Original Article

The prognosis analysis of *RFWD2* inhibiting the expression of *ETV1* in colorectal cancer

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Background: The poor prognosis is partly due to the lack of efficient methods for early diagnosis on colorectal cancer (CRC).

Methods: Bioinformatic analysis and Immunohistochemical analysis were used to evaluate E3 ubiquitin ligase Ring finger and WD domain 2 (*RFWD2*) and ETS variant 1 (*ETV1*) mRNA and protein expression levels.

Results: The abundance of RFWD2 and ETV1 proteins from 76 CRC patients were examined. The relationship between their expression levels and clinic pathological parameters including prognostic significances were also detected. The expression of *RFWD2* and *ETV1* and the relative genes functions in CRC through bioinformatics methods were further analyzed.

Conclusions: In conclusion, *RFWD2* functioning as a tumor suppressor by negative regulating *ETV1*, which might play an important role in the development and progression of CRC. *RFWD2* and *ETV1* have the potential to serve as a pair of molecular biomarkers for the early diagnosis of CRC.

Keywords: E3 ubiquitin ligase Ring finger and WD domain 2 (*RFWD2*); ETS variant 1 (*ETV1*); colorectal cancer (CRC); bioinformatics; prognosis

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Introduction

Colorectal cancer (CRC) is one of the common gastrointestinal malignancies, its incidence ranks third in the world, the tumor-related lethality ranks fourth in the world. CRC is a heterogeneous disease that caused by the interaction of genetic and environmental factors (1,2). CRC is one of the most prevalent and deadly incident cancers worldwide (3,4). In recent years, with the improvement of living standards, the incidence and mortality of CRC in China gradually is increasing (5). With improving diagnostic techniques, and by surgery and chemotherapy for CRC had better effect on early treatment (6). However, for advanced CRC patients, due to the chemotherapy-insensitive and easy to relapse lead to the five year survival rate is only 50% (7). Tumor metastasis and recurrence is the most important factor affecting the survival rate of CRC patients. Therefore, studying the molecular mechanism of CRC metastasis and looking for new targets for prevention and treatment are still the hot topics in the field of cancer

research.

RFWD2 also known as *COP1* (constitutive photomorphogenic 1) protein, comprising RING finger, coiled-coil and WD40 domains, is conserved in both higher plants and vertebrates (8). Scientists have pointed at possible roles for mammalian *COP1* in tumorigenesis and the stress response through regulating the activities of *p53* (9) and *c-Jun* (10). Recent genetic studies have shown that RFWD2 deficiency leads to spontaneous tumor formation in mice, and have identified mutations in *RFWD2* and its substrates in various human cancers (11). *RFWD2* was identified with relevant roles in tumorigenesis process.

The oncoproteins *ETV* family is implicated in melanomas, breast and other types of cancer. Complex post-translational modifications govern the activity of PEA3 factors, which promote cell proliferation, motility and invasion (12). The ETS family is one of the largest families of signal-dependent transcriptional regulators (13). It is involved in tumorigenesis and development of a variety of tumors (14). The ETS family is overexpressed in breast cancer (15), prostate cancer (16), melanoma (17), Ewing's tumor (18) and gastrointestinal stromal tumor (19) and also involved in the development of tumors.

Herein, we determined the expression of *RFWD2* and *ETV1* in CRC through bioinformatic analysis, aiming to ascertain whether they are potential molecular biomarkers for the early diagnosis of CRC and to obtain clues for the pathogenesis of CRC.

Methods

Ethics statement

The research was approved by the Ethics Committee of Guangzhou Medical University (No. 2018-KY-106C), and written informed consent was obtained from each patient involved in the study.

Immunohistochemical analysis

Archived and paraffin-embedded samples were obtained from 76 CRC patients who underwent surgical resection between January 2013 and March 2019 in the Second Affiliated Hospital of Guangzhou University and the Affiliated Shunde Hospital of Guangzhou Medical University. Immunohistochemical staining of *ETV1* or *RFWD2* was carried out according to the manufacture's protocol. In briefly, the sections were incubated overnight in a moist box with antibodies of *ETV1* (1:100; ABCAM, USA) or RFWD2 (1:200; ABCAM, USA) in PBS at 4 ℃. Poly peroxidase anti-mouse/rabbit IgG (GSGB-BIO, China) was subjected to the sections for 30 minutes at room temperature after washing with PBS. Diaminobenzidine was used for colorimetric detection and the sections were counterstained with haematoxylin and mounted with distyrene plasticizer xylene (DPX). Negative controls were performed by replacing the primary antibody with preimmune rabbit serum. Positive controls were conducted according to the manufacture's suggestion. For each run of immunohistochemistry, negative and positive controls were performed. Immunostained tissue array sections were reviewed under a microscope by two pathologists, who were blinded regarding the clinicopathological characteristics and outcome of the patients, while visually scoring each individual tissue core. For positively stained cells: 0 (negative) was denoted for <10% positive cells, 1 (weak) for <25% positive cells, 2 (moderate) for <50% positive cells, and 3 (strong) for >50% positive cells. The staining intensity was defined as: 0 for no stain, 1 for weak-positive (faint yellow), 2 for moderate-positive (yellowish-brown), and 3 for strong-positive (brown). Scores of the proportion and intensity of positively stained tumor cells were added and stratified as having negative (–) expression (0–3 score) and positive (+) expression (4–6 score).

