E2F2/5/8 Serve as Potential Prognostic Biomarkers and Targets for Human Ovarian Cancer

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E2Fs are a family of pivotal transcription factors. Accumulative evidence indicates that aberrant expression or activation of E2Fs is a common phenomenon in malignances, and significant associations have been noted between E2Fs and tumorigenesis or progression in a wide range of cancers. However, the expression patterns and exact roles of each E2F contributing to tumorigenesis and progression of ovarian cancer (OC) have not yet been elucidated. In this study, we investigated the distinct expression and prognostic value of E2Fs in patients with OC by analyzing a series of databases, including ONCOMINE, GEPIA, cBioPortal, Metascape, and Kaplan–Meier plotter. The mRNA expression levels of E2F1/3/5/8 were found to be significantly upregulated in patients with OC and were obviously associated with tumor stage for OC. Aberrant expression of E2F2/5/7/8 was found to be associated with the clinical outcomes of patients with OC. These results suggest that E2F2/5/8 might serve as potential prognostic biomarkers and targets for OC. However, future studies are required to validate our findings and promote the clinical utility of E2Fs in OC.

Keywords: E2Fs, ovarian cancer, database mining, prognostic value, bioinformatics analysis

INTRODUCTION

Ovarian cancer (OC) is a commonly diagnosed gynecological malignancy with the highest cancer-related death rate worldwide (1). According to the development trend, the lifetime incidence for ovarian malignancies is 1 in 72 (1.39%), and the lifetime risk of death from OC is 1 in 96 (1.04%) for women (2). In 2018, approximately 22,240 new cases of OC were diagnosed, and 14,070 OC-related deaths occurred in the United States (3). The high lethality rate can be attributed to the lack of effective biomarkers to detect the disease and to predict the outcome for heterogeneous biological subgroups of patients. Over 75% of patients are not diagnosed until the disease is advanced (stages III and IV), for which the 5-year overall survival (OS) rate is below 30% (2, 4, 5). Thus, identifying reliable predictive biomarkers for early diagnosis and precise prognosis and developing novel molecular-targeted therapeutic strategies for OC are urgently required.

At present, several predictive biomarkers, which might have potential diagnostic, prognostic, or therapeutic values for OC, have been reported. Some of these markers include osteopontin, mesothelin, vascular cell adhesion molecule-1, kallikreins, B7-H4, human prostasin, apolipoprotein A1, interleukin-6/8, glutathione S-transferase polymorphisms, folate receptor alpha miRNA, and aldehyde dehydrogenase (6). Although some of the abovementioned biomarkers of OC have attracted considerable attention, most of them were investigated individually and not as a part of the entire oncogenesis process; related studies are still in the preliminary investigation or clinical
 validation stage. Further, these potential OC biomarkers do not play a significant role in improving the screening, diagnosis, prognosis, prevention, and therapy of OC (3, 4).

E2Fs, a group of genes that encode a family of transcription factors in higher eukaryotes, are widely expressed in many tissues and organs (7). The E2F family includes eight members: E2F1 to E2F8 (8). The members have different homology, which apparently affects their function; hence, the E2F family is divided into the following two subfamilies: E2F1-3 are activators of transcription, whereas E2F4-8 act as repressors (9). The molecular functions of E2Fs are cellular proliferation, differentiation, DNA repair, cell cycle regulation, and cell apoptosis (10). Increased aberrant expression or activation of E2Fs has been reported in several human malignancies; in some studies, E2Fs might act as promising biomarkers to predict tumor prognosis (9–11). Therefore, identifying the underlying mechanisms of E2F-mediated oncogenes or tumor suppressors as predictive biomarkers might provide novel therapeutic strategies. Several E2Fs were shown to be deregulated in OC compared with that in normal tissues, and high expression levels of E2F1, E2F2, E2F4, E2F7, and E2F8 were found to be significantly associated with survival rate in OC (12, 13). More importantly, E2F1 and E2F2 have attracted increasing attention as targeted molecular therapeutic genes for OC (13–16). However, the differences in expression levels, genetic alterations, biological functions, molecular mechanisms, and prognostic significance of the majority of E2Fs in OC have not yet been completely elucidated.

The development of microarray and RNA-sequencing technology has revolutionized RNA and DNA research, which has become a crucial component of biological and biomedical research (17, 18). In this study, we extended the knowledge related to OC based on various large databases for conducting the comprehensive analysis of the relationships between the eight E2F subtypes and the pathogenesis and progression of OC.

**MATERIALS AND METHODS**

**ONCOMINE Analysis**

The gene expression array datasets of ONCOMINE (www.oncomine.org) are a publicly accessible, online cancer microarray database that helps facilitate research from genome-wide expression analyses. ONCOMINE was used to analyze the mRNA levels of E2F family members in OC (19, 20). The thresholds were restricted as follows: P-value = 0.001; fold-change = 1.5; and data type, mRNA. For each gene, comparison between cancer specimen and normal control dataset analysis was performed.

