AEBP1 Predicts Clinical Prognosis and is Associated with Immunocyte Infiltration in Gastric Cancer

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Abstract

AEBP1 is differentially expressed in various tumors. However, the correlation between AEBP1 and immune cell infiltration in gastric cancer remains unclear. Kaplan-Meier survival curves were used to evaluate the relationship between AEBP1 expression and overall survival and progression-free survival. The relationship between AEBP1 expression and infiltrating immune cells, and their corresponding gene marker sets was examined using the TIMER database.The expression of AEBP1 was lower in gastric cancer (GC) tumor tissues than in normal tissues ($P < 0.05$). High AEBP1 gene expression and high levels of AEBP1 gene methylation were correlated with high-grade malignant tumor and old TNM stage. In addition, in gastric cancer, there was a positive correlation between AEBP1 expression and immune infiltrating cells, including neutrophils, CD8 + T cells, and CD4 + T cells, as well as immune-related genes, including CD2, CD3D, and CD3E. Methylation sites such as cg00009293, cg08495088, cg12955216, cg10480062, cg06852744 and cg12978582 were positively correlated with low expression of AEBP1. AEBP1 may be a potential tumor suppressor gene in GC and a potential novel therapeutic target and predictive biomarker for GC. AEBP1 likely plays an important role in immune cell infiltration.

Introduction

Gastric cancer remains an important cancer worldwide and is responsible for over one million new cases in 2020 and an estimated 769,000 deaths (equating to one in every 13 deaths globally), ranking fifth for incidence and fourth for mortality globally[1]. According to estimates by the International Agency for Research on Cancer, 1.1 million people were diagnosed with GC in 2020, and 935,000 people died of CRC, accounting for 5.6 % of cancer diagnoses and 7.7 % of cancer-related deaths, respectively[1]. Moreover, up to 50% of gastro-esophageal cancer patients present with metastatic disease at time of diagnosis[2, 3]. The 5-year survival rate of patients with gastric cancer is < 10%[4]. More and more evidences show that immune infiltrating cells in the tumor microenvironment (TME) play an important role in the occurrence and development of human cancer[5–7]. As a highly heterogeneous internal environment, TME is composed of complex cellular components, including not only TAM, but also other immune infiltrating cells, such as T cells and NK cells. A large number of studies have shown that the prognosis of patients is affected by immune infiltrating cells, and the immune outcome varies with the type of cancer [8],Such as lung cancer[9]breast cancer[10, 11] GBM[12]and hepatocellular carcinoma[13]For GC, immune infiltrating cells have also been reported to have a significant impact on tumor progression. Li and colleagues showed that the polarization of M2 subtype TAM triggered by GC-derived mesenchymal stromal cells promotes EMT and metastasis of GC[13]In addition, the accumulation of Treg cells in GC tumors is the basis for resistance to immune checkpoint blockade (ICB)[14]

Adipocyte enhancer-binding protein 1 (AEBP1) is a transcriptional repressor involved in the regulation of critical biological processes including adipogenesis, mammary gland development, inflammation, macrophage cholesterol homeostasis, and atherogenesis[15–17]. *AEBP1* is involved in the progression of a variety of diseases, such as abdominal aortic aneurysm[18], nonalcoholic steatohepatitis [19], Ehlers-Danlos syndrome[20], and Alzheimer's disease[21]. However, no correlation was found between AEBP1 and immune cell infiltration.

AEBP1 can promote proliferation, metastasis, angiogenesis, and inflammation and suppress apoptosis both in vitro and in vivo; therefore, it acts as an oncogene to promote tumor progression[22–25]. However, the role of AEBP1 as a prognostic marker and the relationship between AEBP1 and immune infiltration in CRC have not been investigated to date. In this study, various databases such as The Cancer Genome Atlas (TCGA) were used to explore whether AEBP1 level can be used as an indicator of poor prognosis and its potential relation to insufficient immune cell infiltration in CRC.

Methods

Data source

TCGA (https://genome-cancer.ucsc.edu/) provides scholars and researchers with clinical and pathological information on 33 types of cancer. Data of COAD patients with RNA-Seq expression and matching clinicopathological information was obtained through the TCGA tool cancer browser. Because the database is publicly available and accessible, approval from the local ethics committee was not necessary.

GEO database

The GEO database is a comprehensive gene expression library in the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/geo/), as one of the largest collections of gene chips in the world.

