

The roles of E2Fs in gastric cancer: the transcription factors E2F1/2/3/4/5 as potential therapeutic targets and E2F6/7/8 as new prognostic biomarkers in gastric cancer

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Abstract

Background

E2F is a family of transcription factor proteins with multiple functions; E2F proteins are involved in cell cycle regulation, cell differentiation, the DNA damage response and cell death. Studies have shown that E2Fs have prognostic significance in many cancers, but the expression patterns and prognostic values of E2Fs in gastric cancer have not been systematically elucidated.

Methods

In this study, we used the ONCOMINE database and UALCAN online analysis website to compare the transcriptional levels and expression of eight E2F family members between gastric cancer and normal samples. UALCAN was also used to analyze the relationship between the expression of 8 E2F members and clinicopathological parameters. The prognostic value of the E2Fs were determined by Kaplan-Meier Plotter. A protein-protein interaction (PPI) network was constructed using the STRING database. The functions and pathways of E2F family and its neighboring 50 frequently changed genes were analyzed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) software. Finally, quantitative PCR was used to verify the expression of E2Fs in gastric cancer cells.

Results

The expression patterns of 8 E2F members were significantly related to the clinical cancer stage and tumor grade of gastric cancer patients. High mRNA expression of E2F1/2/3/4/5 was a prognostic factor for poor OS in gastric cancer patients, while high expression of E2F6/7/8 was associated with better OS. In addition, a high mutation rate (46%) for E2Fs was observed in patients with gastric cancer. The expression levels of E2F1/2/3/5/8 in AGS and HGC27 cell lines were higher than those in normal cells, while E2F7 showed the opposite trend.

Conclusions

In summary, these results indicate that E2F1/2/3/4/5 can be used as potential therapeutic targets in gastric cancer, and E2F6/7/8 can be used as new prognostic markers to improve the survival rate and prognostic accuracy in gastric cancer.

Introduction:

Gastric cancer, the second leading cause of cancer-related deaths, is one of the most common malignant tumors in the world (1). Despite improvements in surgical techniques and chemotherapy regimens, patient treatment results are often disappointing. Most patients with gastric cancer are diagnosed at an advanced stage, and the five-year survival rate is still low (2). Therefore, the identification of prognostic markers related to gastric cancer is crucial to developing the individualized treatment plans for gastric cancer patients and improving the clinical outcomes of patients.

E2F is a family of transcription factor proteins, and E2F proteins are considered to be the main regulators of cell growth and proliferation. The E2F family is usually divided into two categories according to function: transcriptional activators (E2F1, E2F2 and E2F3a) and transcriptional repressors (E2F3b and E2F4-8) (3). The main functions of E2F proteins are to regulate the cell cycle, cell differentiation, the DNA damage response and cell death (4). E2F proteins have been found in several human malignant tumors, including breast cancer (5), ovarian cancer (6), bladder cancer (7), prostate cancer (8), lung cancer (9) and gastrointestinal cancer (10).

To date, eight E2F factors have been identified in mammals, and these proteins (E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7, and E2F8) were numbered according to the order of their discovery (11). E2F family member activators may have carcinogenic effects, and E2F family repressors may be related to tumor suppression (12). In most human tumors, E2F transcription factors undergo transcriptional changes or deregulation through different molecular mechanisms that inactivate the Rb family, and their uncontrolled expression can induce inappropriate S-phase entry and apoptosis (13). Studies have shown that the overexpression of E2F1 in gastric cancer promotes cell death through various mechanisms, demonstrating the role of E2F1 in suppressing gastric cancer tumors (14). However, the expression levels of E2F family members in gastric cancer are unregulated, and their relationships with clinicopathological characteristics and prognosis have not been systematically clarified.

Bioinformatic analysis based on high-throughput sequencing is an important method to explore the molecular mechanisms of tumorigenesis and development and identify biomarkers that can be used for early diagnosis and treatment. With the development of microarray technology, RNA and DNA research has become an important part of biology and biomedicine. By analyzing thousands of published gene expression levels or copy number variations, we studied the expression and mutation of the E2F family in gastric cancer patients in detail to determine the expression patterns, potential functions and unique prognostic values of E2F proteins in gastric cancer.

Materials And Methods:

1. ONCOMINE database

The ONCOMINE database (www.oncomine.org) is an integrated online cancer microarray database containing data from DNA- and RNA-sequencing (RNA-seq) analyses used for differential expression classification of common cancer types and the corresponding normal tissues, as well as clinical and pathological analyses (15). In our study, the transcriptional expression data for 8 different E2F members in different cancer tissue samples and their corresponding adjacent normal samples were obtained from the ONCOMINE database. Differences in transcriptional expression were compared by Student's t test. The p-value cut-off and fold change threshold were as follows: p-value: 0.01, fold change: 1.5, gene grade: 10%, data type: mRNA.

2. UALCAN

UALCAN (<http://ualcan.path.uab.edu>) is an interactive web resource developed based on the grade 3 RNA sequences and clinical data of 31 cancer types in The Cancer Genome Atlas (TCGA) database. It can be used to analyze the relative transcript expression of genes between tumor and normal samples and the correlations between expression and clinicopathological parameters (16). In this study, UALCAN was used to analyze the mRNA expression of eight E2F family members in primary gastric cancer tissue samples and the relationships of these members with clinicopathological parameters. Differences in transcriptional expression were compared by Student's t test ($p < 0.01$).

3. Kaplan-Meier (K-M) Plotter

The online database K-M Plotter (www.kmplot.com) (17) was used to assess the prognostic value of E2F mRNA expression. The database contains gene expression data and survival information for patients with breast, lung, gastric or ovarian cancer. To analyze the overall survival (OS), progression-free survival (FP) and postprogression survival (PPS) of gastric cancer patients, patient samples were divided into two groups according to the median expression level (high expression and low expression) and validated by K-M survival curves. Information on the number of high-risk cases, mRNA expression levels, hazard ratios (HRs), 95% confidence intervals (CIs) and p-values can be found on the K-M plotter webpage. A p-value < 0.05 was considered statistically significant.

4. TCGA data and cBioPortal

The TCGA also contains sequencing and pathological data for 30 different cancers (18). Using cBioPortal (19) (<https://www.cbioportal.org>), the gastric cancer (TCGA, Provisional) dataset (including data from 478 pathological reports) was selected for further E2F analysis. The genome map included mutations, copy number changes from GISTIC (CNA), mRNA expression z-scores (RNA-seq V2 RSEM) and protein expression z-scores (RPPA).