**GEPIA Dataset Analysis**

Gene Expression Profiling Interactive Analysis (GEPIA) is an interactive web server for estimating mRNA expression data based on 9,736 tumors and 8,587 normal samples in the Cancer Genome Atlas (TCGA) and Genotype-tissue Expression dataset projects. GEPIA provides key interactive and customizable functions, including differential expression analysis, profiling plotting, correlation analysis, patient survival analysis, similar gene detection, and dimensionality reduction analysis (21).

**TCGA and CBioPortal Analysis**

The CBioPortal for cancer genomics (http://www.cbioportal.org/) is affiliated with the Memorial Sloan Kettering Cancer Center and provides information regarding the integrative analysis of complex cancer genomics and clinical profiles from 105 cancer studies in the TCGA pipeline (22). The frequency of E2F family gene alterations (amplification, deep deletion, and missense mutations), copy number variance obtained from Genomic Identification of Significant Targets in Cancer(GISTC), and mRNA expression z-scores (RNA Seq V2 RSEM) were assessed using the cbioPortal for Cancer Genomics database and TCGA. In addition, co-expression and network analyses were performed according to the online instructions of cbioPortal (23).

**Functional Enrichment Analysis**

Metascape (http://metascape.org) is a free, well-maintained, user-friendly gene-list analysis tool for gene annotation and analysis. It is an automated meta-analysis tool to understand common and unique pathways within a group of orthogonal target-discovery studies. In this study, Metascape was used to conduct pathway and process enrichment analysis of E2F family members and neighboring genes significantly associated with E2F alterations. For this, the Gene Ontology (GO) terms for biological process, cellular component, and molecular function categories, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, were enriched based on the Metascape online tool. Only terms with P-value < 0.01, minimum count of 3, and enrichment factor of >1.5 were considered as significant. The most statistically significant term within a cluster was chosen as the one representing the cluster. A subset of enriched terms was selected and rendered as a network plot to further determine the relationship among terms, where terms with similarity of >0.3 were connected by edges. Protein–protein interaction enrichment analysis was performed using the following databases: BioGrid, InWeb.IM, and OmniPath. Further, Molecular Complex Detection (MCODE) algorithm was applied to identify densely connected network components.

**The Kaplan–Meier Plotter Analysis**

Kaplan–Meier plotter (www.kmplot.com) is an online database containing microarray gene expression data and survival information derived from Gene Expression Omnibus, TCGA, and the Cancer Biomedical informatics Grid, which contain gene expression data and survival information of 1,816 clinical OC patients (24). In this study, the prognostic value of the mRNA expression of E2F family members was evaluated using the Kaplan–Meier plotter. The OS, progression-free survival (PFS), and post-progression survival (PPS) of patients with OC were determined by dividing the patient samples into two groups based on median expression (high vs. low expression) and assessing using a Kaplan–Meier survival plot, with a hazard ratio with 95% confidence intervals and log rank p-value.
Subgroup analyses were performed by dividing patients based on pathological and histological subtypes.

RESULTS

Transcription Levels of E2Fs in Patients With OC

Eight E2F family members have been identified in human cancers. We compared the transcriptional levels of E2Fs in cancers with those in normal tissue samples by using ONCOMINE databases (Figure 1 and Table 1). ONCOMINE analysis revealed that the mRNA expression of E2F1, E2F4, E2F5, and E2F8 was upregulated in patients with OC. The transcription levels of E2F1 were significantly higher in patients with OC in three datasets (25, 26). In Yoshihara’s dataset (26), E2F1 was overexpressed in ovarian serous adenocarcinoma compared with that in the normal samples, with a fold change of 26.734 and p–value of 6.79E-05. In Bonome’s dataset (25), E2F1 was overexpressed in ovarian carcinoma with a fold change of 1.644 and p–value of 2.60E-07. In the TCGA dataset, E2F1 was overexpressed in ovarian serous carcinoma compared with that in the normal samples, with a fold change of 1.639 and p–value of 1.29E-06. The transcription levels of E2F3 were significantly higher in patients with OC in four datasets (26–28). In the TCGA dataset, the fold change of mRNA expression of E2F3 in ovarian serous carcinoma was 2.013 and p–value of 1.96E-11. In Welsh’s dataset (27), E2F3 was upregulated in ovarian serous carcinoma with a fold change of 2.574 and p–value of 3.40E-07. In Lu’s dataset (28) and Yoshihara’s dataset (26), E2F3 was significantly overexpressed in ovarian serous adenocarcinoma with fold changes of 1.838 (p–value= 1.27E-04) and 1.833 (p–value = 5.66E-04), respectively. The mRNA levels of E2F4 in ovarian carcinoma (fold change = 1.573 and p–value = 7.77E-04) and ovarian serous carcinoma (fold change = 2.574 and p–value =1.01E-06) were significantly higher than those in the normal samples in Bonome’s (25) and Welsh’s datasets (27). The transcriptional levels of E2F5 in ovarian carcinoma (fold change = 4.355 and p–value = 2.71E-08) were significantly different from those in the normal samples.