Bioinformatic analysis of RFWD2 and ETV1

The human patients' samples from The Cancer Genome Atlas (TCGA; https://cancergenome.nih.gov/) were analyzed to investigate the clinical significance of *RFWD2* and *ETV1* expression in CRC patients. Cancer Cell Line Encyclopedia databases (CCLE, https://portals.broadinstitute.org/ccle) was used to investigate the expression levels of *RFWD2* and *ETV1*. In Linkedomics (http://www.linkedomics.org/) we dug out the positively and negatively associated genes regulated by *RFWD2* and *ETV1*. To explore the functional annotation and pathway enrichment of *RFWD2* and *ETV1* genes, the Gene Ontology (GO; http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.kegg.jp/kegg/kegg2.html) database analyses were conducted using a Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 online analysis tool with P<0.05 as the significant threshold to obtain significant gene sets.

Statistical analysis

Data were expressed as the mean ± SD from at least three independent experiments for each group. The differences between *RFWD2* or *ETV1* expression levels with the clinicopathological features groups were analyzed using a factorial model one-way analysis of variance. All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and P less than 0.05 was considered statistically significant.

Results

Association of RFWD2 and ETV1 expression with clinicopathological characteristics in CRC patients

The human patients' samples from TCGA were analyzed to investigate the clinical significance of *RFWD2* and *ETV1* expression levels in CRC patients. In *Figure 1*, the results showed that RFWD2 expression was obviously different in major cancer stage (P<0.001; *Figure 1A*) and individual cancer stages (P=0.0057 normal *vs.* stage 1; P<0.001, normal *vs.* stage 2; P=0.0132 normal *vs.* stage 3; *Figure 1B*). Both gender showed the significance in RFWD2 expression compared with normal (P=0.0066 normal *vs.* male; P<0.001, normal *vs.* female; *Figure 1C*). Interestingly, the patients' weight whether normal or overweight were all associated with RFWD2 protein expression (P=0.0102 normal *vs.* normal weight; P=0.0064 normal *vs.* extreme weight; P=0.0396 normal *vs.* obese; P=0.0028 normal *vs.* extreme obese; *Figure 1D*). The other characteristics, such as age and race were also associated with RFWD2 protein expression (P=0.0010, normal *vs.* Caucasian; P<0.001, normal *vs.* African American; *Figure 1E*. P<0.001, normal *vs.* 41–60 years; P<0.001, normal *vs.* 61–80 years; *Figure 1F*).

As summarized in *Figure 2*, the expression level of ETV1 had significant differences in major cancer stage (P=0.0349; *Figure 2A*) and individual cancer stages (P=0.0072 normal *vs.* stage 3; P=0.0229 stage 1 *vs.* stage 3; *Figure 2B*), female patients had more ETV1 expression than normal (P=0.0313; *Figure 2C*). The ETV1 expression levels of extreme obese patients and Caucasian people were higher compared with normal respectively (P=0.0152; *Figure 2D*; P=0.0166; *Figure 2E*), but not in the patients' age (*Figure 2F*).

Expression and correlation analysis of RFWD2 and ETV1 in CRC tissues and cell lines

In addition, the results of Cancer Cell Line Encyclopedia databases was consistent with that of TCGA, demonstrating the mRNA expression level of *RFWD2* was more than 1.8 times of *ETV1* (*Figure 3A*,*B*), the spearman correlative analysis showed that their expression was negative relevant in colorectal tumor tissue (r^2=0.2015, F=14.13, P<0.001, *Figure 3C*).

Immunohistochemical and prognosis Analysis of RFWD2 and ETV1 in CRC patients

Among the 76 cases of CRC 25 cases (32.9%) were *RFWD2*-positive and 51 (67.1%) were *RFWD2*-negative; whereas 47 cases (61.8%) were *ETV1*-positive and 29 (38.2%) were *ETV1*-negative. Representative images of immunohistochemical analysis of *RFWD2* and *ETV1* in CRC patients were showed in *Figure 4*. *ETV1* expression status according to *RFWD2* expression and their corresponding clinicopathological characteristics were summarized in *Table 1*. Either *ETV1* or *RFWD2* expression status was significantly associated with TNM stages of CRC patients, the number of lymph nodes involved and tumor relapse.

Then, the proteins expression status of RFWD2 or ETV1 in CRC tissues was investigated for associations with overall survival by using logrank test for significance estimates. As indicated by the results, patients with high RFWD2 expression showed a significantly longer cancer-specific survival than those with a low level of *RFWD2* expression (P=0.0479; *Figure 5A*). However, patients with high ETV1 expression had a markedly poor overall survival compared to patients with low ETV1 expression (P=0.0207; *Figure 5B*). These results suggested that *RFWD2* and *ETV1* might have the opposite relationship in tumorigenesis process.

The gene enrichment analysis of RFWD2 and ETV1 in CRC patients

Since *RFWD2* and *ETV1* had the opposite relationship in expression and function as mentioned before, we next performed further exploration on the potential roles of *ETV1* and *RFWD2* in CRC, in connection with other featured biomarkers. In Linkedomics website, the positively and negatively associated genes regulated by RFWD2 and *ETV1* were dug out.

To systematically summarize *RFWD2* and *ETV1* gene function, Gene ontology enrichment analysis was performed to analyze *RFWD2* and *ETV1* associated genes. Nine