5. Protein-protein interaction (PPI) network construction and gene enrichment analyses

The STRING database (<http://string-db.org/>) provides the significant associations of PPIs. (20). In this study, the STRING database was used to analyze the E2F family and its neighboring 50 frequently changed genes. We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (<http://www.DAVID.org>) (21) to conduct agonistic gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of 58 genes, including E2F enrichment analysis. GO enrichment analysis can predict gene function based on biological processes (BPs), cell composition (CC) and molecular function (MF), and KEGG can be used to analyze gene enrichment pathways.

6. Cell culture

The AGS, HGC27 and GES-1 cell lines were purchased from the cell bank of the Chinese Academy of Sciences and cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) (Gibco, NY, USA). The cells were placed in an incubator at 37 °C and 5% CO₂. FBS and RPMI-1640 medium were purchased from Gibco (New York, USA).

7. Real-time quantitative PCR (qPCR)

Total RNA (1 µg) was isolated using a TRIzol (Invitrogen, Carlsbad, California, USA) kit, and its concentration and purity were quantified using an ultraviolet spectrophotometer. Thereafter, cDNA was generated from the isolated RNA by reverse transcription using the Transcriptor First Strand cDNA Synthesis Kit (Roche, USA), and real-time PCR was performed using LightCycler 480 SYBR Green Master Mix (Roche Diagnostics GmbH). The expression level of each E2F gene was normalized to that of GAPDH. The cycle threshold (CT) method for relative mRNA expression was analyzed by $2^{-\Delta\Delta}$. The primer sequences are shown in Table 1. The following cycling conditions were used: 95 minutes or five minutes, followed by 40 cycles of 95 °C for 20 seconds and 60 °C for 30 seconds. QPCR assays were conducted in triplicate in a 10-mL reaction volume for each sample. The primers are shown in Table 1.

Table 1
The primer of hub genes.

Primer name	Sense	Antisense
E2F1	ACGCTATGAGACCTCACTGAA	TCCTGGGTCAACCCCTCAAG
E2F2	CGTCCCTGAGTTCCCAACC	GCGAAGTGTCATACCGAGTCTT
E2F3	GTATGATACGTCTCTTGGTCTGC	CAAATCCAATACCCCATCGGG
E2F4	CACCACCAAGTTCGTGTCCC	GCGTACAGCTAGGGTGTCA
E2F5	GGGCTGCTCACTACCAAGTTC	CCTACACCTTTCCACTGGATACT
E2F6	CCATGAACAGATTGTCATTGCAG	GGTCCTTTGGTGCTCCTAATATG
E2F7	AGGCAGCCCAGACTAGATTTT	GCTGGCAGCACTAATGAGCA
E2F8	ATCTGCCTTGACGAAGTGGC	GGCGTACTTATTCTCCTCCCC

Results:

1. Expression of E2Fs in patients with gastric cancer

Eight E2F family members have been identified in mammals. By using the ONCOMINE database and UALCAN online analysis website, we compared the transcriptional levels and expression of E2Fs between gastric cancer and normal samples. As shown in Fig. 1 and Table 2, E2F2/3/7 mRNA expression was significantly higher in gastric cancer tissue samples than corresponding normal tissue samples in multiple data sets. The DErrico database showed that compared with that in normal tissue, the expression of E2F2 in gastric intestinal type adenocarcinoma was increased, and its fold change was 3.234 ($p = 3.39E-7$), while Cho observed a 1.473-fold increase in E2F2 mRNA expression in diffuse gastric adenocarcinoma ($p = 1.45E-5$). Significant upregulation of E2F3 expression was also found in gastric cancer tissue samples. In the DErrico dataset, the expression levels of GI and G Mix A were increased by 2.862 times ($p = 4.79E-6$) and 2.374 times ($p = 1.50E-10$), respectively. In the Chen and Cho datasets, E2F3

expression also showed a similar trend. Compared with that in normal tissue samples, the expression of E2F7 in gastric cancer tissue samples was increased by 3.234 times in the DErrico dataset ($p = 3.39E-7$) and by 1.473 times in the Cho dataset ($p = 1.45E-5$). Next, through UALCAN, we further explored the mRNA expression patterns of the 8 E2F family members. Unlike the ONCOMINE database, the resources in UALCAN are based on the level 3 RNA-seq and clinical data of 31 cancer types from the TCGA database. As shown in Fig. 2, compared with that in normal samples, the mRNA expression of the 8 E2F members in primary gastric cancer tissue samples was significantly upregulated (all $p < 0.05$).

2. Relationships between the mRNA expression of E2F family members and clinicopathological parameters in patients with gastric cancer

After discovering the mRNA expression patterns of gastric cancer patients, we analyzed the relationships between the mRNA expression of different E2F family members and clinicopathological parameters, including patient cancer stage and tumor grade, through UALCAN. As shown in Fig. 3, the mRNA expression of eight E2F family members was significantly correlated with the stage of cancer development in patients, and patients with advanced cancer had higher E2F mRNA expression. Among the E2F family members, E2F3, E2F4 and E2F7 had the highest mRNA expression in stage 4. Similarly, the mRNA expression of the eight E2F family members was also significantly correlated with tumor grade. As the tumor grade increased, the mRNA expression of E2Fs tended to be higher. The highest mRNA expression of most E2F factors appeared in grade 3 (Fig. 4). In conclusion, the above results indicate that the mRNA expression of eight E2F family members in gastric cancer patients is significantly correlated with clinicopathological parameters.

3. Prognostic value of E2F mRNA expression in gastric cancer patients

K-M Plotter was used to analyze the survival according to each of 8 E2F members. The results showed that the mRNA expression of E2F family members was closely related to the prognosis of gastric cancer patients. High E2F1 (HR = 2, 95% CI: 1.68–2.38, $p = 1.1e-15$), E2F2 (HR = 1.3, 95% CI: 1.09–1.58, $P = 0.0044$), E2F3 (HR = 1.89, 95% CI: 1.57–2.27, $P = 3.5e-12$), E2F4 (HR = 1.97, 95% CI: 1.65–2.36, $P = 5e-14$), and E2F5 (HR = 1.62, 95% CI: 1.35–1.95, $P = 2e-07$) expression was correlated with poor OS, and high E2F6 (HR = 0.77, 95% CI: 0.65–0.92, $P = 0.0033$), E2F7 (HR = 0.59, 95% CI: 0.47–0.75, $P = 1.1e-05$), and E2F8 (HR = 0.53, 95% CI: 0.44–0.65, $P = 1.8e-10$) expression was associated with better OS (Fig. 5). This indicates that E2F family members can be used as effective biomarkers for the survival of gastric cancer patients.