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** | The transcription levels of E2F family members in different types of cancers (ONCOMINE). The graph shows the numbers of datasets with statistically significant mRNA over-expression (red) or down-regulated expression (blue) of the target gene. The threshold was designed with following parameters: p-value of 1E-3 and fold change of 1.5.
samples in Yoshihara's dataset (26). A similar trend was found for E2F8 in Lu's (28) and TCGA datasets: the mRNA levels of E2F8 in ovarian serous adenocarcinoma (fold change = 1.771 and \( p\)-value = 6.04E-05) and ovarian serous carcinoma (fold change = 3.136 and \( p\)-value = 7.97E-06) were significantly higher than those in the normal samples. In addition, no significant difference in E2F2, E2F6, and E2F7 mRNA expression was found between OC and normal controls, according to ONCOMINE analysis. Although the transcription levels of E2F2 were also slightly higher than those in normal ovarian tissues with \( p\)-value of no more than 0.05, the cut-off of fold change was <1.5.

We compared the transcription expression of E2F family members between OC and normal tissues by using the GEPIA dataset (Figure 2). The results showed that the mRNA expression levels of E2F1, E2F2, E2F3, E2F5, and E2F8 were significantly higher in OC tissues than in normal ovarian tissues, whereas the transcription expression levels of E2F4, E2F6, and E2F7 were not significantly different between OC and normal tissues. By using the GEPIA dataset, we also analyzed the relationship between the transcription levels of E2Fs and the tumor stage of patients with OC. The mRNA expression of E2F family members was found to be significantly and negatively associated with the tumor stage for OC (Figure 3).

**Co-expression and Interaction Analyses of E2Fs at the Gene and Protein Levels in Patients With OC**

Pearson correlation analysis was conducted using expression data (RNA Seq V2 RSEM) of E2F family members collected from the cBioPortal online tool for OC (TCGA, Provisional). The results indicated a significant positive correlation among E2F1 and E2F3; E2F3 with E2F1, E2F4, and E2F7; E2F4 with E2F3 and E2F8; and E2F8 with E2F4. However, significant negative correlations were noted for E2F2 with E2F5; E2F5 with E2F2 and E2F8; and E2F8 with E2F2 (Figure 4A).

GeneMANIA was used to conduct correlation analysis of E2F family members at the gene level (Figure 4B). The results revealed relationships in co-expression between E2F1 and E2F2, E2F2 and E2F5, E2F3 and E2F2, E2F6 and E2F5, as well as E2F7 and E2F2. Relationships were noted in co-localization between E2F1 and E2F3, as well as E2F3 and E2F2. Further, relationships were noted between E2F3 and E2F4 in genetic interactions, and E2F1 and E2F2 participated in a network group. In addition, the same pathway was shared between E2F1

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**TABLE 1** | The transcription levels of E2F family members in between different types of OC and ovarian normal tissues (ONCOMINE).