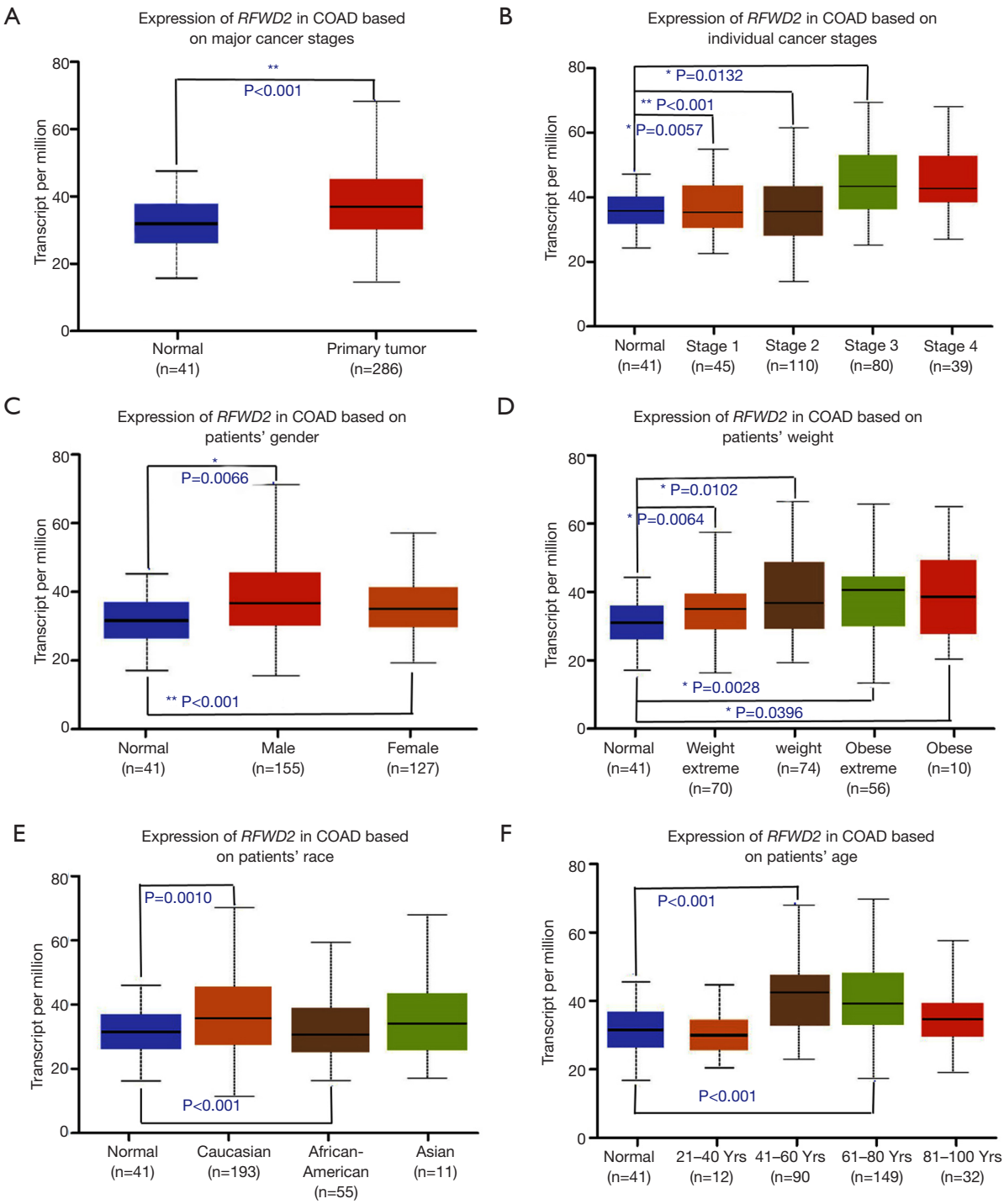


Figure 1 *RFWD2* expression data from TCGA. (A) Expression of *RFWD2* in COAD based on major cancer stages; (B) expression of *RFWD2* in COAD based on individual cancer; (C) expression of *RFWD2* in COAD based on patient's gender; (D) expression of *RFWD2* in COAD based on patient's weight; (E) expression of *RFWD2* in COAD based on patient's race; (F) expression of *RFWD2* in COAD based on patient's weight. *, P<0.05; **, P<0.001. *RFWD2*, E3 ubiquitin ligase Ring finger and WD domain 2.