4. E2F family genomic changes in patients with gastric cancer and the prediction of 50 frequently changed neighboring genes

We used the cBioPortal online analysis tool to perform mutational analysis of the E2F family. As shown in Fig. 6A, the E2F family members had mutations in all four types of gastric cancer, with the highest

mutation rate found in tubular stomach adenocarcinoma (63.29%). The mutation rates in stomach adenocarcinoma, mucinous stomach adenocarcinoma, and diffuse-type stomach adenocarcinoma were 46.64%, 40.91% and 29.71%, respectively. Figure 6B shows that among 360 gastric cancer samples, E2Fs were mutated in 183 samples (51%). E2F1, E2F5, E2F3 and E2F6 ranked the highest four genes of genetic alterations, and their mutation rates were 15%, 15%, 11% and 9%, respectively. To further study the potential connections among E2F family members, the STRING tool was used to mine 50 frequently changed genes in the vicinity of E2F family genes. We found that genes related to the cell cycle, including CCNE1, CCNE2, CDK2, CDK4, CDKN1B, CDKN2A, etc., were closely related to E2F changes (Fig. 6C).

5. Biological function and pathway enrichment analyses of E2Fs and their 50 neighboring genes

The functions of E2Fs and their 50 neighboring genes were analyzed by GO and KEGG analyses in DAVID. GO enrichment analysis predicts gene function from three aspects, namely, biological process, cell composition and molecular function. As shown in Table 3, we found that in biological processes, target genes were mainly enriched in transcription, the G1/S transition of the mitotic cell cycle, and DNA replication initiation, and in cell components, target genes were enriched in the nucleus, nucleoplasm and cytoplasm. The molecular functions were mainly zinc ion binding, DNA binding and transcription factor activity. KEGG enrichment analysis showed that 58 genes were enriched in cancer-related pathways, such as the p53 signaling pathway, TGF-beta signaling pathway, and cellular senescence (Fig. 7).

Table 2

Significant changes of E2Fs expression in transcription level between GC and normal gastric tissues (ONCOMINE).

	Type of GC VS. Normal	Fold Change	P value	t-test	Ref
E2F2	Gastric Intestinal Type Adenocarcinoma	3.234	3.39E-7	5.611	Derrico
	Diffuse Gastric Adenocarcinoma	1.473	1.45E-5	4.620	Cho
E2F3	Gastric Mixed Adenocarcinoma	2.862	4.79E-6	9.767	Derrico
	Gastric Intestinal Type Adenocarcinoma	2.374	1.50E-10	7.842	Derrico
	Diffuse Gastric Adenocarcinoma	1.416	2.56E-4	4.134	Chen
E2F7	Gastric Intestinal Type Adenocarcinoma	3.234	3.39E-7	5.611	Derrico
	Diffuse Gastric Adenocarcinoma	1.473	1.45E-5	4.620	Cho

Table 3
Gene ontology analysis of E2Fs and their 50 neighboring genes in gastric cancer.

Category	Term	Count	P value
GOTERM_BP_DIRECT	transcription, DNA-templated	15	2.51E-12
	negative regulation of transcription from RNA polymerase II promoter	10	1.25E-05
	cell cycle	8	7.22E-12
	positive regulation of transcription from RNA polymerase II promoter	8	0.003713285
	G1/S transition of mitotic cell cycle	6	4.30E-07
	regulation of transcription from RNA polymerase II promoter	6	0.001687836
	DNA replication initiation	5	1.16E-06
	Ras protein signal transduction	5	5.16E-06
	regulation of cell cycle	5	1.15E-04
	positive regulation of transcription, DNA-templated	5	0.011586847
GOTERM_MF_DIRECT	zinc ion binding	10	0.006592598
	DNA binding	9	0.000481922
	transcription factor activity, sequence-specific DNA binding	7	0.005258945
	RNA polymerase II core promoter proximal region sequence-specific DNA binding	6	0.003172364
	cyclin-dependent protein serine/threonine kinase activity	5	1.24E-08
	core promoter binding	5	1.48E-05
	transcription corepressor activity	5	0.000602344
	promoter-specific chromatin binding	4	4.19E-06
	transcription coactivator activity	4	0.009993704
	cyclin-dependent protein serine/threonine kinase regulator activity	3	0.001064817
GOTERM_CC_DIRECT	nucleus	22	2.75E-05

Note: Top 10 terms were selected according to count and P value <0.05. Count: the number of enriched genes in each term.

Category	Term	Count	P value
	nucleoplasm	18	9.35E-07
	transcription factor complex	14	6.00E-15
	cyclin-dependent protein kinase holoenzyme complex	8	9.43E-14
	nucleolus	7	0.012387791
	nuclear chromatin	5	0.001619265
	MLL1 complex	3	0.003318566
	transcriptional repressor complex	3	0.009041742
	PML body	3	0.017212989
	chromatin	3	0.022127818

Note: Top 10 terms were selected according to count and P value <0.05. Count: the number of enriched genes in each term.

6. Expression of E2Fs in gastric cancer cells

We conducted qPCR experiment to confirm the mRNA expression levels of E2Fs in gastric cancer (Fig. 8). The results of the qPCR analyses confirmed that the expression of E2F1/2/3/4/5/6 in AGS and HGC27 cell lines was higher than that in GES-1 cell lines, while that of E2F7 showed the opposite trend. However, in our current study, no differential expression of E2F8 in gastric cancer was found, which suggests that we may need to conduct more experiments to study the role of E2Fs.

Discussion:

As transcription factors that regulate the cell cycle, E2F family members are involved in the development of various cancers (12). Although the role of E2Fs in tumorigenesis and their prognostic value have been confirmed (6–9), it is still necessary to clarify the different roles of E2F family members in gastric cancer. In this study, we analyzed the expression, mutation and prognostic value of different E2F family members in gastric cancer.