Expression Profile and Correlation Analysis

The functional expression of AEBP1 gene in colon carcinoma is analyzed using UALCAN, a public server to analyze the cancer OMICS data (TCGA and MET500), built upon PERL-CGI with high-quality graphics through javascript and CSS to provide graphs and plots depicting gene expression, survival information, epigenetic regulation, and also correlation among gene[26]. It is used here to analyze the expression and promoter methylations of the AEBP1 gene in colon adenocarcinoma based on clinicopathological features including sample type, individual cancer stage, patients' sex and age, histological subtype, nodal metastasis status, and TP53 mutation status.

Immunochemistry

The Human Protein Atlas (www.proteinatlas.org) shows a map of protein expression in 32 human tissues. It not only measures RNA levels, but also uses antibody profiling to precisely locate the corresponding protein[27]. Using the Human Protein Atlas (www.proteinatlas.org)[27], we detected the expression of AEBP1 protein in colorectal tissue and normal colorectal tissue.

Statistical analysis survival

According to the median expression of the AEBP1 gene, patients in the experimental group and the validation groups were divided into two subgroups: high AEBP1 expression and low AEBP1 expression. The effect of AEBP1 expression level on the clinical outcomes of GC patients was investigated using Kaplan-Meier (KM) survival curves, and a prognostic classifier was constructed to compare survival differences. The KM survival curve was implemented with the survminer package. Kaplan-Meier plotter (https://kmplot.com/analysis/)) verified the association between AEBP1 expression and total cancer survival. Kaplan-Meier plotter system consists of gene chips and RNA-seq data.

TIMER database analysis

(http://timer.cistrome.org/). The association between the expression of AEBP1 and the pregnancy of five infiltrating immune cells (neutrophils, macrophages, CD4 + T cells, CD8 + T cells) and immune-related genes (CD2, DC3D and CD3E) in GC patients was evaluated using the TIMER database.

Meta-analysis

A meta-analysis of data from TCGA, GEO, and ICGC databases was performed to evaluate the significance of AEBP1 expression for GC prognosis. Heterogeneity among the included studies was determined by the I^2-value obtained from the Cochrane Q test and the P-value obtained from the chi-square test. In cases of heterogeneity ($I^2 \geq 50\%$ or $P < 0.05$), the results were summarized using a random-effects model. Otherwise, a fixed-effect model was used for analysis. The ´meta' R package (R version 4.0.0) was used to perform the meta-analysis.

GO and KEGG enrichment analyses

GO and KEGG enrichment analyses were performed using R 4.0.2 and the R packages "org. Hs.eg.db," "ggplot2," "Cluster Profiler" and "enrich plot." Only terms with P-value <0.05 were considered significantly enriched.

Results

Patient characteristics

The RNA sequencing data of 378 samples from TCGA database and detailed clinical prognostic information were included in the analysis. Patients were divided into a low expression group (n =169) and a high expression group (n =169). Age, gender, lymphatic metastasis, metastasis did not differ significantly between the high and low expression groups (P = 0.5002, 0.7321, 0.5366, 0.6563). Grade, tumor size and stage was significantly different between the high and low expression groups (P = 0.0383, 1.00E-04, 0.017) (Table 1).

Table. 1

Correlation between AEBP1 expression in tumor and clinicopathological characteristics of patients with colorectal cancer.

Covariates	Type	Total	High	Low	Pvalue
Age	<=65	155(45.86%)	82(48.52%)	73(43.2%)	0.5002
Age	>65	179(52.96%)	87(51.48%)	92(54.44%)	
Age	unknow	4(1.18%)	0(0%)	4(2.37%)	
Grade	G1	8(2.37%)	3(1.78%)	5(2.96%)	0.0383
Grade	G2	123(36.39%)	51(30.18%)	72(42.6%)	
Grade	G3	198(58.58%)	110(65.09%)	88(52.07%)	
Grade	unknow	9(2.66%)	5(2.96%)	4(2.37%)	
M	M0	301(89.05%)	151(89.35%)	150(88.76%)	0.6563
M	M1	19(5.62%)	8(4.73%)	11(6.51%)	
M	unknow	18(5.33%)	10(5.92%)	8(4.73%)	
N	N0	103(30.47%)	53(31.36%)	50(29.59%)	0.5366
N	N1	87(25.74%)	40(23.67%)	47(27.81%)	
N	N2	71(21.01%)	33(19.53%)	38(22.49%)	
N	N3	69(20.41%)	39(23.08%)	30(17.75%)	
N	unknow	8(2.37%)	4(2.37%)	4(2.37%)	
T	T1	18(5.33%)	1(0.59%)	17(10.06%)	1.00E-04
T	T2	67(19.82%)	29(17.16%)	38(22.49%)	
T	T3	160(47.34%)	81(47.93%)	79(46.75%)	
T	T4	93(27.51%)	58(34.32%)	35(20.71%)	
T	unknow	0(0%)	0(0%)	0(0%)	
Gender	female	118(34.91%)	57(33.73%)	61(36.09%)	0.7321
Gender	male	220(65.09%)	112(66.27%)	108(63.91%)	
Stage	stage I	1(0.3%)	0(0%)	1(0.59%)	0.017
Stage	Stage I	45(13.31%)	13(7.69%)	32(18.93%)	
Stage	Stage II	108(31.95%)	62(36.69%)	46(27.22%)	
Stage	Stage III	145(42.9%)	76(44.97%)	69(40.83%)	
Stage	Stage IV	29(8.58%)	13(7.69%)	16(9.47%)	
Stage	unknow	10(2.96%)	5(2.96%)	5(2.96%)	
expression	High	169(50%)	169(100%)	-	0