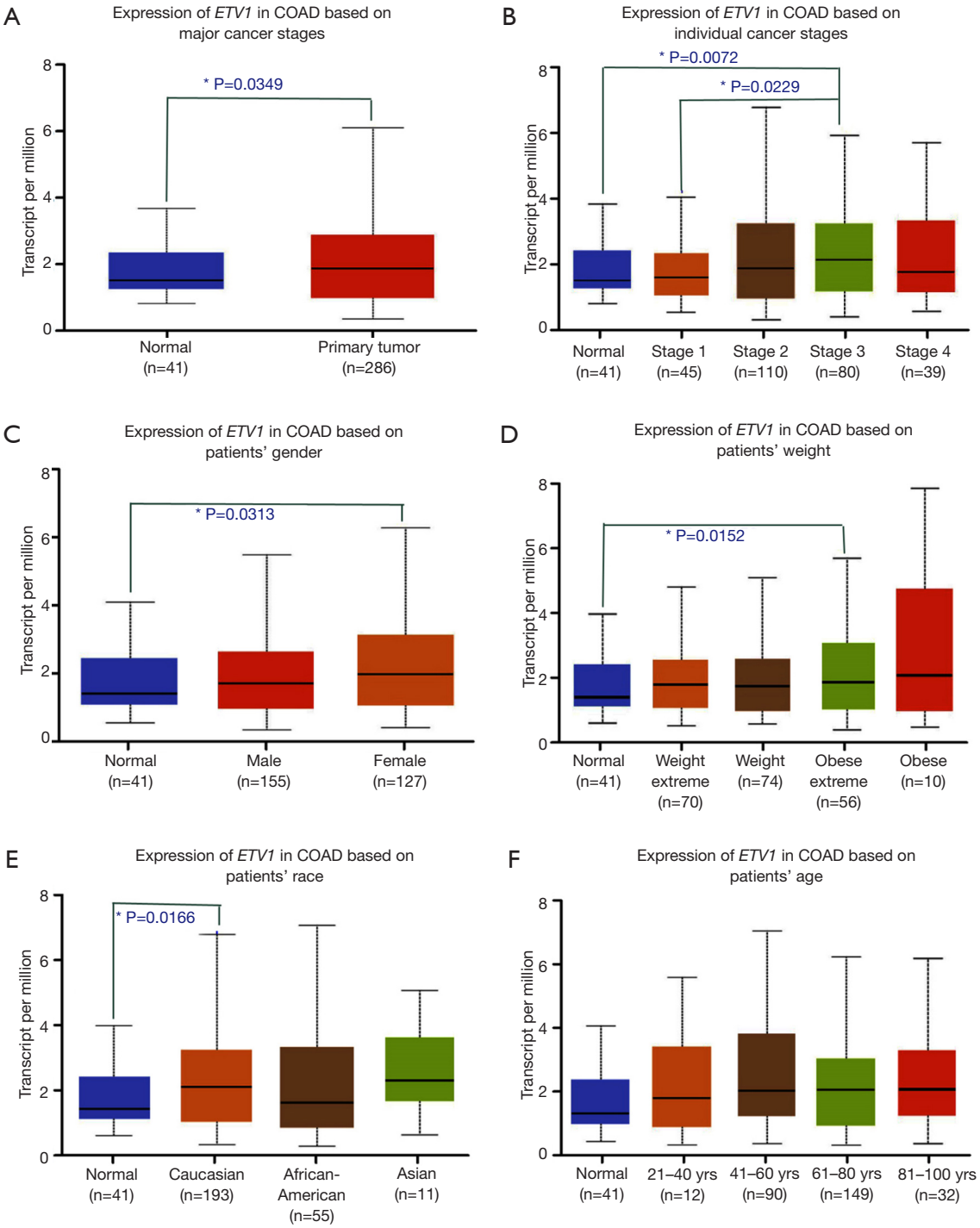
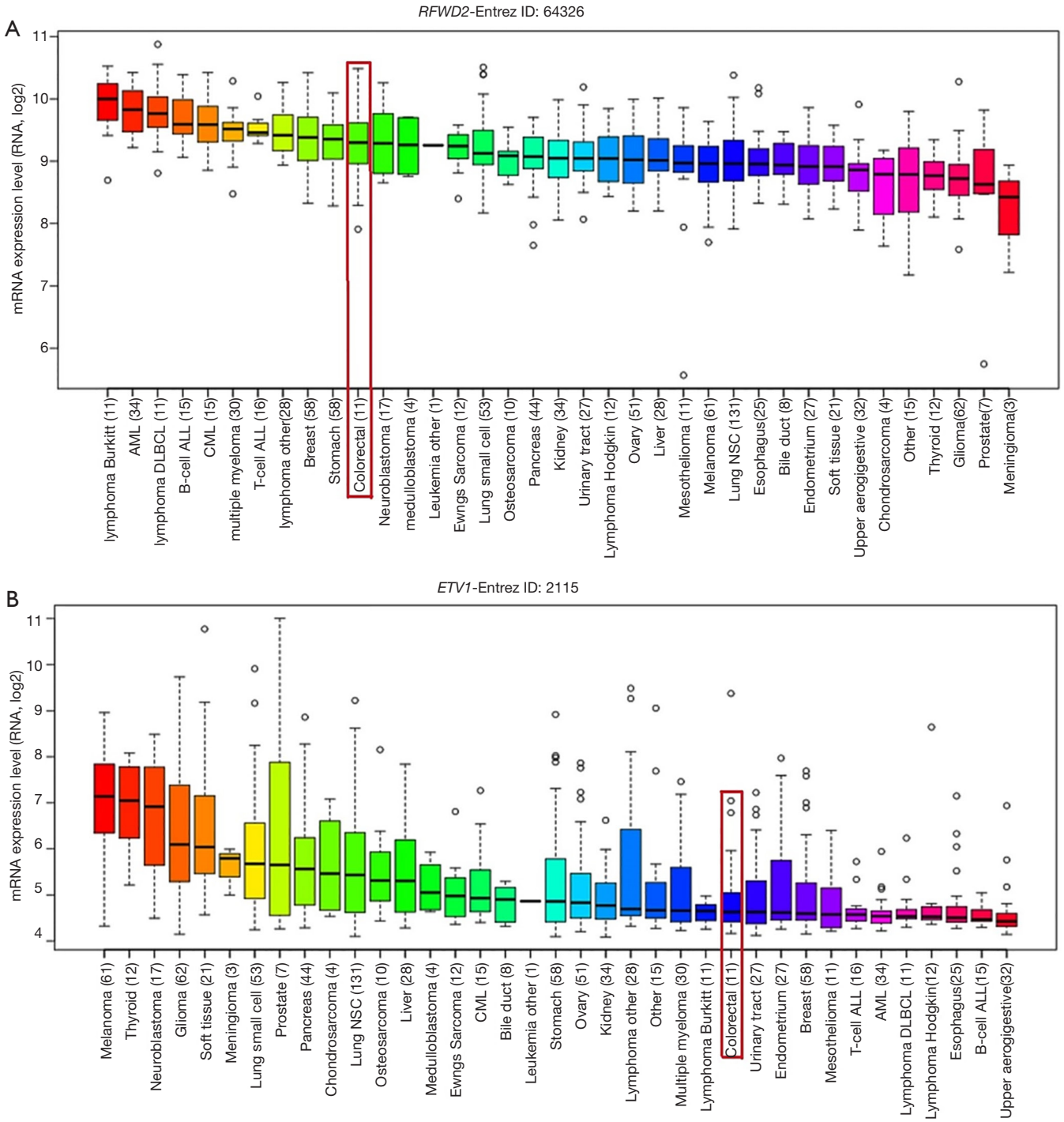


Figure 2 *ETV1* expression data from TCGA. (A) Expression of *ETV1* in COAD based on major cancer stages; (B) expression of *ETV1* in COAD based on individual cancer. (C) expression of *ETV1* in COAD based on patient's gender; (D) expression of *ETV1* in COAD based on patient's weight; (E) expression of *ETV1* in COAD based on patient's race; (F) expression of *ETV1* in COAD based on patient's weight. *, P<0.05; **, P<0.001. *ETV1*, ETS variant 1.