| Types of ovarian cancer vs. norma | Fold change | t-test | \( p\)-value | Ref | PMID |
|----------------------------------|-------------|--------|-------------|-----|------|
| E2F1 Ovarian serous carcinoma vs. normal | 26.734 | 6.95 | 6.79E-05 | Yoshihara ovarian | 19486012 |
| Ovarian carcinoma vs. normal | 1.644 | 10.596 | 2.60E-07 | Bonome ovarian | 18593951 |
| Ovarian serous carcinoma vs. normal | 1.218 | 8.593 | 7.69E-06 | Hendrix ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.195 | 7.539 | 1.26E-05 | Hendrix ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.118 | 4.551 | 8.56E-04 | Hendrix ovarian | 16452189 |
| Ovarian serous carcinoma vs. Normal | 1.639 | 11.563 | 1.29E-06 | TCGA ovarian | |
| E2F2 Ovarian carcinoma vs. normal | 1.091 | 3.188 | 3.00E-03 | Bonome ovarian | 18593951 |
| E2F3 Ovarian serous carcinoma vs. normal | 2.013 | 22.413 | 1.96E-11 | TCGA ovarian | |
| Ovarian serous carcinoma vs. normal | 2.574 | 11.888 | 3.40E-07 | Welsh ovarian | 11158614 |
| Ovarian carcinoma vs. normal | 1.838 | 5.143 | 1.27E-04 | Lu ovarian | 15161682 |
| Ovarian serous carcinoma vs. normal | 1.833 | 3.901 | 5.66E-04 | Yoshihara Ovarian | 19486012 |
| Ovarian serous carcinoma vs. normal | 1.176 | 8.187 | 1.04E-04 | Hendrix Ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.138 | 6.236 | 2.13E-04 | Hendrix Ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.429 | 5.945 | 1.88E-04 | Hendrix Ovarian | 16452189 |
| E2F4 Ovarian carcinoma vs. normal | 1.573 | 4.417 | 7.77E-04 | Bonome ovarian | 18593951 |
| Ovarian serous carcinoma vs. normal | 1.203 | 9.917 | 1.25E-04 | Hendrix Ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.231 | 11.123 | 6.90E-05 | Hendrix Ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.228 | 8.276 | 8.32E-06 | Hendrix Ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.074 | 5.971 | 1.96E-07 | Hendrix Ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 2.574 | 11.888 | 1.01E-06 | Welsh ovarian | 11158614 |
| Ovarian carcinoma vs. normal | 4.355 | 8.753 | 2.71E-08 | Yoshihara ovarian | 19486012 |
| Ovarian serous carcinoma vs. normal | 1.948 | 3.696 | 4.05E-04 | Hendrix ovarian | 16452189 |
| Ovarian carcinoma vs. normal | 1.118 | 4.551 | 8.56E-04 | Hendrix ovarian | 16452189 |
| Ovarian serous carcinoma vs. normal | 1.195 | 7.539 | 1.26E-05 | Hendrix ovarian | 16452189 |
| Ovarian serous carcinoma vs. Normal | 1.639 | 11.563 | 1.29E-06 | TCGA ovarian | |
| E2F5 Ovarian carcinoma vs. normal | 1.431 | 5.429 | 1.11E-05 | Lu ovarian | 15161682 |
| Ovarian serous carcinoma vs. normal | 1.148 | 5.357 | 6.56E-06 | Hendrix ovarian | 16452189 |
| E2F6 | | | | | |
| E2F7 | | | | | |
| E2F8 Ovarian serous carcinoma vs. normal | 1.771 | 5.173 | 6.04E-05 | Lu ovarian | 15161682 |
| Ovarian serous carcinoma vs. normal | 3.136 | 9.63 | 7.97E-06 | TCGA ovarian | |
and E2F3, E2F1, E2F4, E2F5, and E2F7, as well as E2F4 and E2F2. Physical interactions were noted between E2F1 and E2F4 as well as E2F7 and E2F2. Moreover, relationships were noted between E2F1 and E2F4, E2F3 and E2F4, E2F3 and E2F4, and E2F4. Shared protein domains were noted among E2F1 with E2F5, E2F7, and E2F8, and E2F7 with E2F4, E2F5, E2F6, and E2F8; as well as E2F8 with E2F5 and E2F6.

STRING analysis was conducted to identify interactions of E2F gene family members at the protein expression level. E2F1 was shown to interact with E2F2, E2F4, and E2F8, and E2F7 was found to interact with E2F8 with regard to co-expression, text-mining, and protein homology. Detailed results are shown in Figure 4C.

**E2F Genetic Alteration and Neighbor Gene Network in Patients With OC**

Alteration frequency of E2F mutations in OC was analyzed using cBioPortal. A total of 839 patients from three datasets of ovarian serous carcinoma were analyzed. Among the 3 OC datasets analyzed, gene set/pathway was altered in 389 (22.2%) of the queried samples, and alterations ranging from 31.02% (188/606) to 13.7% (83/606) were found for the gene sets submitted for analysis (Figure 5A). The percentages of genetic alterations in E2F family members for OC varied from 3 to 14% for individual genes based on the TCGA Provisional dataset (E2F1, 9%; E2F2, 4%; E2F3, 16%; E2F4, 10%; E2F5, 14%; E2F6, 10%; E2F7, 3%; E2F8, 4%; Figure 5B). The results of Kaplan–Meier plotter and log-rank test indicated no significant difference in OS and disease-free survival (DFS) between the cases with alterations in one of the query genes and those without alterations in any query genes (P-values, 0.224 and 0.874, respectively; Figures 5C,D). Next, we constructed the network for E2Fs and the 50 most frequently altered neighbor genes by using the cBioPortal. The results showed that ATR, CBX4, CCND2, CCNE1, CCNE2, CDK6, CDKN1A, CDKN1B, CEBPA, CEBPB, CTBP1, DNMT1, EED, EZH2, GSK3B, HDAC1, HES1, LIN37, LIN9, MAML2, MAML1, MAPK11, MCL1, MYC, NFATC2, PCNA, POLD3, POLG, PRMT5, RB1, RBBP4, RBL2, RPS6KB1, RRMM, SMAD2, SMARCA2, SNW1, SUV39H1, SUZ12, TBP, TERT, TFDP2, TF3, TK2, TOPBP1, TP53, TRRAP, UXT, XRCC1, and YY1 were closely associated with E2F alterations and functions (Figure 5E).
Zhou et al. E2Fs and Ovarian Cancer

FIGURE 3 | Correlation between E2F expression and tumor stage in OC patients (GEPIA). Violin plot derived from correlation between the expression of a specific E2F family member and tumor stage in patients with OC; the p-value was set at 0.05. The distribution of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8 mRNA expression correlated with tumor stage.

