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

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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 samples (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).
The expression levels
Log2 (TPM +1 )

**E2F1**  **E2F2**  **E2F3**  **E2F4**  **E2F5**  **E2F6**  **E2F7**  **E2F8**

**Analysis type by cancer**

| Analysis type by cancer | **Cancer** vs. **normal** | **Cancer** vs. **normal** | **Cancer** vs. **normal** | **Cancer** vs. **normal** | **Cancer** vs. **normal** | **Cancer** vs. **normal** |
|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Bladder cancer          | 2                        | 1                        | 2                        | 3                        |                          |                          |
| Brain and CNS cancer    | 3                        | 1                        | 1                        | 10                       | 1                        | 3                        |
| Breast cancer           | 10                       | 13                       | 1                        | 5                        | 2                        | 8                        |
| Cervical cancer         | 2                        | 1                        | 3                        |                          |                          |                          |
| Colorectal cancer       | 4                        | 8                        | 5                        | 12                       | 5                        | 14                       |
| Esophageal cancer       | 1                        | 1                        | 3                        |                          |                          |                          |
| Gastric cancer          | 1                        | 2                        | 5                        |                          |                          |                          |
| Head and Neck cancer    | 1                        | 1                        | 7                        | 1                        | 1                        | 4                        |
| Kidney cancer           | 1                        | 1                        | 1                        |                          |                          |                          |
| Leukemia                | 3                        | 4                        | 1                        | 5                        | 3                        | 2                        |
| Liver cancer            | 1                        | 1                        | 3                        |                          |                          |                          |
| Lung cancer             | 4                        | 6                        | 11                       |                          |                          |                          |
| Lymphoma                | 4                        | 1                        | 3                        | 5                        | 1                        | 2                        |
| Melanoma cancer         | 1                        |                          |                          |                          |                          |                          |
| Myeloma cancer          |                          |                          |                          |                          |                          |                          |
| Other cancer            | 3                        | 2                        | 1                        | 4                        | 1                        | 1                        |
| Ovarian cancer          | 1                        | 2                        |                          |                          |                          |                          |
| Pancreatic cancer       | 1                        | 2                        |                          |                          |                          |                          |
| Prostate cancer         | 1                        | 1                        | 1                        | 3                        | 2                        | 1                        |
| Sarcoma cancer          | 1                        |                          |                          |                          |                          |                          |

Significant unique analyses: 36 9 34 20 60 4 5 3 38 7 7 1 39 1 28 5
Total unique analyses: 449 403 438 455 457 206 256 376

**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
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 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.
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 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.
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
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.
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-\(\beta\) 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 gene thus 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 systematically to 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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**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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