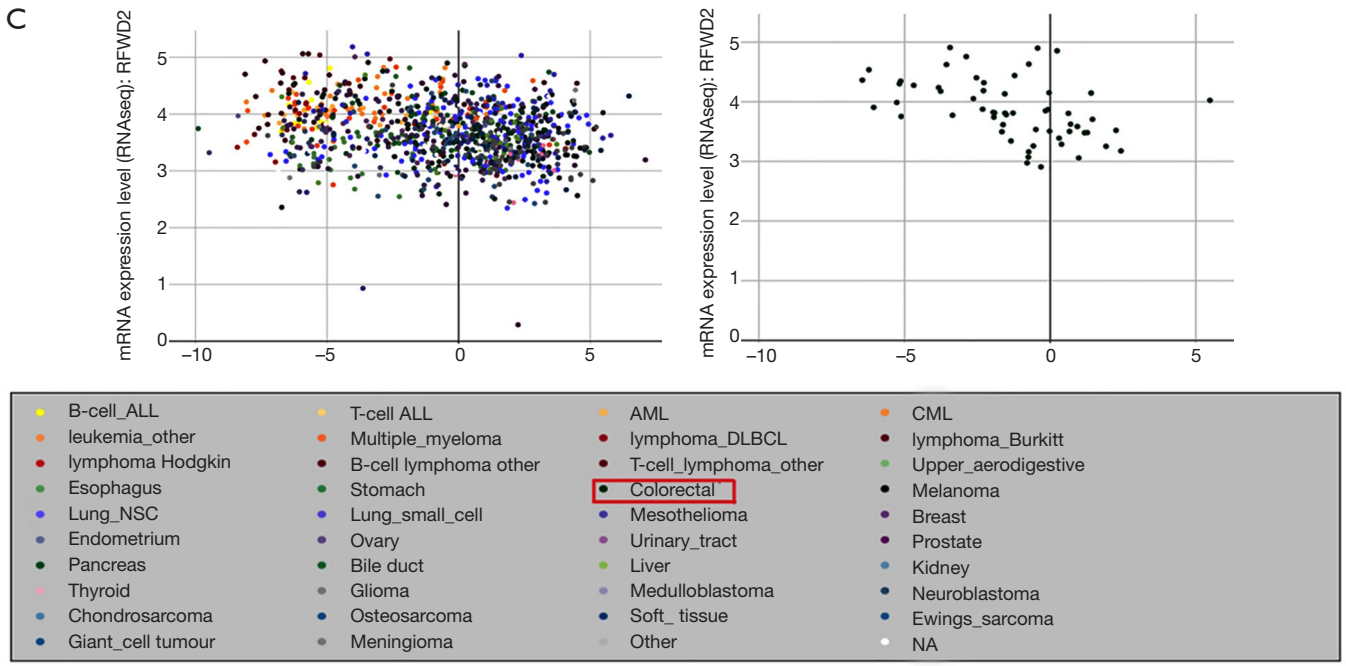


Figure 3 *RFWD2* and *ETV1* expression and correlation analysis in CRC cells lines. The expression of *RFWD2* (A) and *ETV1* (B) in CRC cells from CCLE showed that the mRNA expression level of *RFWD2* was more than 1.8 times of *ETV1* (red boxes). (C) The spearman correlative value of *RFWD2* and *ETV1* in lung cancer cell lines. *RFWD2*, E3 ubiquitin ligase Ring finger and WD domain 2; *ETV1*, ETS variant 1; CRC, colorectal cancer.

cellular component GO terms of *RFWD2* biological process focused on: interleukin-17 production; peptide cross-linking; amino sugar metabolic process; ATP hydrolysis coupled transmembrane transport; type 2 immune response; dopamine receptor signaling pathway; post-translational protein modification and protein localization to Golgi apparatus; oligosaccharide-lipid intermediate biosynthetic process (*Table 2*). And the biological process of *ETV1* may involve in nucleotide phosphorylation; secondary metabolic process; columnar/cuboidal epithelial cell differentiation; platelet-derived growth factor receptor signaling pathway; mesenchymal cell proliferation; spinal cord development; odontogenesis; regulation of behavior; central nervous system neuron differentiation; interleukin-13 production and response to osmotic stress (*Table 3*).

Analysis of cellular components enrichment analysis suggested that *RFWD2* may be expressed in telomerase holoenzyme complex; ATPase complex; cornified envelope; PcG protein complex and membrane region (*Table 4*). *ETV1* may be expressed in GTPase complex; sperm part; replication fork; cell projection membrane; cytoplasmic region; secretory vesicle; intrinsic component of organelle membrane; cytoplasmic vesicle membrane; endoplasmic reticulum lumen and cell surface furrow (*Table 5*).

Analysis of Molecular Function demonstrated that *RFWD2* may participate in ephrin receptor binding; sulfur compound transmembrane transporter activity; extracellular matrix structural constituent and virus receptor activity (*Table 6*), and *ETV1* may participate in taste receptor activity; Wnt-activated receptor activity; GTP-dependent protein binding; macromolecule transmembrane transporter activity; carbohydrate kinase activity; semaphorin receptor binding; drug transporter; E-box binding; actinin binding; cyclin binding; phosphotransferase activity, phosphate group as acceptor; cyclin-dependent protein kinase activity and oxidoreductase activity, acting on single donors with incorporation of molecular oxygen (*Table 7*).