Our results showed that mRNA expression of all eight E2F factors was found and that the mRNA expression of E2Fs was closely related to the cancer stage and tumor grade of gastric cancer patients. The prognostic analysis results showed that high expression of E2F1/2/3/4/5 was associated with poor OS in gastric cancer patients, while high expression of E2F6/7/8 was associated with better OS. In addition, a high mutation rate (51%) for E2Fs was observed in gastric cancer patients. Adjacent genes closely related to E2Fs were predicted, and genes related to the cell cycle were found to include CCNE1,

CCNE2, CDK2, CDK4, CDKN1B, and CDKN2A. Related enrichment pathways included the p53 signaling pathway and TGF-beta signaling pathway.

E2F1 is the most studied transcription factor in the E2F family (10). E2F1 can function as an oncogene or tumor suppressor gene to regulate tumorigenesis according to the cellular environment (22). A large number of studies have shown that E2F1 overexpression is of great significance in the poor prognoses of various cancers, including lung cancer (23), breast cancer (24), esophageal cancer (25), hepatocellular carcinoma (26) and pancreatic cancer (27). According to previous studies, the functional role of E2F1 in gastric cancer is different. Studies have shown that E2F1 overexpression inhibits gastric cancer progression in vitro (28). However, in a study by Xu et al., compared with that in noncancerous tissue samples, the expression of E2F1 in gastric cancer tissue samples was significantly upregulated, and its overexpression promoted cell proliferation and tumorigenicity. Patients with higher E2F1 levels have larger tumor sizes, more advanced tumor stages, and poorer survival rates than patients with lower levels (29). This is similar to the results of our study. Overexpression of E2F1 indicated a poor prognosis and was associated with a more advanced clinical stage (stage 3).

E2F2 plays dual roles in the development of tumors. On the one hand, E2F2 can inhibit tumorigenesis by inhibiting cell cycle regulators. On the other hand, E2F2 can act as an "activator" to increase target expression and cause cancer (30). Previous studies have shown that changes in E2F2 protein expression are closely related to the occurrence of different cancers (31). It has been reported that knocking down the expression of E2F2 significantly reduces the metastatic ability of breast cancer cells, and mutations in E2F2 are related to tumor proliferation and survival in breast cancer patients (32). E2F2 functions as an oncogene in liver cancer (33), while in prostate cancer, E2F2 inhibits tumor cell proliferation by targeting miRNAs (34). A study by Wang et al. showed that E2F2 was overexpressed in gastric cancer tissue samples. High levels of E2F2 were positively correlated with poor tumor differentiation, lymph node metastasis, more advanced stages and poorer OS. Silencing E2F2 significantly reduced cell proliferation, invasion and migration (35). This suggests that E2F2 plays a carcinogenic role in gastric cancer. Our research also supports this finding, which shows that E2F2 can be used as an effective biomarker for the diagnosis and treatment of gastric cancer.

E2F3 is considered an oncogene involved in the apoptosis and proliferation of cancer cells and related to cell invasion and migration (36). It has been found to be amplified in a variety of human tumors, including lung cancer (37), bladder cancer (38), liver cancer (39), ovarian cancer (40), breast cancer (41), pancreatic cancer (42), etc. A study by Li et al. found that silencing E2F3 had an inhibitory effect on proliferation and inducing effect on apoptosis in gastric cancer cells (43). There are also reports in the literature indicating that E2F3 can function as a direct target of miRNA to play a carcinogenic role in gastric cancer (44, 45). Our research shows that overexpression of E2F3 is associated with a poor prognosis in patients and occurs in gastric cancer cells, suggesting that E2F3 may be a candidate therapeutic target for gastric cancer patients.

E2F4 is abundant in nonproliferating and differentiated cells and plays an important role in inhibiting proliferation-related genes (46). A recent study showed that overexpression of E2F4 in the breast cancer cell nucleus was associated with various advanced clinical pathological features and a poor clinical prognosis in breast cancer patients (47). Sun et al. found that high E2F4 expression was significantly associated with poor OS, FP and PPS in lung cancer patients (48), while in digestive tract tumors, E2F4 was found to promote the development of liver cancer, colorectal cancer and gastric cancer (10). In our study, high expression of E2F4 was associated with a poor prognosis in gastric cancer patients.

Previous data have shown that E2F5 is overexpressed in various types of human cancer, including breast cancer, ovarian epithelial cancer, prostate cancer, hepatocellular carcinoma, and colorectal cancer, and is closely related to cancer progression and prognosis (49–53). In a study by Li et al., knocking out E2F5 had a significant inhibitory effect on the growth rate of gastric cancer cells, suggesting that E2F5 may be an oncogene in gastric cancer (54). Our results indicate that elevated E2F5 mRNA expression levels are found in gastric cancer and that high expression is associated with an advanced cancer stage and tumor grade and a poor survival rate.

E2F6-8 have similar functions as a repressor group, but they have completely different molecular mechanisms (55). Compared with E2F1-5, E2F6-8 lack the transactivation domain and Rb binding domain, so they can function as independent protein transcriptional repressors (56). In addition, it has been shown that E2F6 plays a repressive role by interacting with the multicomplex, while E2F7 and E2F8 can form homodimers or heterodimers to inhibit the transcription of target genes (57). It has been reported that the expression of E2F6 is related to the prognosis of malignant tumors such as pancreatic cancer (58), breast cancer (59) and nasopharyngeal cancer (60). In gastric cancer, Li et al. found that downregulation of E2F6 expression inhibited the proliferation and invasion of gastric cancer cells, suggesting that E2F6 may play a carcinogenic role in gastric cancer. E2F7 has been found to be involved in the development of breast cancer (61), gallbladder cancer (62), pancreatic cancer (63), cervical cancer (64) and other cancers. E2F8 has also been shown to be involved in the development of various cancers, including breast cancer (65), lung cancer (66), and liver cancer (67), but the roles of E2F7 and E2F8 in gastric cancer have not been reported. K-M plotter analysis found that high expression of E2F6/7/8 was associated with better OS. QPCR results confirmed that compared with normal cells, gastric cancer cell lines had high expression of E2F6, while E2F7 showed the opposite trend. The differential expression of E2F8 in gastric cancer cells was not found., and it is necessary to further explore the biological roles E2F6/7/8 play in gastric cancer.

Our research had some limitations. Most of the data in the study come from online databases, and we need to conduct more research to explore whether E2Fs can be used as diagnostic markers or therapeutic targets. The potential molecular mechanisms of different E2Fs in gastric cancer are also worthy of further research in the future.

