The E2F family as potential biomarkers and therapeutic targets in colon cancer

Haibo Yao 1, Fang Lu 2, Yanfei Shao Corresponding Author: Yanfei Shao
Email address: syf2019509@163.com

Background: The E2F family is a group of genes encoding a series of transcription factors in higher eukaryotes and participating in the regulation of cell cycle and DNA synthesis in mammals. This study was designed to investigate the role of E2F family in colon cancer.

Methods: In this study, the transcriptional levels of E2F1-8 in patients with colon cancer from GEPIA was examined. Meanwhile, the immunohistochemical data of the 8 genes were also obtained in the The Human Protein Atlas website. Additionally, we re-identified the mRNA expression levels of these genes via real time PCR. Furthermore, the association between the levels of E2F family and stage plot as wells overall survival of patients with colon cancer were analyzed.

Results: We found that the mRNA and protein levels of E2F1, E2F2, E3F3, E2F5, E2F7 and E2F8 were significantly higher in colon cancer tissues than in normal colon tissues while the expression levels of E2F4 and E2F6 displayed no significant difference between colon cancer tissues and normal tissues. Additionally, E2F3, E2F4, E2F7 and E2F8 were significantly associated with the stages of colon cancer. The Kaplan-Meier Plotter showed that the high levels of E2F3 conferred a worse overall survival and disease free survival of patients with colon cancer. And high level of E2F4 resulted in a worse overall survival.

Conclusion: Our study implied that E2F3, E2F4, E2F7 and E2F8 are potential targets of precision therapy for patients with colon cancer while E2F1, E2F2, E3F3, E2F5, E2F7 and E2F8 are potential biomarkers for the diagnosis of colon cancer.
Title Page

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**Running Title:**
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**Authors:**
Haibo Yao¹, Fang Lu², Yanfei Shao³, *

**Affiliations:**
¹Department of Gastrointestinal and Pancreatic Surgery, Zhejiang Provincial People’s Hospital (People’s Hospital of Hangzhou Medical College, Key Laboratory of Gastroenterology of Zhejiang Province), Hangzhou 310014, China
²Department of Neurology, Second People's Hospital of Yuhang District, Hangzhou 310014, China
³Department of Pharmacy, Zhejiang Provincial People’s Hospital (People’s Hospital of Hangzhou Medical College), Hangzhou 310014, China

*Correspondence to: Yanfei Shao, Department of Pharmacy, Zhejiang Provincial People’s Hospital (People’s Hospital of Hangzhou Medical College), 158 Shangtang Road, Hangzhou 310014, China; Tel: +86-13588702943, Email: syf2019509@163.com

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None.
Abstract

Background: The E2F family is a group of genes encoding a series of transcription factors in higher eukaryotes and participating in the regulation of cell cycle and DNA synthesis in mammals. This study was designed to investigate the role of E2F family in colon cancer.

Methods: In this study, the transcriptional levels of E2F1-8 in patients with colon cancer from GEPIA was examined. Meanwhile, the immunohistochemical data of the 8 genes were also obtained in the The Human Protein Atlas website. Additionally, we re-identified the mRNA expression levels of these genes via real time PCR. Furthermore, the association between the levels of E2F family and stage plot as well as overall survival of patients with colon cancer were analyzed.

Results: We found that the mRNA and protein levels of E2F1, E2F2, E3F3, E2F5, E2F7 and E2F8 were significantly higher in colon cancer tissues than in normal colon tissues while the expression levels of E2F4 and E2F6 displayed no significant difference between colon cancer tissues and normal tissues. Additionally, E2F3, E2F4, E2F7 and E2F8 were significantly associated with the stages of colon cancer. The Kaplan-Meier Plotter showed that the high levels of E2F3 conferred a worse overall survival and disease free survival of patients with colon cancer. And high level of E2F4 resulted in a worse overall survival.