Functional Enrichment Analysis of E2Fs in Patients With OC

The functions of E2F family members and their neighboring genes were predicted by analyzing GO and KEGG in Metascape. The top 20 GO enrichment items were classified into three functional groups: biological process group (11 items), molecular function group (5 items), and cellular component group (4 items; Figures 6A,B and Table 2). The E2F family members and their neighboring genes were mainly enriched in cell cycle, apoptosis, and transcriptional regulation biological processes such as G1/S transition of mitotic cell cycle, negative regulation of G0 to G1 transition, negative regulation of cell proliferation, DNA biosynthetic process, DNA replication, telomere maintenance, negative regulation of cell differentiation, negative regulation of transcription involved in G1/S transition of mitotic cell cycle, intrinsic apoptotic signaling pathway by p53 class mediator, liver development, and apoptotic signaling pathway. The molecular functions for these genes were mainly transcription regulation by sequence-specific DNA binding, transcription co-regulator activity, promoter-specific chromatin binding, DNA-binding transcription repressor activity, RNA polymerase II-specific, and RNA polymerase II transcription factor binding; the cellular components that these genes were involved in were the nuclear chromosome, transferase complex, SWI/SNF superfamily-type complex, and nuclear body.

The top 12 KEGG pathways for the E2F family members and their neighboring genes are shown in Figures 6C,D and Table 3. Among these pathways, the cell cycle signaling pathway, viral carcinogenesis signaling pathway, TGF-beta signaling pathway, Wnt signaling pathway, and Jak-STAT signaling pathway were found to be related to multiple tumor development and were involved in OC tumorigenesis and pathogenesis. In addition, to better understand the relationship between E2F family members and OC, we performed a Metascape protein–protein interaction enrichment analysis. The protein–protein interaction network and MCODE components identified in the gene lists are shown in Figures 6E,F. The four most significant MCODE components were extracted from the protein–protein interaction network. After pathway and process enrichment analysis was independently applied to each MCODE component, the results showed that biological function was mainly related to cell cycle, prostate cancer, hepatitis B, HTLV-I infection, Epstein–Barr virus infection, and pathways in cancer.

Prognostic Values of E2Fs in Patients With OC

By using Kaplan–Meier plotter analysis, we initially assessed the prognostic significance of the E2F family members in all OC patients. The Kaplan–Meier survival curves are shown in Figure 7 and Table 4. The increased mRNA levels of E2F7 and E2F8 were strongly associated with poor OS; the remaining E2F family members were not related with OS in OC. The high expression of E2F5, E2F6, and E2F8 mRNA was predicted to have worse PFS, whereas high E2F4 mRNA expression was correlated to longer PFS in OC patients. In addition, increased E2F1, E2F2, E2F4, and E2F7 mRNA expression levels were associated with poor PPS.

Further, we assessed the correlation of individual E2F family members with different pathological and histological subtypes of OC, including serous and endometrioid. The high mRNA
expression levels of E2F1, E2F3, E2F7, and E2F8 were correlated to poor OS in serous OC patients, whereas increased E2F2 mRNA expression was associated with better OS in serous OC patients. Further, increased E2F2 and E2F4 mRNA expression levels were associated with poor PFS in serous OC patients. High mRNA expression of E2F1, E2F2, E2F3, E2F4, and E2F7 was significantly associated with worse PFS. However, increased E2F6 mRNA expression level was correlated with better PFS. In endometrioid OC, none of the E2F family members were related with prognosis in endometrioid OC. High E2F1, E2F3, and E2F8 mRNA expression levels were associated with poor PFS, whereas increased E2F2 and E2F5 mRNA expression levels were associated with superior PFS in endometrioid OC patients. Data to calculate PPS in patients with endometrial OC based on Kaplan–Meier online tool were not sufficient. The prognostic value of mRNA level of E2F family members in OC patients using Kaplan–Meier plotter ($p > 0.05$) are shown in Appendix.

**DISCUSSION**

Numerous studies have suggested that E2Fs are involved in not only proliferation and differentiation but also apoptosis and tumorigenesis (7, 10). Accumulative evidence indicated that aberrant expression or activation of E2Fs is a common phenomenon in malignances, and significant associations between E2Fs and tumorigenesis or progression of patients with cancer has been partially confirmed (10, 29). However, the patterns of expression and the exact roles of distinct E2F family members in OC are not yet known. In this study, we attempted to systematically explore the expression patterns, prognostic values, genetic alteration, correlation, and potential functions of different E2Fs in OC.

