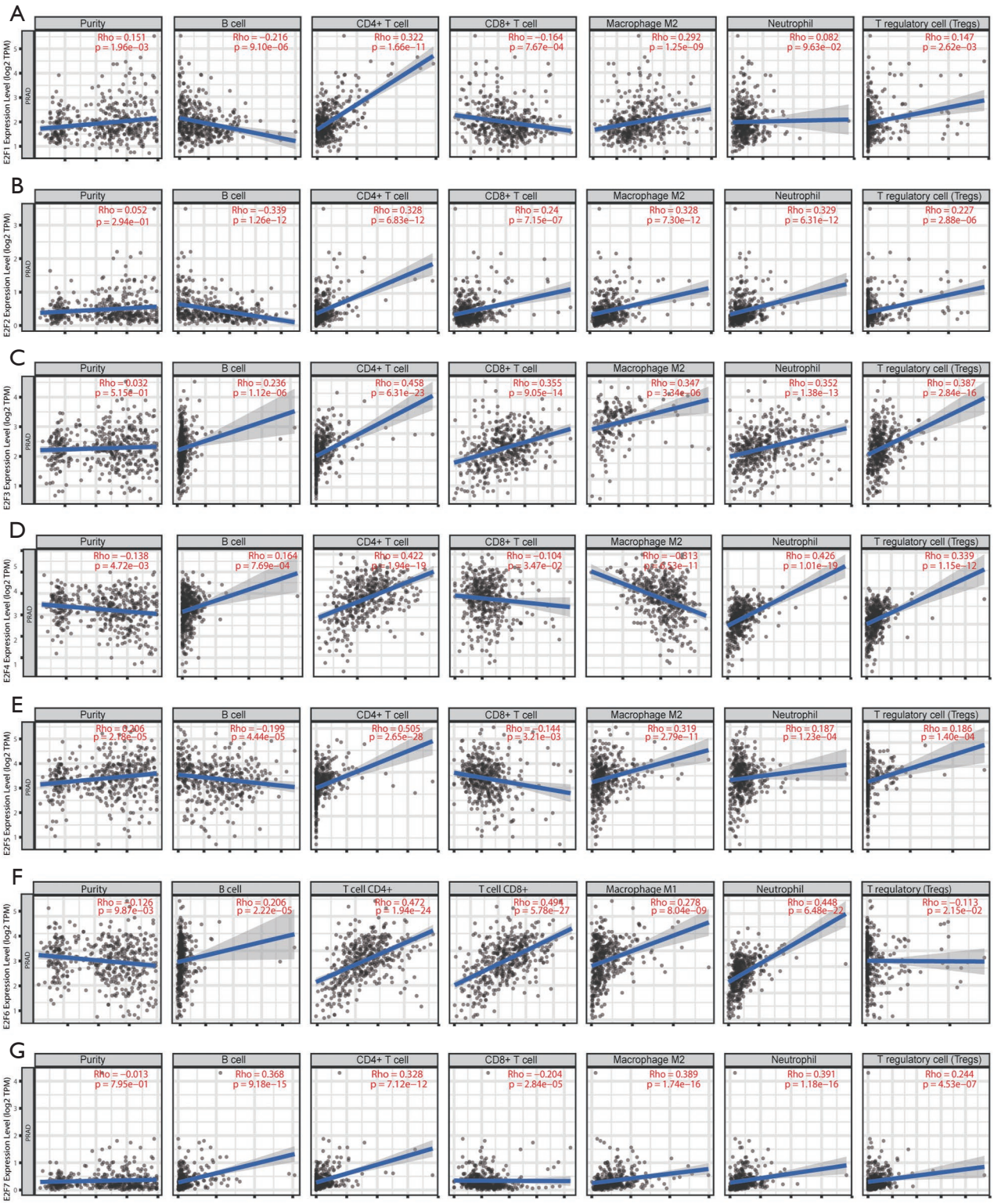


Figure 6 (A) Gene Ontology analysis and (B) Kyoto Encyclopedia of Gene and Genomes pathway analysis.