Conclusion: Our study implied that E2F3, E2F4, E2F7 and E2F8 are potential targets of precision therapy for patients with colon cancer while E2F1, E2F2, E3F3, E2F5, E2F7 and E2F8 are potential biomarkers for the diagnosis of colon cancer.

Key words colon cancer; E2F family; prognosis; biomarkers; bioinformatics
1. Introduction

The E2F family is a group of genes that encode a family of transcription factors in higher eukaryotes[1], which are currently subdivided into 2 groups based on their functional characteristics: namely transcriptional activators including E2F1, E2F2, and E2F3a and transcriptional repressors including E2F3b, E2F4, E2F5, E2F6, E2F7 and E2F8 [2]. The E2F family was originally regarded as a cellular activity that is required for the early region 1A transforming protein of adenovirus to mediate the transcriptional activation of the viral E2 promoter[3]. Subsequent studies unveiled that E2F family also controls the transcription of cellular genes that are responsible for cell division[4]. The expression pattern of E2F activators is abnormal in multiple human malignancies, such as ovarian cancer[5], breast cancer[6], bladder cancer[7], prostate cancer[8], lung adenocarcinoma[9] and colon cancer[10].

Colon cancer is a common malignancy, which is currently ranked as the third most prevalent cancer and the third leading cause of cancer death in the United States[10]. Despite that considerable advancements in diagnostic and treatment methods, the 5-year overall survival rate of colon cancer remains no more than 21%[11]. Hence, novel biomarkers and potential therapeutic targets should be screened to enhance prognosis and individualized treatments in patients with colon cancer. Of the 8 members in E2F family, many genes have been disclosed to participate in the development of colon cancer in vitro and in vivo. For instance, E2F1 could upregulate the expression of c-Myc and p14ARF, therefore inducing apoptosis in colon cancer cells[12]. E2F2 was also proved to act as a potential new molecular biomarker for colon carcinogenesis[13]. But to the best of our knowledge, bioinformatics analysis has yet been applied to investigate the roles of E2F gene family in colon cancer. Based on the analyses of thousands of gene expression or variation in copy numbers published online, we analyzed and validated the expression and different E2F transcription factors in patients with colon cancer in detail to explore the expression patterns, potential functions, and distinct prognostic values of transcription factors in colon cancer.

2. Methods

2.1 Ethics statement

Our study was conducted based on the principles expressed in the Declaration of Helsinki, and approved
by the Academic Committee of Zhejiang Provincial People’s Hospital (Ethical Application Ref: IR2019001002).

The datasets mentioned in our study were retrieved from the free and publicly available database, indicating that it was confirmed that all written informed consent was acquired.

2.2 Comparison of the hub genes expression level

GEPIA is a newly developed interactive web server for analyzing the RNA sequencing expression data including 9736 tumors and 8587 normal samples from the TCGA database and the GTEx projects in a standard processing manner[14]. GEPIA provides customizable functions including tumor/normal differential expression analysis, profiling according to cancer types or pathological stages, patient survival analysis, similar gene detection, correlation analysis, and dimensionality reduction analysis. In present study, we mainly employed the boxplot to visualized the expression of E2F genes in colon cancer tissues and adjacent tissues. Additionally, GEPIA was also employed to provide the transcripts per million (TPM) of E2F genes to display their relative expression level.

2.3 The overall survival (OS) and stage plot of E2F

Similarly, we used the GEPIA database to get the overall survival (OS) and stage plot information of E2F genes of 135 patients with high level of E2Fs and 135 patients with low levels of E2Fs. The log rank P value and hazard ratio (HR) with 95% confidence intervals were showed on the plot. P < 0.05 was statistically significant.

2.4 Immunohistochemistry staining

The Human Protein Atlas (HPA, https://www.proteinatlas.org/) is a Swedish-based program initiated in 2003 with the aim to map all human proteins in cells, tissues, and organs using the integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology. By acquiring immunohistochemical data of patients with or without colon cancer based on HPA, we further verified the expression of E2F genes.