expression	Low	169(50%)	-	169(100%)	
methylation	High	169(50%)	80(47.34%)	89(52.66%)	0.3841
methylation	Low	169(50%)	89(52.66%)	80(47.34%)	

AEBP1 expression is higher in normal tissues than in tumor samples

Analysis of the mRNA expression levels of AEBP1 in TCGA samples showed that AEBP1 expression was lower in tumor samples than in normal tissues ($P < 0.022$) (Figure. 1A). Analysis of the mRNA expression levels of AEBP1 in TCGA samples showed that AEBP1 expression was higher in tumor samples than in normal tissues (Fig. 1B).

Transcriptional Expression and Epigenetic Regulation of AEBP1 Across Various Clinicopathological Parameters

The expression of AEBP1 in GC was analyzed based on the different clinicopathological parameters like sample type, individual cancer stage, cancer grades, patient's sex and age, histological subtype, nodal metastasis status, and TP53 mutation status using the UALCAN server (Figure. 2 and Table. 2). The results support the inference depicted in the earlier section by demonstrating that AEBP1 expression is higher in the colorectal cancer tissue at different clinical stages than in normal tissue (Figure. 2A-F). It tends to increase the expression of AEBP1 at advanced stages of cancer (Stage 4 > Stage 3 > Stage 1) (Figure. 2B) and decrease along with the increase in the age group of patients (Figure 2C). It was also found that the expression of the COL11A1 gene increases along with the nodal metastasis status (N3 > N2 > N1) (Figure. 2E).

Table. 2

The AEBP1 mRNA expression for gastric cancer based on different clinicopalthoogical parameters using UALCAN.

Variables	Different stages	N	Comparisons	Statistical significance
Statistical significance	Normal	41	Normal vs. primary tumor	3.64E-05
	Primary tumor	286		
Individual cancer stages	Stage 1	34	Normal-vs-Stage1	1.02E-02
	Stage 2	123	Normal-vs-Stage2	2.47E-06
	Stage 3	169	Normal-vs-Stage3	1.64E-04
	Stage 4	41	Normal-vs-Stage4	2.38E-03
Patients age	21-40 yrs	34	Normal-vs-Age(21-40Yrs)	8.88E-01
	41-60 yrs	4	Normal-vs-Age(41-60Yrs)	1.54E-06
	61-80 yrs	128	Normal-vs-Age(61-80Yrs)	1.21E-04
	81-100 yrs	253	Normal-vs-Age(81-100Yrs)	3.83E-01
Histological subtype	Adenocarcinoma NOS	155	Normal-vs-Adenocarcinoma(NOS)	2.01E-06
	Adenocarcinoma Diffuse	69	Normal-vs-Adenocarcinoma(Diffuse)	6.91E-07
	Adenocarcinoma SignetRing	12	Normal-vs-Adenocarcinoma(Signet Ring)	7.74E-02
	IntestinalAdenocarcinoma NOS	73	Normal-vs-IntestinalAdenocarcinoma(NOS)	5.13E-02
	IntestinalAdenocarcinoma Tubular	76	Normal-vs-IntestinalAdenocarcinoma(Tubular)	6.85E-01
	IntestinalAdenocarcinoma Mucinous	20	Normal-vs-IntestinalAdenocarcinoma(Mucinous)	8.20E-04
	IntestinalAdenocarcinoma Papillary	7	Normal-vs-IntestinalAdenocarcinoma(Papillary)	7.73E-01
Nodal metastasis status	N0-No regional lymph node metastasis	123	Normal-vs-N0	9.88E-05
	N1- Metastases in 1 to 3 axillary lymph nodes	112	Normal-vs-N1	8.79E-04
	N2-Metastases in 4 to 9 axillary lymph nodes	79	Normal-vs-N2	2.92E-03
	Metastases in 10 or more axillary lymph nodes	82	Normal-vs-N3	1.38E-04
TP53 mutation status	TP53 mutation status	178	Normal-vs-TP53-Mutant	2.58E-03
	TP53 non-mutant	235	Normal-vs-TP53-NonMutant	2.21E-06