KEGG datasets revealed that RFWD2 might involve in Asthma; Fat digestion and absorption and Starch and sucrose metabolism (*Table 8*), and *ETV1* might involve in Neomycin, kanamycin and gentamicin biosynthesis; Sulfur metabolism; Mismatch repair; Selenocompound metabolism; Mannose type O-glycan biosynthesis; Galactose metabolism; Tryptophan metabolism; Glycine,

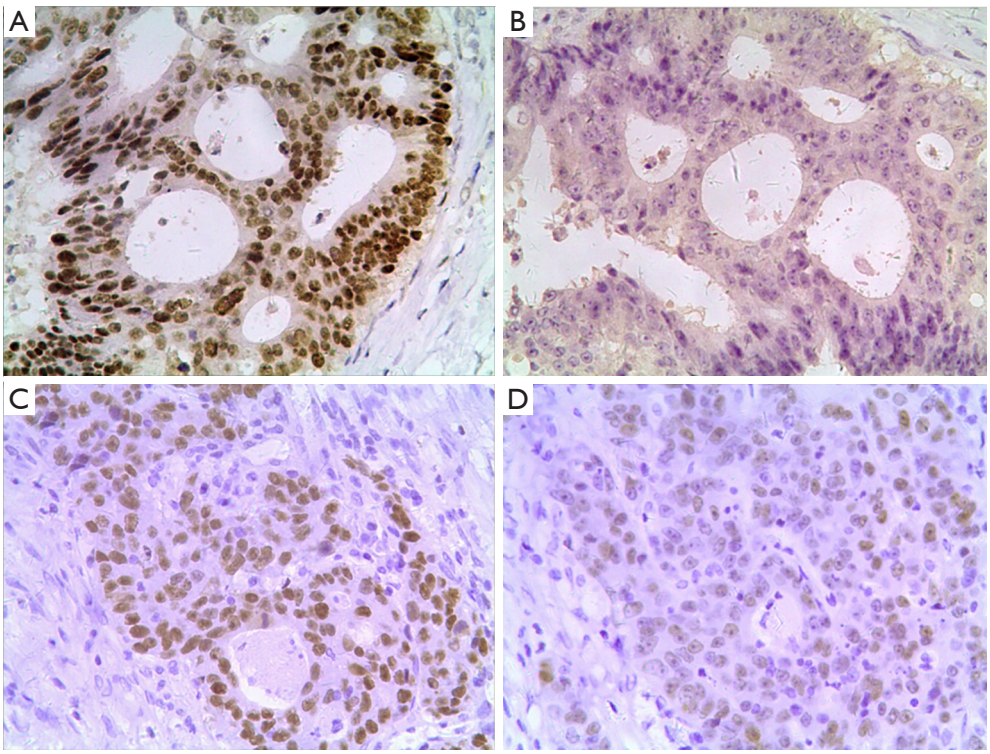


Figure 4 Representative images of *RFWD2* and *ETV1* protein expression in CRC surgical specimens shown by immunohistochemistry. (A) Strong expression of *ETV1* in CRC; (B) weak expression of *ETV1* in CRC; (C) positive expression of *RFWD2* in CRC; (D) negative expression of *RFWD2* in CRC. All of these four pictures were taken under the same magnification (200×). *RFWD2*, E3 ubiquitin ligase Ring finger and WD domain 2; *ETV1*, ETS variant 1; CRC, colorectal cancer.

serine and threonine metabolism and Viral myocarditis (*Table 9*). Therefore, our next step research will focus on the above bioinformatics data to investigate the function and mechanism of *RFWD2* and *ETV1* in CRC cells.

Discussion

Colon and rectum cancer (CRC) is the second most lethal type of cancer in United States (20). Therefore, identification of the CRC-specific biomarkers involved in these procedures is very important for diagnosis, therapy and prognostic prediction in clinics.

RFWD2 overexpression had been found in many tumor types. High expression of *RFWD2* was found in promoting cell proliferation, cell transformation, and tumor progression, manifesting its role as cancer promoter (21). These results suggested that *RFWD2* might play an important role in promoting tumorigenesis or progression. These results supported an earlier hypothesis that *RFWD2* might be an oncogene. However, recent study showed that *RFWD2* might be a tumor suppressor in patients with Triple-negative breast cancer (15). Evidence also showed that loss of *COP1* (*RFWD2*) expression determines poor prognosis in patients with gastric cancer (22). *COP1* was downregulated in renal cell carcinoma (RCC) and inhibited the migration of RCC ACHN cells *in vitro* (23). All above data suggested that *RFWD2* was a potent tumor-suppressor or oncogene in different kinds of human cancers, and the expression and clinical significance of *RFWD2* in CRC had not been explored.

ETV1 was found significantly associated with lymphatic metastasis of CRC (22). miR-17-5p acts as a tumor suppressor in Triple-negative breast cancer by targeting *ETV1*, and a low-abundance of miR-17-5p may be involved in the pathogenesis of Triple-negative breast cancer (24). *ETV1* and other Pea3-ETS transcription factors are critical nuclear effectors of MAPK signaling that are regulated through protein stability. *COP1-DET1-ETS* axis played an important role in regulating ERK transcriptome and sensitivity to MAPK inhibitors (25).

Table 1 Comparison between *RFWD2* and *ETV1* expression, and clinicopathologic parameters in 76 colorectal cancer cases

Characteristics	*RFWD2*		P value	*ETV1*		P value
	Negative	Positive		Negative	Positive	
Tumor size (cm)						
≤3	11	4	0.3346	4	13	0.3821
>3	50	11		10	49	
Pathological type						
COAD	44	23	0.3769	27	39	0.1816
Other	7	2		2	8	
TNM stage						
I	4	2	0.0007	1	2	0.0093
II	10	6		10	19	
III	52	2		3	41	
Lymph node metastasis						
0–4	37	5	0.0004	10	23	0.0478
5–8	12	14		2	29	
≥9	7	1		2	10	
Relapse						
No	2	50	0.0000	11	15	0.0003
Yes	15	9		3	47	

RFWD2, E3 ubiquitin ligase Ring finger and WD domain 2; *ETV1*, ETS variant 1.