2.5 Gene Ontology and KEGG Pathway Analysis of E2F genes

Gene Ontology (GO) analysis is a common framework which can annotate genes and gene products including functions of Cellular Components (CC), Biological Pathways (BP) and Molecular Function (MF). Kyoto Encyclopedia of Genes and Genomes (KEGG) contains a set of genomes and biological pathways related
with disease and drugs online database, which essentially is a resource for systematic understanding of biological system and certain high-level genome functional information. The Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.ncifcrf.gov, version DAVID 6.7) is an online bioinformatics database. It has covered a great many biological data and relevant analysis tools, then provide tools for the biological function annotation information for plenty of genes or proteins. P < 0.05 was considered as the cut-off criterion with significant difference. We could visualize the key biological processes, molecular functions, cellular components and pathways of DEGs by using DAVID online database. And further the scatter plot was performed by ImageGP (http://www.ehbio.com/ImageGP/index.php/Home/Index/index.html) according to the results of GO and KEGG pathway.

2.5 Quantitative Real-Time PCR (qPCR)

Thirty-two pairs of colon tissues and surrounding adjacent tissues were obtained from patients diagnosed with colon cancer in Zhejiang Provincial People’s Hospital. Total RNA (1μg) was isolated using the TRIzol (Invitrogen, Carlsbad, CA, USA) kit, the concentrations and purities of which were quantified using an ultraviolet spectrophotometer. After that, cDNA was generated from RNA via reverse-transcribing using Transcriptor First Strand cDNA Synthesis Kit (Roche, USA) and followed real-time PCR was administrated using LightCycler 480 SYBR Green Master Mix (Roche Diagnostics GmbH)[32]. The expression levels of E2F genes were normalized to GAPDH. Relative mRNA expression levels were analyzed by the $2^{-\Delta\Delta CT}$ cycle threshold (CT) method. The primer sequences are displayed in Table 1.

2.6 TCGA data and cBioPortal

The Cancer Genome Atlas possessed both pathological data and sequencing of 30 different cancers. The liver colon carcinoma (TCGA, Provisional) dataset involving data from 618 samples with pathology reports was chosen for comprehensive analyses of E2Fs using cBioPortal. The genomic profiles included mutations, putative copy-number alterations (CNA) from GISTIC, mRNA expression z-scores (RNASeq V2 RSEM) and protein expression Z-scores (RPPA).

2.7 Statistical analysis

All values were reported as means ± SD. A paired, two-sided Student’s t test was used to compare differences between two groups. Statistical significance was analyzed by SPSS 19.0 software. Differences
were considered significant when P < 0.05.

3. Results

3.1 Expression levels of E2F genes in patients with colon cancer

By analyzing 349 normal colon tissues and 275 colon cancer tissues in GEPIA online website, we found the mRNA expression levels of E2F1, E2F2, E2F3, E2F5, E2F7 and E2F8 were significantly higher in patients with colon cancer than normal control (P < 0.05) while E2F4 and E2F6 displayed no significant difference between colon cancer group and control group (P > 0.05) (Figure 1 A-H). Meanwhile, the above results were further identified using Oncomine database (Supplementary Figure 1).

3.2 Transcripts per million (TPM) of E2F genes

Transcripts per million (TPM) serves as a measurement of the proportion of transcripts in the pool of RNA. In our study, the higher TPM levels of E2F1, E2F2, E2F3, E2F5, E2F7 and E2F8 in colon cancer group were observed (P < 0.05), suggesting that the above 6 genes possessed more transcripts in colon cancer tissues. Consistent with the mRNA levels of E2F4 and E2F6, no significant difference was found between the 2 groups (P > 0.05) (Figure 2).

3.3 Correlation between E2F genes expression and tumor stage in patients with colon cancer

Subsequently, we analyzed the correlation between E2F genes expression and tumor stage in patients with colon cancer based on GEPIA online website. The results demonstrated that the expression levels of E2F3, E2F4, E2F7 and E2F8 displayed significant correlation with the tumor stage in patients with colon cancer while other members in E2F family in normal group and tumor group did not significantly differ (Figure 3A-H).