E2F1, the most classic member of the E2F family, was found to play roles in both proliferation and apoptosis and exhibited a complex role in tumor development regulation (30). E2F1 has been shown to exhibit dual properties and can act as a tumor suppressor or oncogene in the same cancer (11, 13, 31). However, E2F1 overexpression is known to contribute to the development and progression of OC, and this role is mediated by the p53-dependent apoptotic pathway and PI3K/AKT signaling pathway and microRNA activity (13, 31). More and more studies revealed that E2F1 overexpression could produce more aggressive tumors with a high proliferation rate and chemoresistance (32–34). In our study, ONCOMINE and GEPIA datasets revealed that the
Expression of \( E2F1 \) was up-regulated in human OC, and \( E2F1 \) expression was linked with the clinical characteristics of patients with OC. By using Kaplan–Meier plotter, we found increased \( E2F1 \) RNA expression level, which was associated with poor PPS in all patients with OC, which seemed consistent with the role of \( E2F3 \) as an oncogene.

\( E2F2 \) regulates many cell processes such as cell cycle, DNA synthesis, proliferation, and tumorigenesis (35). Previous studies indicated that like \( E2F1 \), \( E2F2 \) exhibited oncogenic or tumor suppressive activity, and overexpression of \( E2F2 \) contributed to the development of several solid tumors, indicating worse patient outcome (36, 37). However, the predictive roles of \( E2F2 \) for oncogenesis, prognosis, and prediction of therapeutic in human OC are not yet completely understood. In this study, \( E2F2 \) expression was found to be higher in OC tissues than in normal tissues and was significantly and negatively correlated with tumor stage in patients with OC. In addition, high \( E2F2 \) expression was significantly correlated with worse PPS in all patients with OC.

The \( E2F3 \) transcription factor is known to play a role in controlling cell cycle progression. Recently, the clear oncogenic role of \( E2F3 \) was revealed in several human cancers (38). Amplification and overexpression of \( E2F3 \) has been shown to be closely associated with clinical stage, pathological grading, proliferation index, and tumor aggression (39). Interestingly, \( E2F3a \) was found to be essential in EGFR-mediated proliferation in ovarian cancer cells (40). An in vitro study showed that siRNA for \( E2F3 \) facilitated the silencing of \( E2F3 \) overexpression and protected against breast cancer. Therefore, \( E2F3 \) might be a newly identified diagnostic and potential therapeutic target for solid human cancers (41). In this study, the expression of \( E2F3 \) in OC tissues was higher than that in normal tissues. We also found that \( E2F3 \) expression was significantly and negatively correlated with tumor stage in patients with OC. Further, although no significant association was observed between \( E2F3 \) and clinical outcomes in all OC patients, subgroup analysis revealed that \( E2F3 \) overexpression was associated with reduced OS and PPS in serous OC patients, as well as worse PFS in endometrioid OC patients.

\( E2F4 \) is a key regulator of cell transformation, proliferation, and cell cycle progression, and a recent study showed that patients exhibiting high expression of \( E2F4 \) target genes exhibited more severe cancer and shorter survival (42). In OC, \( E2F4 \) is involved in cell cycle arrest at the G0 phase in TOV21G and SKOV3 cells, and this role is enhanced by deregulated cyclin-dependent kinase inhibitors such as p27, p130/Rb2, and p130/Rb2 (43). Lawrenson et al. (44) confirmed that \( E2F4 \) variants are associated with OC pathogenesis by conducting genome-wide association studies. Reimer et al. (14, 15) found that the expression level of \( E2F4 \) was lower in tissues of platinum-resistant OC patients than in

![FIGURE 5](image-url)
tissues of platinum-sensitive patients, which indicated a tumor suppressor function and prognostic value for E2F4. In our study, the expression of E2F4 was slightly lower in OC tissues than in normal ones and was markedly and negatively correlated with tumor stage in patients with OC. Survival analysis results showed that increased E2F4 expression was significantly correlated with longer PFS in all OC patients.

E2F5 is an important member of the E2F family. It has growth-repressive characteristics that have been observed in several solid cancers such as osteosarcoma, colon cancer, breast cancer, and OC (4). A recent study showed E2F5 overexpression in early as well as advanced stages of OC, and E2F5 status was shown to significantly improve malignancy diagnosis of epithelial OC (12). Moreover, silencing of E2F5 by using miR-132 inhibited the proliferation, colony formation, migration, and invasion of OC cells (45). Thus, E2F5 has been suggested to have a putative role in OC pathogenesis. In this study, E2F5 expression was higher in OC tissues than in normal ones and was significantly and negatively correlated with tumor stage in patients with OC. Furthermore, an elevated level of E2F5 was significantly associated with a worse PFS in all patients with OC.

E2F6, one of the unique E2F family members, is known to be a pRb-independent transcription repressor of E2F-target
genes (46). Although the possible links between E2F6 and cell growth control are intriguing, little is known about the regulation mechanism, and the expression pattern and prognostic role of E2F6 in OC are not yet known. In this study, no significant difference in E2F6 expression was noted between OC tissues and normal ones, but E2F6 expression was negatively correlated with tumor stage in patients with OC. Interestingly, the overexpression of E2F6 was significantly correlated with worse PFS in all patients with OC.