E2F1 (29). Hagiwara *et al.* indicated that through the integration of *E2F1* and esBAF, MUC1-C facilitates the progression of neuroendocrine PCa (30). Altayyar *et al.* reported that *E2F1* is a translational target of *WDR77* and is reactivated during PCa (31). Yang *et al.* demonstrated that high expression of *E2F1* is associated with unfavorable prognosis in PCa cells (32). The study by Xu *et al.* showed that *E2F1* was markedly up in cancer and plays a key role in cellular inhibition when it is down-regulated (33). Qi *et al.* showed that in PCa cells, *E2F1* participates in epithelial mesenchymal transition (EMT) (34). Wang *et al.* showed that the *E2F1* pathway, which contributes to cell cycle arrest at the G0/G1 phase, promoted the radiosensitivity of PCa cells (35). Koushyar *et al.* demonstrated that *E2F1* leads to cell cycle progression in PCa (36). It was also reported that *RB* loss can result in *E2F1* cistrome up-regulation and different binding specificity (37). In our study, the database analysis showed that the transcription level of *E2F1* in PCa was significantly higher than that in normal prostate tissues both in paired samples or unpaired samples. Moreover, the expression level of *E2F1* increased with the progression of tumors, and significant differences existed in various stages of patients with PCa. There were also significant differences in the expression levels of *E2F1* in patients with distinct ages and serum levels of PSA. Kaplan-Meier analysis found that high *E2F1* transcription levels were markedly related to the OS of patients with PCa.

Like *E2F1*, *E2F2* can play opposing roles in causing and suppressing cancer. On the one hand, down-regulated expression of *E2F2* can induce cell cycle arrest at the G1/S phase and thus suppress cellular proliferation (38). On the other hand, through the inhibition of cell growth via G1 arrest, silibinin can lead to differentiation of androgen-dependent LNCaP cells (39). In our report, we found that the expression of *E2F2* in human PCa was higher in both paired and unpaired samples. Furthermore, the expression of *E2F2* was associated with tumor stage and lymph node stage in patients with PCa.

High expression of *E2F3* is a cancer-promoting event for many cancers including PCa, and is pivotal to tumor cell proliferation and the cell cycle (40). Altayyar *et al.* identified that *E2F3* is a translational target of *WDR77* and is reactivated during PCa (31). Previous studies have indicated that compared with tissues adjacent to PCa, the *E2F3* protein is overexpressed in clinical PCa samples, and the silencing of *E2F3* suppresses the proliferation, migration, and invasion of PCa cells (41). Sun *et al.* showed that through targeting *E2F3*, GA suppresses the growth of PCa cells (42), while O'Bryant *et al.* found that through inhibiting *E2F3*, prostate-specific deletion of *WDR77* inhibited prostate tumorigenesis (43). The data analysis showed that the *E2F3* expression in PCa was significantly higher than that in normal tissues, and it was also correlated



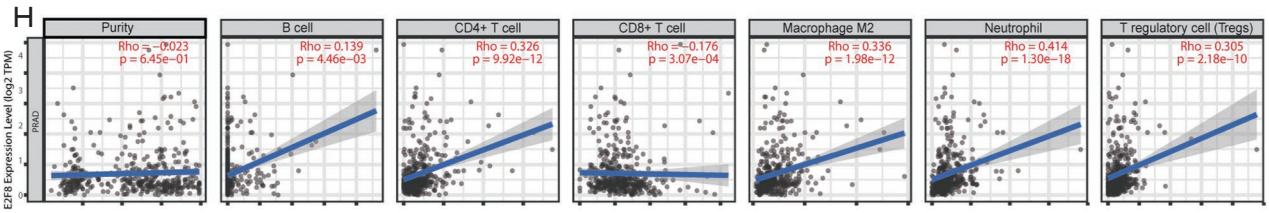


Figure 7 The correlation between E2F transcription factors and immune cell infiltration. (A) E2F1 was negatively associated with the infiltration levels of B cells, $CD8^+$ T cells, and neutrophils, and positively associated with $CD4^+$ T cells, T regulatory cells, and macrophages; (B) E2F2 was negatively associated with B cells, but positively associated with the other 5 kinds of immune cells; (C) E2F3 was positively associated with all 6 kinds of immune cells; (D) there existed a negative association between the expression levels of E2F4 and infiltration levels of $CD8^+$ T cells and T regulatory cells, while the expression levels of E2F4 were positively associated with the other 4 kinds of cells; (E) E2F5 and (F) E2F6 were negatively associated with B cells and $CD8^+$ T cells, while they were positively associated with the other 4 kinds of cells. (G) E2F7 and (H) E2F8 were both negatively associated with $CD8^+$ T cells and positively associated with the other 5 kinds of cells.