3.4 GO and KEGG enrichment Analysis

The results (Table 2) from GO term enrichment analysis varied from expression levels and GO classification of the DEGs. By analyzing GO enrichment of these E2F genes via DAVID, we found that the E2F genes in BP were mainly enriched in transcription, DNA-templated, positive regulation of DNA endoreduplication, hepatocyte differentiation, negative regulation of cytokinesis and chorionic trophoblast cell differentiation. As for CC, the E2F genes were principally enriched in transcription factor complex. MF analysis uncovered that the E2F genes were mainly enriched in core promoter binding, transcription factor activity, sequence-specific DNA
binding, DNA binding and transcription corepressor activity.

To acquire a more comprehensive information regarding to the critical pathways of those selected DEGs, KEGG pathways analysis were also carried out via DAVID. The results in Table3 unveiled the most vital KEGG pathways of the E2F genes, which were mainly enriched in cell cycle and various malignant tumors including bladder cancer, non-small cell lung cancer and pancreatic cancer.

3.5 Correlation between E2F genes expression and overall survival in patients with colon cancer

Meanwhile, we further explored the potential association between the expression levels of E2F genes and the overall survival of patients with colon cancer (Figure 4A-H). The Kaplan-Meier showed that E2F3 and E2F4 displayed significantly correlation with the overall survival of patients with colon cancer. To be more specific, the high levels of E2F3 and E2F4 may contribute to worse prognosis of colon cancer (P<0.05). Also, we explored the disease free survival of these genes in patients with colon cancer. The results (Supplementary Figure2) showed that lower level of E2F3 may contribute to better disease free survival in patients with colon cancer. Furthermore, we analyzed the E2F alterations by using the cBioPortal online tool for colon cancer (http://www.cbioportal.org/). E2Fs were altered in 109 samples out of 618 patients with lung adenocarcinoma (17.7%). Two or more alterations were detected in almost one fifth of the samples (Supplementary Figure3).

3.6 The immunohistochemical staining of E2F genes

Additionally, we detected the protein expression levels of E2F genes based on the Human Protein Atlas online database. In accordance with the mRNA expression levels obtained from GEPIA, the protein expression of E2F1, E2F2, E2F3, E2F5, E2F7 and E2F8 were significantly higher in carcinoma tissues (Figure 5).

3.7 Re-identification of the mRNA expression levels of E2F genes

To further valid the expression patterns of E2F genes in colon cancer, we detected the mRNA levels of E2F genes in 32 patients with colon cancer using real time PCR. The results (Figure 6) showed that the mRNA levels of E2F1, E2F2, E2F3, E2F5, E2F7 and E2F8 were significantly lower in adjacent tissues compared with those in carcinoma tissues (Figure 6).

4. Discussion

The dysregulation of E2F genes has been reported in a great many cancers, including gastric cancer, lung
cancer, hepatocellular carcinoma, pancreatic cancer and colon cancer[15][33,34]. Further bioinformatics analysis of colon cancer has to be further performed although the roles of E2F factors in the tumorigenesis and prognosis of certain cancers have been elaborated. To our knowledge, our study was the first time to investigate the mRNA and protein expression, and prognostic values of different E2F genes in patients with colon cancer. We sincerely hope our bioinformatics analysis could contribute to extra proof, optimize treatment strategy, and improve the accuracy of diagnosis and prognosis for patients with colon cancer.