E2F7 is an atypical E2F family member that acts as a transcriptional repressor of E2F target genes, thereby contributing to cell cycle arrest for DNA repair and genomic integrity (47). One study showed that the down-regulation of E2F7 might contribute to platinum resistance, and high expression of E2F7 predicted favorable DFS and OS in OC (14). Clements et al. (48) revealed that BRCA2-deficient cells are less sensitive to PARP inhibitor and cisplatin treatment after E2F7 depletion, thereby indicating that E2F7 could be a putative biomarker for tumor response to PARP inhibitor therapy. In this study, like that of E2F6, no significant difference in E2F7 expression was noted between OC and normal tissues, but E2F7 expression was significantly and negatively correlated with tumor stage in patients with OC. Further, high E2F7 expression was significantly correlated with poor PFS and PPS in all and serous OC patients, indicating its oncogenic role in OC.

### TABLE 2 | The GO function enrichment analysis of E2F family members and neighbor genes in OC (GeneMANIA).

| GO          | Category                                | Description                                | Count | %      | Log_{10}(P) | Log_{10}(q) |
|-------------|-----------------------------------------|--------------------------------------------|-------|--------|-------------|-------------|
| GO:0000082  | GO biological processes                 | G1/S transition of mitotic cell cycle      | 20    | 35.09  | -24.16      | -19.82      |
| GO:0070317  | GO biological processes                 | Negative regulation of G0 to G1 transition| 8     | 14.04  | -13.25      | -10.65      |
| GO:0008285  | GO biological processes                 | Negative regulation of cell proliferation | 17    | 29.82  | -11.84      | -9.31       |
| GO:0071997  | GO biological processes                 | DNA biosynthetic process                  | 10    | 17.54  | -10.36      | -7.85       |
| GO:0006260  | GO biological processes                 | DNA replication                            | 10    | 17.54  | -9.09       | -6.60       |
| GO:0000723  | GO biological processes                 | Telomere maintenance                      | 8     | 14.04  | -8.35       | -5.89       |
| GO:0045596  | GO biological processes                 | Negative regulation of cell differentiation| 13    | 22.81  | -7.90       | -5.48       |
| GO:0071930  | GO biological processes                 | Regulation of transcription involved in G1/S transition of mitotic cell cycle | 3    | 5.26  | -7.85      | -5.44       |
| GO:0072332  | GO Biological processes                 | Intrinsic apoptotic signaling pathway by p53 class mediator | 6    | 10.53  | -7.53      | -5.13       |
| GO:0001889  | GO biological processes                 | Liver development                          | 7     | 12.28  | -7.38       | -5.01       |
| GO:0097190  | GO biological processes                 | Apoptotic signaling pathway                | 11    | 19.30  | -6.89       | -4.57       |
| GO:0000228  | GO cellular components                  | Nuclear chromosome                         | 23    | 40.35  | -21.41      | -17.68      |
| GO:1990234  | GO cellular components                  | Transferase complex                        | 21    | 36.84  | -16.20      | -13.23      |
| GO:0070603  | GO cellular components                  | SWI/SNF superfamily-type complex           | 7     | 12.28  | -9.24       | -6.74       |
| GO:0016604  | GO cellular components                  | Nuclear body                               | 13    | 22.81  | -7.47       | -5.08       |
| GO:0000976  | GO molecular functions                  | Transcription regulatory region sequence-specific DNA binding | 25    | 43.86  | -21.41      | -17.68      |
| GO:0003712  | GO molecular functions                  | transcription coregulator activity         | 18    | 31.58  | -15.31      | -12.52      |
| GO:0001981  | GO molecular functions                  | Promoter-specific chromatin binding        | 6     | 10.53  | -8.96       | -6.48       |
| GO:0001227  | GO molecular functions                  | DNA-binding transcription repressor activity, RNA polymerase II-specific | 9    | 15.79  | -7.82      | -5.40       |
| GO:00001085 | GO molecular functions                  | RNA polymerase II transcription factor binding | 7    | 12.28  | -7.25      | -4.90       |