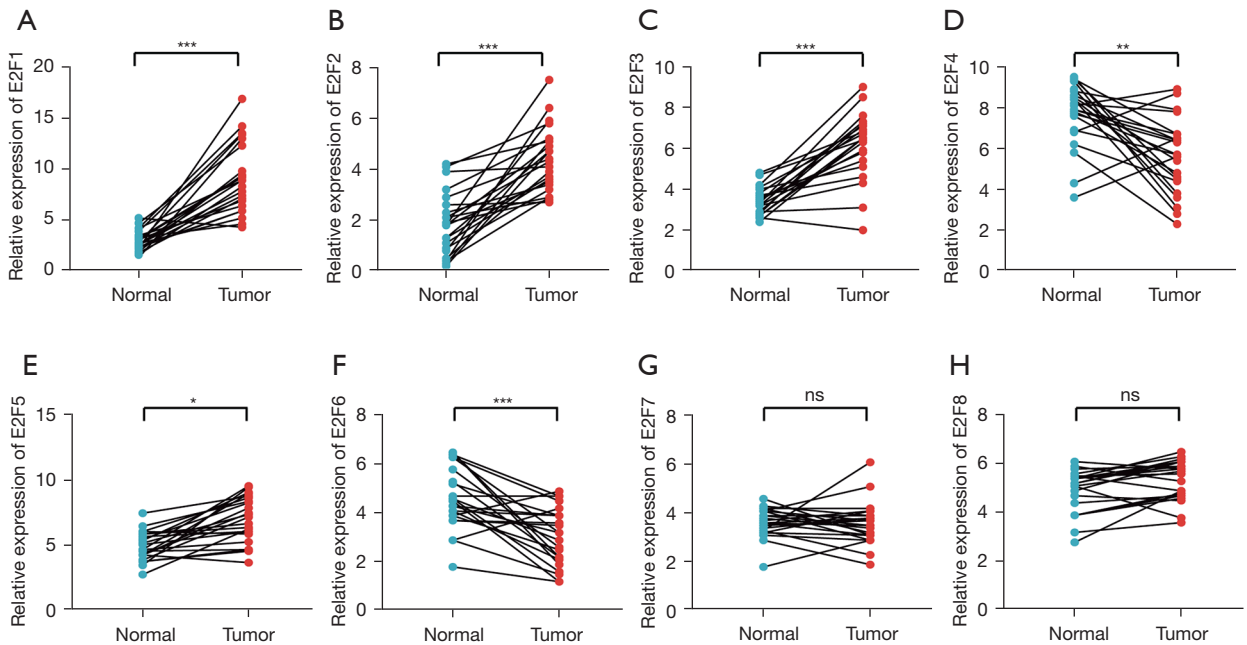


Figure 8 The expression levels of E2F transcription factors in 23 paired samples of PCa. The expression levels of E2F1-E2F3 and E2F5 were higher than those of adjacent tissues (P<0.05) (A,B,C,E), while the expression levels of E2F4 (D) and E2F6 (F)were lower than those of adjacent tissues. There was no statistically significant difference in the expression of E2F7 (G) and E2F8 (H) between cancer and adjacent normal tissues. *P<0.05; **P<0.01; ***P<0.001. ns, not statistically significant.

with tumor stage.

E2F4, enriched in differentiated and nonproliferating cells, plays critical roles in the suppression of proliferation-associated genes (44). The results of the above study show that colon cancer, kidney cancer, and lung cancer are associated with high levels of *E2F4*. Li *et al.* demonstrated that through translocating from the cytoplasm to the nucleus, *E2F4* subsequently suppresses the transcription of cyclin B1 and the progression of the cell cycle (45). DuPree *et al.* found that the levels of *E2F4* protein increased significantly in the nuclei of PCa cells (46). However, Yang *et al.* showed that TGF-β reduces survivin expression in PCa epithelial cells by a mechanism of transcriptional suppression of *E2F4* (47). Also, Crosby *et al.* showed that *E2F4*, in response

to radiation, enhances stable G2 arrest by repressing the target genethus affording increased cell survival ability in PCa (48). In this study, we found that the mRNA level of *E2F4* was markedly lower in PCa tissues than in normal tissues, yet it was obviously related to stages of PCa. The expression level of *E2F4* is inversely related to OS.