Conclusion

In conclusion, our results indicate that the overexpression of eight E2F members was significantly correlated with the clinical cancer stage and pathological tumor grade in gastric cancer patients. In addition, relatively high E2F1/2/3/4/5 mRNA expression was found to be significantly associated with poor OS in gastric cancer patients, while relatively high E2F6/7/8 mRNA expression was associated with better OS. In addition, a high mutation rate (51%) for E2Fs was observed in gastric cancer patients. These results indicate that E2F1/2/3/4/5 are potential therapeutic targets and in gastric cancer, and E2F6/7/8 can be used as potential prognostic markers to improve the survival rate and prognostic accuracy in gastric cancer. The focus of this study is to provide new ideas for the clinical diagnosis and prognostic evaluation of gastric cancer through bioinformatic analysis. Our results provide an important bioinformatic foundation and related theoretical foundation for guiding follow-up research on gastric cancer.

Abbreviations

E2F1

E2F transcription factor 1

E2F2

E2F transcription factor 2

E2F3

E2F transcription factor 3

E2F4

E2F transcription factor 4

E2F5

E2F transcription factor 5

E2F6

E2F transcription factor 6

E2F7

E2F transcription factor 7

E2F8

E2F transcription factor 8

GC

gastric cancer

TCGA

The Cancer Genome Atlas

DAVID

Database for Annotation, Visualization, and Integrated Discovery

K-M Plotter

Kaplan-Meier Plotter

OS

over survival

PPI

protein-protein interaction

GO

gene ontology

KEGG

Kyoto encyclopedia of genes and genomes

BP

biological processes; CC:cellular components; MF:molecular functions

OS

over survival

Declarations

Availability of data and materials

The data that support the findings of this study come from the public free-charged database, and some or all data, models, or code generated or used during the study are available from the corresponding author by request.

Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Note

Top 10 terms were selected according to count and P value < 0.05. Count: the number of enriched genes in each term.

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Authors' contributions

HL and SZ analyzed the data and wrote the manuscript. LS, PW and SL assisted in editing the manuscript. YM, ZL and GW contributed to the design of the study. WQ and JL are the corresponding authors of the paper. All authors read and approved the final manuscript.

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Figures

Analysis Type by Cancer	Cancer vs. Normal							
	E2F1	E2F2	E2F3	E2F4	E2F5	E2F6	E2F7	E2F8
Bladder Cancer	2	1	1					3
Brain and CNS Cancer				1	5	1	3	3
Breast Cancer	4	9	2		2		4	4
Cervical Cancer	1	1	3				1	2
Colorectal Cancer	3		2		11	5	13	1
Esophageal Cancer			3					
Gastric Cancer		1	2				1	
Head and Neck Cancer			6		1		1	
Kidney Cancer	1							
Leukemia	1		2	1	2	3		4
Liver Cancer	1	1	3					2
Lung Cancer	2	2	4		2		2	5
Lymphoma		1	1	2	1	1	1	3
Melanoma	1		2					
Myeloma								
Other Cancer	1	2	4				3	2
Ovarian Cancer	1		2		1			1
Pancreatic Cancer			1				1	1
Prostate Cancer					2			
Sarcoma		1	2			1		1
Significant Unique Analyses	18 4	18 8	37 3	3 1	27 6	5	30 1	27 4
Total Unique Analyses	449	403	438	455	457	206	256	376

Figure 1

Transcriptional expression of E2Fs in 20 different types of cancer diseases (ONCOMINE database). Note: Difference of transcriptional expression was compared by students' t-test. Cut-off of p value and fold change were as following: p value: 0.01, fold change: 1.5, gene rank: 10%, data type: mRNA.

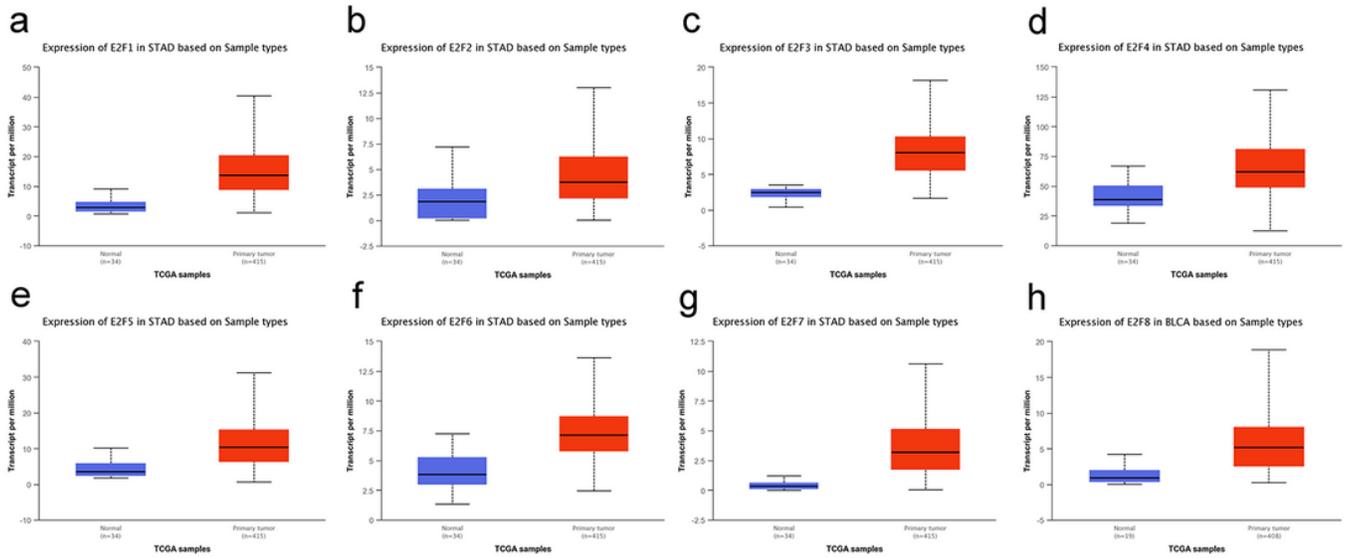


Figure 2

mRNA expression of distinct E2Fs family members in GC tissues and adjacent normal gastric tissues (UALCAN).