### TABLE 3 | The KEGG function enrichment analysis of E2F family members and neighbor genes in OC (GeneMANIA).

| GO          | Category                                | Description                                | Count | %      | Log_{10}(P) | Log_{10}(q) |
|-------------|-----------------------------------------|--------------------------------------------|-------|--------|-------------|-------------|
| hsa04110    | KEGG pathway                            | Cell cycle                                 | 21    | 36.84  | -33.24      | -30.55      |
| hsa05166    | KEGG pathway                            | HTLV-I infection                           | 17    | 29.82  | -19.54      | -17.15      |
| hsa05203    | KEGG pathway                            | Viral carcinogenesis                        | 12    | 21.05  | -13.20      | -11.51      |
| hsa05169    | KEGG pathway                            | Epstein-Barr virus infection               | 10    | 17.54  | -10.20      | -8.88       |
| hsa04330    | KEGG pathway                            | Notch signaling pathway                    | 5     | 8.77   | -9.32       | -5.86       |
| hsa04068    | KEGG pathway                            | FoxO signaling pathway                     | 6     | 10.52  | -6.06       | -4.73       |
| hsa04310    | KEGG pathway                            | Wnt signaling pathway                      | 6     | 10.52  | -8.64      | -4.55       |
| hsa04350    | KEGG pathway                            | TGF-beta signaling pathway                 | 5     | 8.77   | -7.05      | -4.09       |
| hsa03410    | KEGG pathway                            | Base excision repair                       | 3     | 5.26   | -4.10       | -2.90       |
| hsa04137    | KEGG pathway                            | Mitophagy—animal                           | 3     | 5.26   | -3.26      | -2.11       |
| hsa04630    | KEGG pathway                            | Jak-STAT signaling pathway                 | 4     | 7.01   | -3.23      | -2.10       |
| hsa00240    | KEGG pathway                            | Pyrimidine metabolism                      | 3     | 5.26   | -2.65      | -1.59       |
**E2F8** is a recently identified member of the E2F family with a duplicated DNA-binding domain feature discriminated from that in E2F1-6 (49). Accumulating evidence indicates that E2F8 is indispensable for angiogenesis, lymphangiogenesis, and embryonic development. E2F8 is highly expressed in various tumors and promotes tumor progression, and serves...
as a therapeutic target in lung and liver cancers (50, 51). Unfortunately, there is little research evidence between E2F8 and ovarian cancer diagnosis, staging, prognosis, and targeted drug therapy. In this study, E2F8 was significantly overexpressed in OC tissues, and its expression was markedly and negatively correlated with the tumor stage of patients with OC. Interestingly, high E2F8 expression was significantly correlated with poor OS and PFS in all patients with OC.

Growing evidence suggests that the cross-talk of the eight members of the E2F family is causatively involved in cell cycle control, cell proliferation, apoptosis, and carcinogenesis (8, 9, 31, 52). In this study, co-expression and correlation analyses of the E2F family were performed at both the gene and protein levels. These findings are similar to those of previous studies. For example, E2F1 and E2F3 were shown to be target genes involved in the p53 and p73 pathways for inducing apoptosis in a transgenic mouse model (53). Reimer et al. (14, 15) showed that deregulation of both proliferation-promoting and proliferation-inhibiting E2F transcription factors and their cross-talk is crucial for tumor progression of OC and influence clinical outcome; thus, they could be possible useful targets in anti-cancer therapy. Although we partially recognized the important role of E2F interactions in the pathogenesis and development of OC, the cross-talk and specific molecular mechanisms of E2F family members remain to be further investigated.

To further clarify the genetic alteration, potential function, and carcinogenic mechanism of the E2F family members, we calculated the percentages of genetic alterations in E2F family members for OC and found that they varied from 3 to 14% for individual genes based on TCGA Provisional dataset. Further, cases with alterations in one of the query gene had worse OS and DFS than those without any alterations in the query genes, but the difference was not statistically significant. We constructed a network for E2F family members and 50 neighboring genes. The results of functional analysis indicated that these genes are mainly enriched in tumor-related pathways related to the development of multiple tumors. Our study adds to the growing evidence regarding the complexity of the E2F family members and their associated signaling pathways, which offer clues into the rational development of multi-targeted and E2F-mediated targeted therapy.

CONCLUSIONS

In summary, our results indicated that the mRNA expression levels of E2F1, E2F3, E2F5, and E2F8 were significantly upregulated, and obvious and negatively associated with tumor stage for OC. Furthermore, aberrant expression of E2F2, E2F5, E2F7, and E2F8 were found to be associated with the clinical outcomes of patients with OC. These results suggest that E2F2, E2F5, and E2F8 may serve as potential prognostic biomarkers and targets for OC. These results may be beneficial to better understand the molecular underpinning of OC and may be useful to develop tools for more accurate OC prognosis and for promoting the development of E2F-mediated drug for OC treatment. However, future studies are required to validate our findings and thus promote the clinical utility of E2Fs serving as prognostic indicators or therapeutic targets in OC.

AUTHOR CONTRIBUTIONS

QZ and M-ZZ developed the idea and designed the research. FZ and ZH analyzed the data. QZ and ZH wrote the draft of the manuscript. QZ and M-ZZ obtained copies of studies and revised the writing. All authors read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/onc.2019.00161/full#supplementary-material

Appendix | The prognostic value of mRNA level of E2F family members in OC patients using Kaplan-Meier plotter (p > 0.05).

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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