Previous studies have found that *E2F5* shows higher expression in certain kinds of tumors, including PCa (49,50). Li *et al.* reported that the up-regulation of microRNA-132 causes the down-regulation of *E2F5*, which may contribute to the tumorigenesis of PCa (10). Zhao *et al.* indicated that the overexpression of the *E2F5* protein was obviously correlated with a higher Gleason score, positive metastasis, advanced clinical stage, and PSA failure (51). Li *et al.* found that through suppressing *E2F5*, the tumor repressor miR-1-3p regulates the aggressiveness of PCa cells (52). Qi *et al.* showed that through enhancing *CDK13* transcription, *E2F5* leads to the up-regulation of its expression and the proliferation of PCa cells (49). Karmakar *et al.* provided strong evidence that by regulating the level and activity of its downstream targets, *E2F5* overexpression accelerates cell invasion and migration in PCa (50). In the present study, we showed that the mRNA level of *E2F5* in PCa is obviously distinct from that in normal tissues.

Some studies have reported the role of *E2F6* in PCa. Knockdown of *E2F6* enhances the sensitivity of PCa cells to apoptosis induced by docetaxel (53). Similarly, Bhatnagar *et al.* reported that miR-205 and miR-31 down-regulate *E2F6* to enhance the PCa cell apoptosis induced by chemotherapeutics (54). The results from our analysis found that the mRNA level of *E2F6* in human PCa is markedly different from that in normal tissues. However, survival analysis found that high mRNA expression of *E2F6* resulted in worse OS in PCa patients but was not associated with tumor staging in PCa patients.

Recent studies have shown that *E2F7* functions as a transcriptional repressor and is up-regulated in many tumors. *E2F7* was mostly expressed both in the nuclei of poorly differentiated PCa tissues and in the cytoplasm of moderately or highly differentiated PCa tissues. In PCa cell lines, inhibiting the expression of *E2F7* reduces the cell proliferation rate, increases the proportion of cells in the G1 phase of the cell cycle, and boosts the apoptosis rate (12). He *et al.* indicated the cell cycle gene *E2F7*, expression of ARV-PBS target genes, was significantly associated with poor survival and tumor progression (55). However, this study found that there was no difference in the transcription level of *E2F7* between PCa and normal tissues, although it had an influence on the stage of tumors. Also, survival analysis found that the expression of *E2F7* had no effect on OS in PCa patients.

As for *E2F8*, little is currently known about its expression and role in PCa. Lee *et al.* indicated that overexpression of *E2F8* was related to PCa metastasis and that the down-regulation of *E2F8* was able repress cell growth by enhancing G2/M arrest (56). In our report, as with *E2F7*, there was no difference in the transcription level of *E2F8* between PCa and normal tissues despite it having an influence on tumor stage. However, survival analysis found that high *E2F8* mRNA expression resulted in worse OS in PCa patients.

Conclusions

This is the first study to systematically perform a comprehensive analysis of the expression and prognostic value of *E2Fs* in PCa. The aim of this research was to provide a better understanding of the *E2F* family in the diversity of PCa from various aspects, such as the clinical, histopathological, and biomolecular characteristics. Our results suggest that the up-regulation of *E2F1/2/3/5* and the down-regulation of *E2F4/6* in PCa tissues may play important roles in PCa tumorigenesis. Highly expressed *E2F1/2/3/5/7* can be regarded as a molecular marker to identify high-risk PCa patients. Our findings revealed that *E2F1/4/6/8* are potential treatment targets for PCa. In conclusion, the above results indicate that *E2Fs* may act as promising biomarkers for PCa. However, it is necessary to further study the molecular mechanisms, focusing on a single *E2F* or a combination of several *E2Fs*, in order to promote the clinical application of *E2Fs* as prognostic indicators or treatment target for PCa.

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