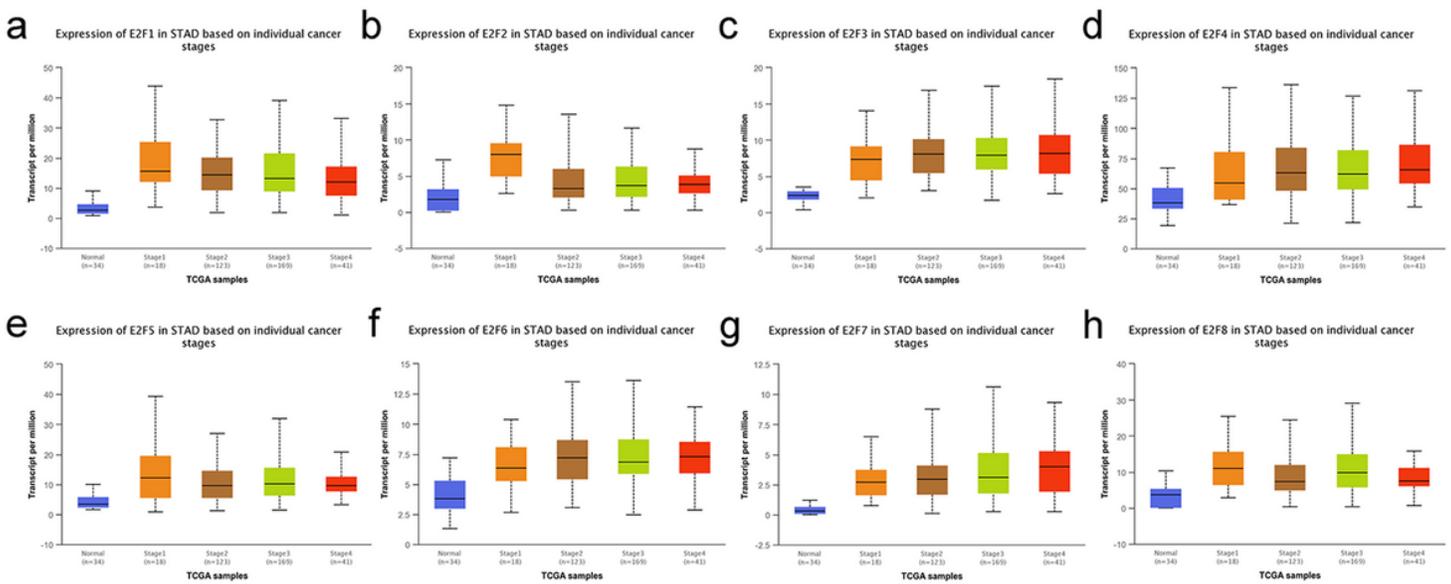


Figure 3

Association of mRNA expression of E2Fs with individual cancer stages of GC patients.

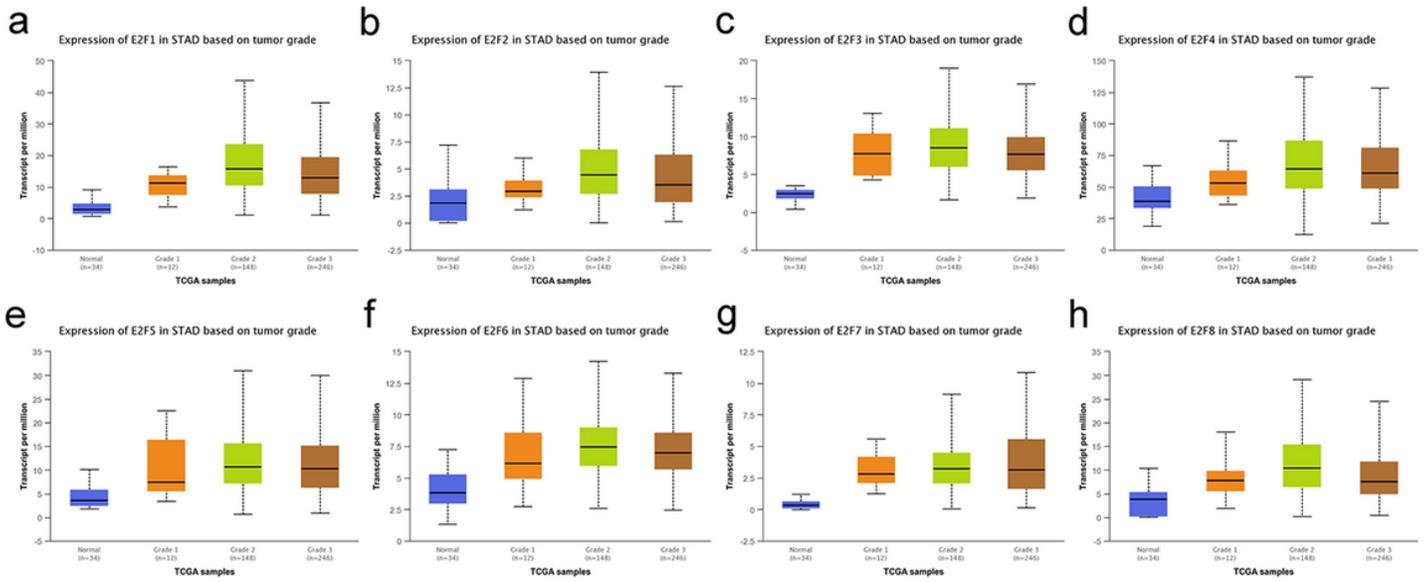


Figure 4

Relationship between mRNA expression of E2F family members and tumor grades of GC patients.