E2F1 is the most explored in colon cancer among the 8 genes of E2F family. E2F1 regulates G1/S-phase transition of cell cycle by transactivating multiple genes including chromosomal DNA replication and its own promoter[16]. E2F1 itself could be regulated in a cell cycle-dependent manner, mainly through temporal association with retinoblastoma, a pocket protein family member. In turn, pocket proteins could be regulated by phosphorylated cyclin-dependent kinase[17]. The transcription of the thymidylate synthase gene was regulated by E2F1 in primary colon cancer specimens, the regulatory pattern of which from E2F1 to thymidylate synthase may be highly conserved during malignant progression of colon cancer[18]. Additionally, in p53 deficient human colon cancer cells, Mdm2 inhibition could trigger cell apoptosis by activating E2F1-and p73-mediated protein expression of Siva-1 and PUMA[19]. In our study, we found that the mRNA and protein expression of E2F1 was significantly higher in colon carcinoma, however, the expression level of which did not affect tumor stage and overall survival, suggesting that E2F1 may just serve as a tumor “switch” to induce tumorigenesis.

E2F2 plays dual roles in the occurrence and development of tumor. On the one hand, E2F2 can indeed serve as an “activator” to increase the expression of its targets[20]. On the other hand, E2F2 can also be a suppressor by repressing cell cycle regulators to maintain quiescence and suppressing myc-induced proliferation and tumorigenesis[21]. For example, E2F2 could suppress the proliferation of T lymphocytes[22]. In fact, the expression level of E2F2 was very low level in colon cancer and E2F2 inhibition could enhance proliferation and cell cycle of colon cancer cells by suppressing the expression of surviving and regulating the expression of C-MYC, CCNA2, MCM4[23] as well as CDK2 [24]. Our analysis unveiled that the mRNA and protein expression levels of E2F2 were significantly higher in colon cancer tissues. Similarly, the expression level of E2F2 was not associated with tumor stage and overall survival in patients with colon cancer.

E2F3 is critical for the transcriptional activation of various oncogenes controlling the rate of proliferation
of both tumor and primary cells. For example, miR-125b could block the colonies form of bladder cancer cells in vitro and to develop tumors in nude mice by targeting E2F3[25]. In colon cancer, E2F3 serves as a direct target of miR-503 that is responsible for the proliferation and cell cycle distribution. The inhibition of E2F3 not only suppressed proliferation but also triggered apoptosis and G0/G1 arrest in SW480 colon cancer cells[25]. In our report, the expression of E2F3 in colon cancer tissues was higher than that in normal tissues. We also disclosed that E2F2 expression was significantly correlated with tumor stage in patients with colon cancer. A high E2F2 expression was significantly correlated with poor survival in patients with LC.

E2F4 as well as its target cyclin A were significantly up-regulated and mostly nuclear in human colon tumor cells compared with the corresponding benign epithelium[26]. The mutations of E2F4 also enhanced the capacity of colon cancer cells to grow without anchorage, therefore giving rise to tumor progression[27]. In our study, although there was no significant difference of the expression between colon cancer tissues and normal tissues, the level of E2F4 was associated with tumor stage as well as overall survival in patients with colon cancer.

E2F5 was a bona fide target gene of miR-34a and the restoration of E2F5 significantly antagonized the suppression of colon cancer cell proliferation and invasion[28]. Our study demonstrated that the expression of E2F5 was significantly increased in colon cancer tissues, however, the level of which did not affect the tumor stage and survival rate of patients with colon cancer.

Up to now, there were no studies on the role of E2F6 in colon cancer published, which was consistent with our bioinformatic analysis, as evidenced by the undifferentiated expression levels of E2F6. Similarly, the expression levels of E2F6 had no effect on the tumor stage and overall survival of patients with colon cancer based on our findings.

The E2F7 gene, located at chromosome 12q21.2, contains 14 exons and acts as a tumor suppressor in the regulation of cell cycle progression[29]. A significant association between E2F7 missense variant rs3829295 and CRC susceptibility, especially in males, was found in a Chinese Han population[30]. Additionally, miR-520a could regulate inflammatory reactions by targeting E2F7 in colon cancer[31]. Our findings disclosed that the expression of E2F3 had an association with colon cancer stage, meanwhile the expression of E2F7 in colon cancer tissues were significantly increased in patients with colon cancer.