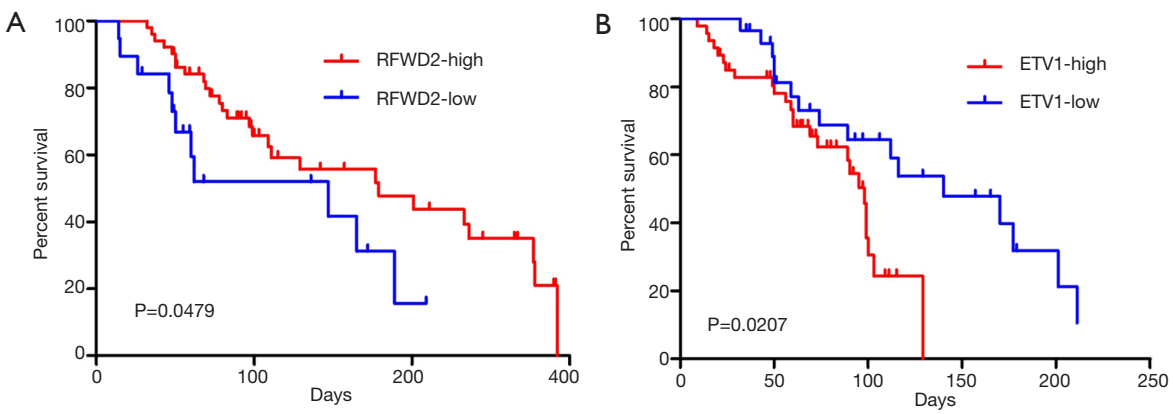


Figure 5 Overall survival of 76 patients with CRC according to RFWD2 and ETV1 protein expression statuses. (A) RFWD2-high CRC patients had longer overall survival time than that of RFWD2-low CRC patients (P=0.0479); (B) significant difference in overall survival time was observed between ETV1-high and ETV1-low group (P=0.0207). RFWD2, E3 ubiquitin ligase Ring finger and WD domain 2; ETV1, ETS variant 1; CRC, colorectal cancer.

In our study, CRC patients with high *RFWD2* expression showed a significantly longer cancer-specific survival than those with a low level of *RFWD2* expression. While patients with high *ETV1* expression had a markedly poor overall survival compared to patients with low *ETV1* expression. These results indicated that *RFWD2* and *ETV1* might have the opposite relationship in function. Results from our collected data showed that among the 76 cases

Table 2 GO gene function (biological process) analysis of *RFWD2*

Gene set	Description	Size	P value
GO:0032620	Interleukin-17 production	25	0e+00
GO: 0018149	Peptide cross-linking	56	0e+00
GO:0006040	Amino sugar metabolic process	38	4.06e-03
GO:0090662	ATP hydrolysis coupled transmembrane transport	34	2.04e-03
GO:0042092	Type 2 immune response	33	3.06e-02
GO:0007212	Dopamine receptor signaling pathway	37	3.25e-02
GO:0043687	Post-translational protein modification	39	0e+00
GO:0034067	Protein localization to Golgi apparatus	34	0e+00
GO:0006490	Oligosaccharide-lipid intermediate biosynthetic process	21	4e-02

RFWD2, E3 ubiquitin ligase Ring finger and WD domain 2.

Table 3 GO gene function (biological process) analysis of *ETV1*

Gene set	Description	Size	P value
GO:0046939	Nucleotide phosphorylation	88	8e-03
GO:0019748	Secondary metabolic process	55	1.6e-02
GO:0002065	Columnar/cuboidal epithelial cell differentiation	97	2e-03
GO:0060021	Palate development	85	8e-03
GO:0048008	Platelet-derived growth factor receptor signaling pathway	46	3.41e-02
GO:0010463	Mesenchymal cell proliferation	47	2.4e-02
GO:0021510	Spinal cord development	93	8e-03
GO:0042476	Odontogenesis	113	0e+00
GO:0050795	Regulation of behavior	60	2.4e-02
GO:0021953	Central nervous system neuron differentiation	168	0e+00
GO:0032616	Interleukin-13 production	20	0e+00
GO:0006970	Response to osmotic stress	65	3.2e-02

ETV1, ETS variant 1.

Table 4 GO gene function (cellular component) analysis of *RFWD2*

Gene set	Description	Size	P value
GO:0005697	Telomerase holoenzyme complex	21	4.19e-02
GO:1904949	ATPase complex	23	1.03e-02
GO:0001533	Cornified envelope	46	0e+00
GO:0031519	PcG protein complex	23	2.42e-02
GO:0098589	Membrane region	347	1.8e-02

RFWD2, E3 ubiquitin ligase Ring finger and WD domain 2.

Table 5 GO gene function (cellular component) analysis of E*TV1*

Gene set	Description	Size	P value
GO:1905360	GTPase complex	31	6e-03
GO:0097223	Sperm part	151	0e+00
GO:0005657	Replication fork	63	4.2e-02
GO:0031253	Cell projection membrane	289	2.8e-02
GO:0099568	Cytoplasmic region	286	4.2e-02
GO:0099503	Secretory vesicle	454	6e-03
GO:0031300	Intrinsic component of organelle membrane	154	4.2e-02
GO:0030659	Cytoplasmic vesicle membrane	465	2e-03
GO:0005788	Endoplasmic reticulum lumen	195	2.4e-02
GO:0097610	Cell surface furrow	46	3.8e-02

ETV1, ETS variant 1.