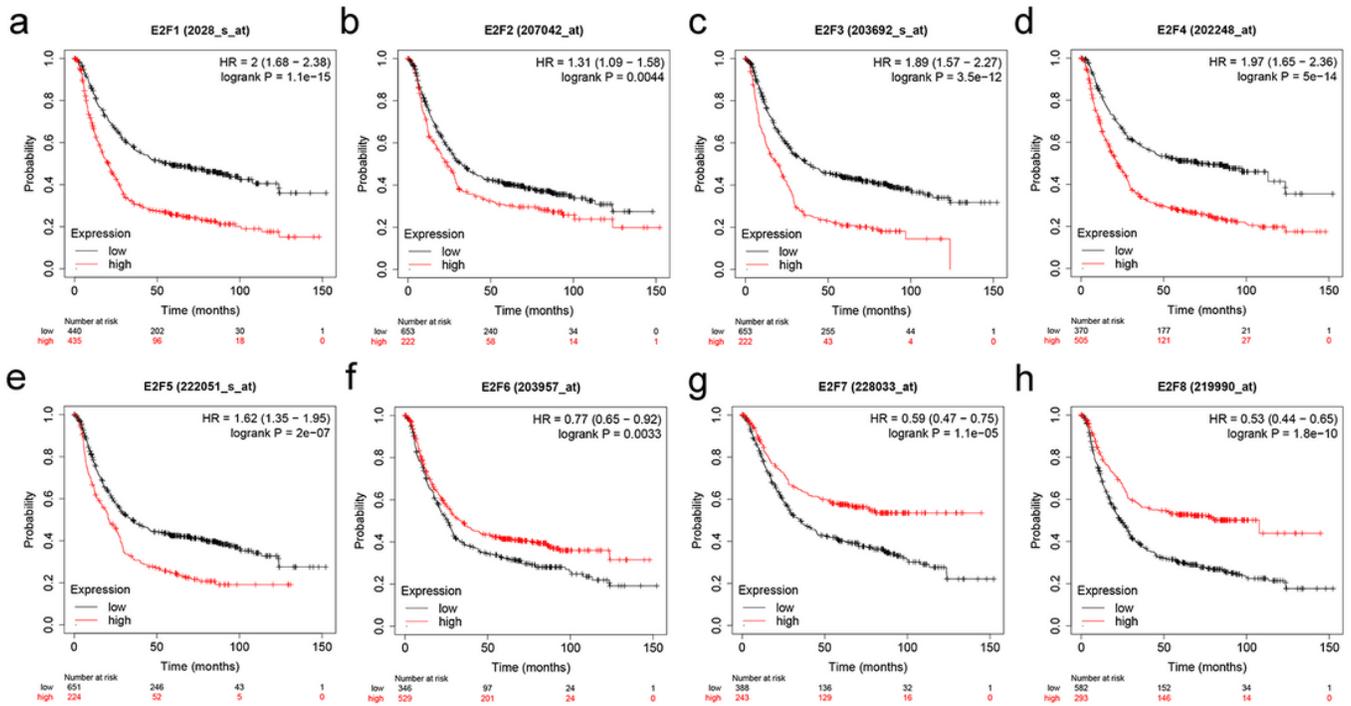


Figure 5

The prognostic value of mRNA level of E2F factors in GC patients (Kaplan-Meier plotter).

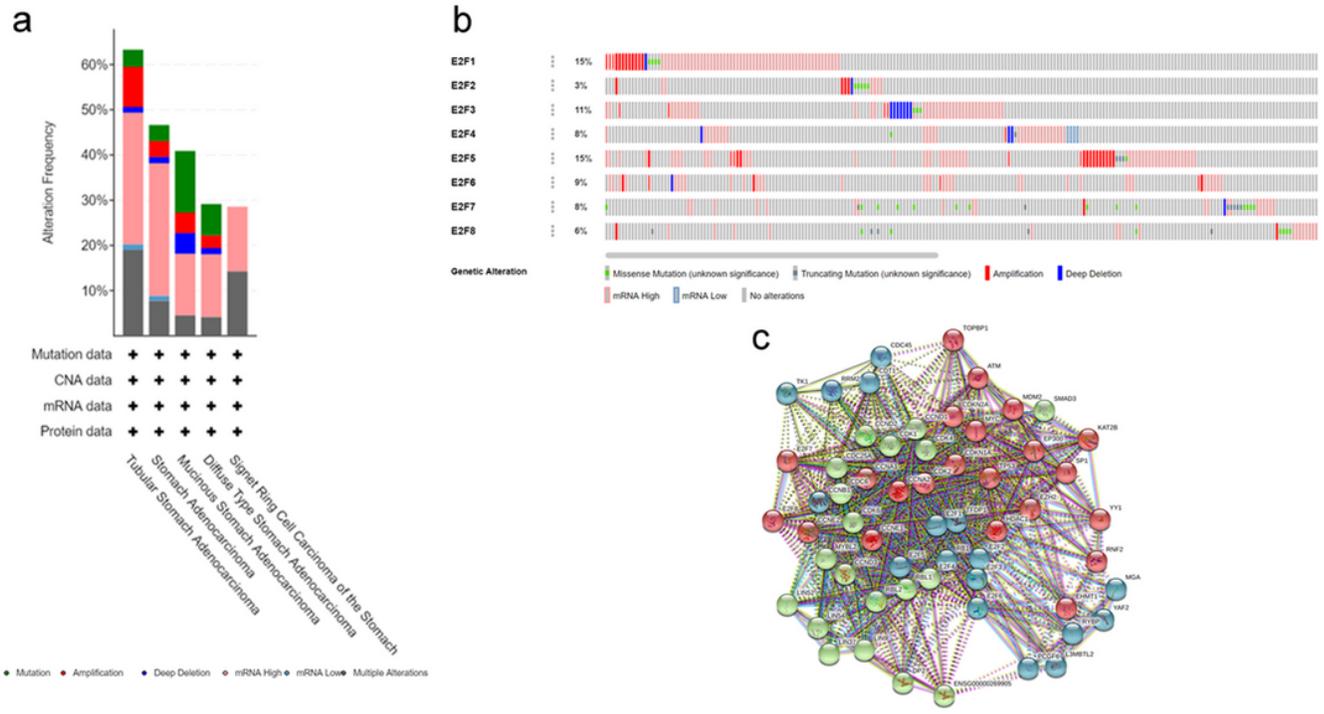


Figure 6

E2F family genomic changes in patients with gastric cancer and the prediction of 50 frequently changed neighboring genes. (A) Genome-specific changes in E2Fs in 5 gastric cancer data sets. (B) High mutation rate (51%) of E2Fs was observed in GC patients. (C) Network of E2Fs mutations and their 50 frequently altered neighbor genes was constructed.