As for E2F8, although there were no studies reporting its roles in colon cancer, our findings unveiled its
different expressions between normal tissues and colon cancer tissues, apart from its association with tumor stage. Hence, more experiments should be performed to explore the potential roles of E2F8 in colon cancer.

5. Conclusion

Our studies indicated that the deregulation of E2F1, E2F2, E2F3, E2F5, E2F7 and E2F8 in colon cancer tissues might play a vital role in colon cancer oncogenesis, which could be promising diagnostic biomarkers for LUAD. In addition, the expression of E2F3, E2F4, E2F7 and E2F8 were significantly associated with tumor stages and overall survival of the patients with colon cancer, suggesting that they may serve as potential therapeutic targets for colon cancer. Altogether, we hope our study may be helpful to potential prognostic markers for the improvement of colon cancer survival and prognostic accuracy in the future.
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Figure 1: The mRNA expression level of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7 and E2F8. (A) E2F1; (B) E2F2; (C) E2F3; (D) E2F4; (E) E2F5; (F) E2F6; (G) E2F7 and (H) E2F8;
Figure 2

Figure 2: Transcripts per million (TPM) of E2F1, E2F2, E2F3, E2F4, E2F5, E2F6, E2F7 and E2F8. (A) E2F1; (B) E2F2; (C) E2F3; (D) E2F4; (E) E2F5; (F) E2F6; (G) E2F7 and (H) E2F8.

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Figure 3

Figure 3: The relationship between the level of E2F genes and tumor stages in patients with colon cancer. (A) E2F1; (B) E2F2; (C) E2F3; (D) E2F4; (E) E2F5; (F) E2F6; (G) E2F7 and (H) E2F8.
Figure 4

Figure 4: The relationship between the level of E2F genes and overall survival in patients with colon cancer. (A) E2F1; (B) E2F2; (C) E2F3; (D) E2F4; (E) E2F5; (F) E2F6; (G) E2F7 and (H) E2F8.
Figure 5

Figure 5: The immunohistochemical staining data obtained from HPA database
Figure 6

Figure 6: Validation of E2F genes in the carcinoma tissues and adjacent tissues from patients with colon cancer using real time PCR. *P < 0.05 vs carcinoma tissue.
Table 1. Primer sequences used in this study
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| Gene | Species | Forward             | Reverse               |
|------|---------|---------------------|-----------------------|
| E2F1 | Human   | AGCATGATCCGAGATGGA  | TGCTCGCAGATGCTAGCCT   |
| E2F2 | Human   | ACGATGAGATGCTAGCGT  | CGCGTGACCTGACACTGCT   |
| E2F3 | Human   | ACGTCGATAGCTGATGGC  | CCGTGTACGTACAAAAACTG  |
| E2F4 | Human   | ACAATTGATCGGTGAGCGA | GACATGCGCTGGAGAAAGC   |
| E2F5 | Human   | ACGTGAGAGGTGCAAGTCC | GACATGCGCTGGAGAAAGC   |
| E2F6 | Human   | CGCGTAGCTACGCTACAG  | ACGTGATCGATGCTAGGC    |
| E2F7 | Human   | CACACAGTCAAACCAACC | CGTGTGGGCGACGTGGCAAAC |
| E2F8 | Human   | ACAAGTGCGGTACGTTTCA | ACGATCGATGCTGATCGGA   |
Table 2 (on next page)