Table 6 GO gene function (molecular function) analysis of *RFWD2*

Gene set	Description	Size	P value
GO:0046875	Ephrin receptor binding	26	2.06e-03
GO:1901682	Sulfur compound transmembrane transporter activity	27	4.53e-02
GO:0005201	Extracellular matrix structural constituent	75	1e-02
GO:0001618	Virus receptor activity	65	1.4e-02

RFWD2, E3 ubiquitin ligase Ring finger and WD domain 2.

of CRC 25 cases (32.9%) were *RFWD2*-positive and 51 (67.1%) were negative; whereas 47 cases (61.8%) were *ETV1*-positive and 29 (38.2%) were *ETV1*-negative. The spearman correlative analysis showed that their expression was negative relevant in colorectal tumor tissue. Either *ETV1* or *RFWD2* expression status was significantly associated with TNM stages of CRC patients, the number of lymph nodes involved and tumor relapse.

Our study provides a new insight on functional roles of *ETV1* or *RFWD2* gene in CRC. According to the predicting results obtained from GO bioinformatics, we detected that *RFWD2* could involve in interleukin-17

Table 7 GO gene function (molecular function) analysis of *ETV1*

Gene set	Description	Size	P value
GO:0008527	Taste receptor activity	29	0e+00
GO:0042813	Wnt-activated receptor activity	22	2.21e-02
GO:0030742	GTP-dependent protein binding	24	1.42e-02
GO:0022884	Macromolecule transmembrane transporter activity	21	2.43e-02
GO:0019200	Carbohydrate kinase activity	20	3.23e-02
GO:0030215	Semaphorin receptor binding	23	4.86e-02
GO:0090484	Drug transporter	21	3.64e-02
GO:0070888	E-box binding	33	6.04e-03
GO:0042805	Actinin binding	29	4.61e-02
GO:0030332	Cyclin binding	20	3.22e-02
GO:0016776	Phosphotransferase activity, phosphate group as acceptor	39	2.2e-02
GO:0097472	Cyclin-dependent protein kinase activity	32	4.01e-02
GO:0016701	Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	26	0e+00

ETV1, ETS variant 1.

Table 8 Pathways enrichments analysis of *RFWD2* from KEGG

Gene set	Description	Size	P value	Leading edge gene
hsa05310	Asthma	16	1.98e-02	*FCER1A; FCER1G; IL10*
hsa04975	Fat digestion and absorption	38	0e+00	*PLA2G2D; PLA2G2E; APOA1; APOA4; PLA2G2C; PLA2G2A; PLA2G5; PLA2G2F; MOGAT2; DGAT2*
hsa00500	Starch and sucrose metabolism	35	0e+00	*TREH; AGL; GBE1; AMY1A; AMY1B; AMY1C; AMY2A; AMY2B; PGM2L1; PGM1; PYGM*

RFWD2, E3 ubiquitin ligase Ring finger and WD domain 2.

production; peptide cross-linking; amino sugar metabolic process; ATP hydrolysis coupled transmembrane transport; type 2 immune response; dopamine receptor signaling pathway; post-translational protein modification and protein localization to Golgi apparatus; oligosaccharide-lipid intermediate biosynthetic process, suggested that *RFWD2* could participate in ephrin receptor binding; sulfur compound transmembrane transporter activity; extracellular matrix structural constituent and virus receptor activity. In addition, Kyoto Encyclopedia of Genes and Genomes datasets (KEGG) suggested that *RFWD2* could target on Asthma; Fat digestion and absorption and Starch and sucrose metabolism. Meanwhile *ETV1* could target on Neomycin, kanamycin and gentamicin biosynthesis; Sulfur metabolism; Mismatch repair; Selenocompound metabolism; Mannose type O-glycan biosynthesis; Galactose metabolism; Tryptophan metabolism; Glycine, serine and threonine metabolism and Viral myocarditis. These pathways predicted from *RFWD2* and *ETV1* were related to metabolism, survival, proliferation and transcription regulation, respectively, which might involve in the potential regulating function in CRC.

Table 9 Pathways enrichments analysis of *ETV1* from KEGG

Gene set	Description	Size	P value	Leading edge gene
hsa00524	Neomycin, kanamycin and gentamicin biosynthesis	5	2.42e-03	*GCK*
hsa00920	Sulfur metabolism	10	2.36e-02	*CYCS*
hsa03430	Mismatch repair	23	6.05e-03	*PMS2; POLD2; RFC2; RPA3; SSBP1*
hsa00450	Selenocompound metabolism	15	1.65e-02	*INMT*
hsa00515	Mannose type O-glycan biosynthesis	18	2.42e-02	*ISPD*
hsa00052	Galactose metabolism	30	2.2e-02	*AKR1B1; GCK; AKR1B10; MGAM*
hsa00380	Tryptophan metabolism	38	4.01e-03	*INMT; DDC; AOC1; OGDH*
hsa00260	Glycine, serine and threonine metabolism	39	0e+00	*DLD; PGAM2; PSPH; BPGM*
hsa05416	Viral myocarditis	38	1e-02	*CYCS; RAC1; ACTB; CAV1; CD40*

ETV1, ETS variant 1.

Conclusions

In conclusion, we demonstrated that *RFWD2* functioning as an oncogene might be a tumor suppressor in negatively regulating *ETV1* in patients with CRC. By our data suggested that *RFWD2* and *ETV1* could have the potential to serve as molecular biomarkers for the development and progression of CRC. It provides important message about effective therapeutic targets towards changing CRC patients' outcome and is worth to be further explored.

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Footnote

Conflicts of Interest: The authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.11.35). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This submission has been received explicitly from all co-authors. And authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results. Approval for this study was obtained by Ethics Committee of Guangzhou Medical University and the ethics committee waived the use of the inform consent (these CRC tumor specimens are the remaining tissues after pathological examination. This study does not cause any secondary damage to the patients).

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