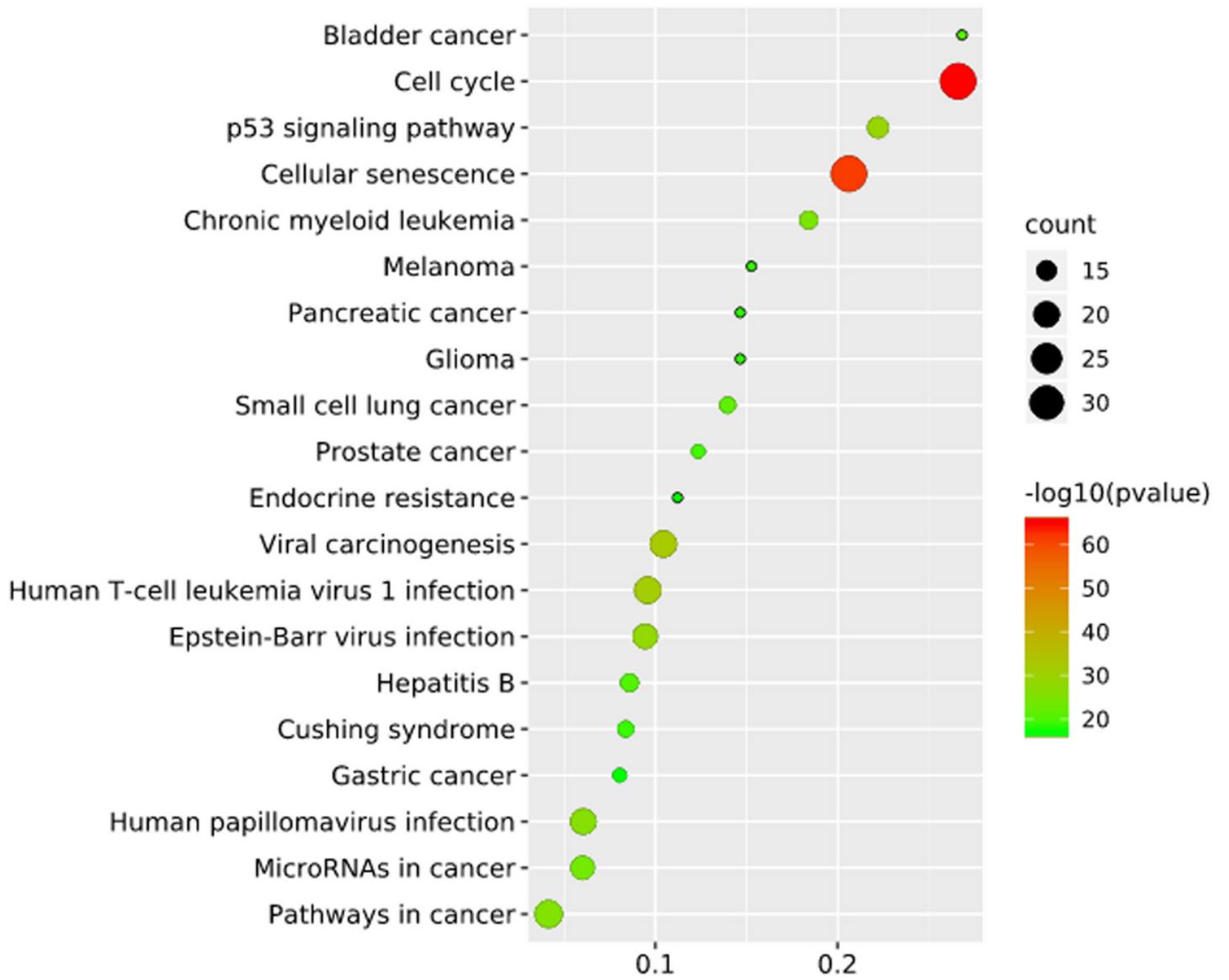


Figure 7

Pathways enrichment map of E2Fs and their 50 neighboring genes.

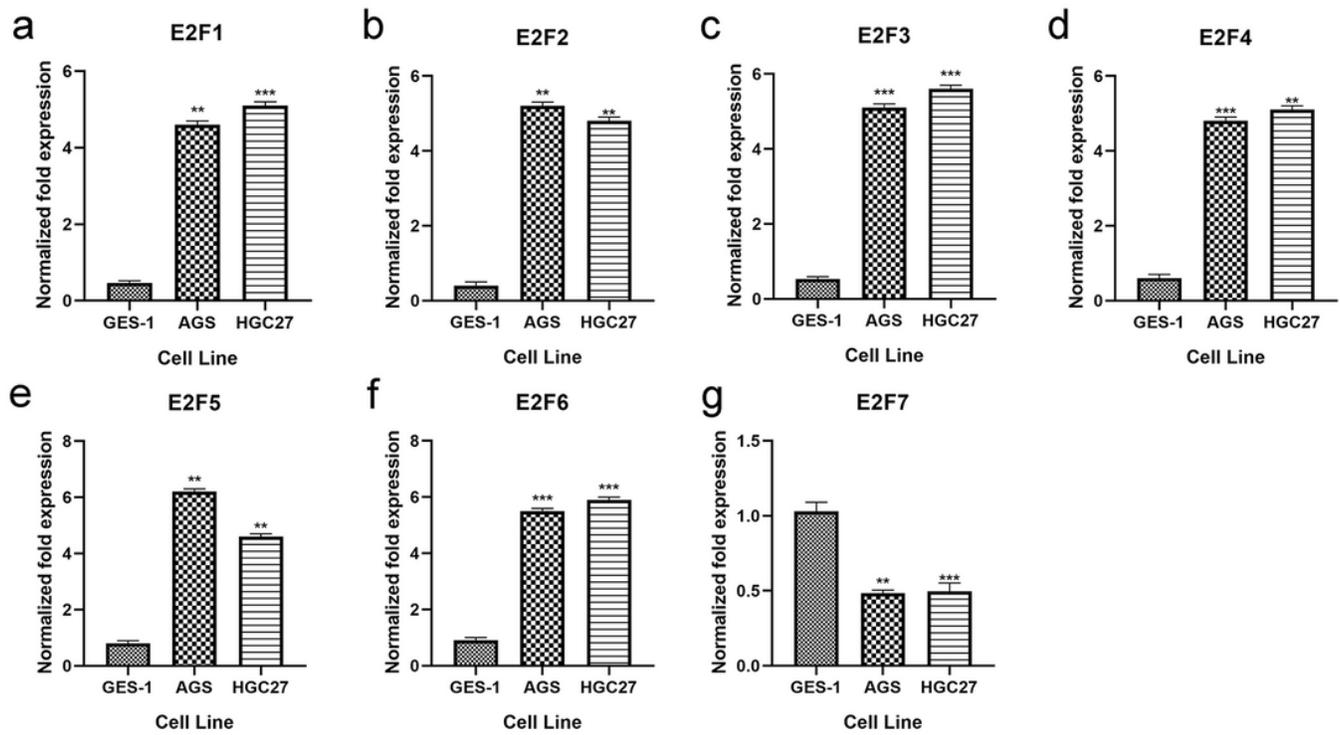


Figure 8

Expression levels of E2Fs in gastric cancer cells. (A-F) qRT-PCR analysis of mRNA expression of E2F1/2/3/4/5/6/7 in GES-1, MGC803 and AGS cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.