Table 2: Gene ontology analysis of E2F family associated with colon cancer

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| Category                | Term                                                                 | Count | %        | P-Value   | FDR       | Genes                  |
|-------------------------|----------------------------------------------------------------------|-------|----------|-----------|-----------|------------------------|
| GOTERM_BP_DIRECT        | GO:0006351–transcription, DNA-templated                            | 6     | 0.423429781 | 6.74E-09  | 5.53E-06  | E2F3, E2F4, E2F5, E2F6, E2F7, E2F8 |
| GOTERM_BP_DIRECT        | GO:0032877–positive regulation of DNA endoreduplication             | 2     | 0.14114326  | 8.11E-04  | 0.663544451 | E2F7, E2F8               |
| GOTERM_BP_DIRECT        | GO:0070365–hepatocyte differentiation                                | 2     | 0.14114326  | 0.001622454 | 1.322792672 | E2F7, E2F8               |
| GOTERM_BP_DIRECT        | GO:0032466–negative regulation of cytokinesis                       | 2     | 0.14114326  | 0.002027739 | 1.65081418  | E2F7, E2F8               |
| GOTERM_BP_DIRECT        | GO:0060718–chorionic trophoblast cell differentiation                | 2     | 0.14114326  | 0.002432892 | 1.977771789  | E2F7, E2F8               |
| GOTERM_CC_DIRECT        | GO:0005667–transcription factor complex                             | 6     | 0.423429781 | 1.61E-10  | 8.27739543574068E-08 | E2F3, E2F4, E2F5, E2F6, E2F7, E2F8 |
| GOTERM_MF_DIRECT        | GO:0001047–core promoter binding                                    | 3     | 0.211714891 | 1.15E-04  | 0.068932147  | E2F3, E2F7, E2F8          |
| GOTERM_MF_DIRECT        | GO:0003700–transcription factor activity, sequence-specific DNA binding | 4    | 0.282286521 | 4.73E-04  | 0.283673732  | E2F3, E2F4, E2F5, E2F6    |
| GOTERM_MF_DIRECT        | GO:0003677–DNA binding                                              | 3     | 0.211714891 | 0.016757148 | 9.649556019  | E2F4, E2F5, E2F6          |
| GOTERM_MF_DIRECT        | GO:0003714–transcription corepressor activity                      | 2     | 0.14114326  | 0.04355609  | 23.46393428  | E2F7, E2F8               |
Table 3: KEGG pathway analysis of E2F family associated with colon cancer
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| Category       | Term                                      | Count | %       | P-Value   | FDR        | Genes                        |
|----------------|-------------------------------------------|-------|---------|-----------|------------|------------------------------|
| KEGG_PATHWAY   | cfa04110:Cell cycle                       | 5     | 0.352858151 | 1.05E-07 | 7.14E-05  | E2F1, E2F2, E2F3, E2F4, E2F5 |
| KEGG_PATHWAY   | cfa05219:Bladder cancer                   | 3     | 0.211714891 | 2.14E-04 | 0.145507524 | E2F1, E2F2, E2F3            |
| KEGG_PATHWAY   | cfa05223:Non-small cell lung cancer       | 3     | 0.211714891 | 4.16E-04 | 0.282144998 | E2F1, E2F2, E2F3            |
| KEGG_PATHWAY   | cfa05212:Pancreatic cancer                | 3     | 0.211714891 | 5.25E-04 | 0.355786534 | E2F1, E2F2, E2F3            |
| KEGG_PATHWAY   | cfa05214:Glioma                           | 3     | 0.211714891 | 5.41E-04 | 0.366990838 | E2F1, E2F2, E2F3            |
| KEGG_PATHWAY   | cfa05218:Melanoma                         | 3     | 0.211714891 | 6.46E-04 | 0.437793075 | E2F1, E2F2, E2F3            |
| KEGG_PATHWAY   | cfa05220:Chronic myeloid leukemia         | 3     | 0.211714891 | 6.64E-04 | 0.450188061 | E2F1, E2F2, E2F3            |
| KEGG_PATHWAY   | cfa05222:Small cell lung cancer           | 3     | 0.211714891 | 8.82E-04 | 0.597682584 | E2F1, E2F2, E2F3            |
| KEGG_PATHWAY   | cfa05215:Prostate cancer                  | 3     | 0.211714891 | 9.69E-04 | 0.656358939 | E2F1, E2F2, E2F3            |