DNA methylation is relatively associated with the development of cancer within the human body[28]. From our data, it was evident that the promoter methylation of the AEBP1 gene is overexpressed in the colon cancer tissue than that of the normal tissue, and is negatively regulated for all other clinicopathological parameters (Figures. 2G–L and Table. 3). It is reflected that along with the development of cancer stages and nodal metastasis status, the expression of promoter methylation decreases in the tissues (N0 > N1 > N3) (Figures. 2K). These results indicate that the promoter methylation is negatively associated with the expression of AEBP1 mRNA, and the hypermethylation of the promoter of AEBP1 may inhibit AEBP1 in upgrading cancer development.

Table. 3

The AEBP1 promoter methylation for gastric cancer based on different clinicopathological parameters using UALCAN.

Variables	Different stages	N	Comparisons	Statistical significance
Statistical significance	Normal	2	Normal vs. primary tumor	3.64E-05
	Primary tumor	235		
Individual cancer stages	Stage 1	34	Normal-vs-Stage1	6.43E-01
	Stage 2	123	Normal-vs-Stage2	7.49E-01
	Stage 3	169	Normal-vs-Stage3	7.67E-01
	Stage 4	41	Normal-vs-Stage4	9.80E-01
Patients age	21-40 yrs	4	Normal-vs-Age(21-40Yrs)	2.77E-01
	41-60 yrs	127	Normal-vs-Age(41-60Yrs)	8.62E-01
	61-80 yrs	237	Normal-vs-Age(61-80Yrs)	6.98E-01
	81-100 yrs	22	Normal-vs-Age(81-100Yrs)	6.49E-01
Histological subtype	Grade1	9	Normal-vs-Grade1	6.32E-01
	Grade2	142	Normal-vs-Grade2	7.18E-01
	Grade3	235	Normal-vs-Grade3	7.81E-01
Nodal metastasis status	N0-No regional lymph node metastasis	124	Normal-vs-N0	7.72E-01
	N1- Metastases in 1 to 3 axillary lymph nodes	101	Normal-vs-N1	6.94E-01
	N2-Metastases in 4 to 9 axillary lymph nodes	79	Normal-vs-N2	9.36E-01
	Metastases in 10 or more axillary lymph nodes	28	Normal-vs-N3	5.99E-01
TP53 mutation status	TP53 mutation status	170	Normal-vs-TP53-Mutant	5.87E-01
	TP53 non-mutant	225	Normal-vs-TP53-NonMutant	8.82E-01

Gastric cancer AEBP1 expression analysis

The expression of AEBP1 in GC was confirmed by immunohistochemistry (Figure. 3A–F). The results confirmed that AEBP1 expression was higher in GC tissues than in normal gastric tissues.

Higher AEBP1 mRNA expression in GC is associated with shorter OS

According to the KM chart, GC cases with higher AEBP1 mRNA expression had a shorter OS (*P* = 0.007, 0.003, 0.011) (Figure. 4A-C), methylation sites cg27493928, cg25289803, cg08739576, cg06128448, cg02126753 GC cases with lower AEBP1 mRNA expression had a shorter OS (*P* = 0.007, 0.003, 0.04, 0.046, 0.034) (Figure. 4D), methylation sites cg12978582 GC cases with higher AEBP1 mRNA expression had a shorter OS (*P* = 0.043), methylation sites cg1083171, cg25289803, cg27493928 GC cases with higher AEBP1 mRNA expression had a shorter progression-free survival (*P* = 0.038, 0.017, 0.040) in the test cohort (Figure. 4E). The results showed that the expression of AEBP1 was lower in gastric cancer than in normal gastric tissues. A meta-analysis of the above three datasets was performed to evaluate the correlation between overall survival (OS) and expression AEBP1 gene and obtain more objective conclusions. Because there was no statistically significant difference between the three datasets (*P* = 0.54, I^2 = 0%), a fixed effects model was used to evaluate the combined hazard ratio (HR) and 95% confidence interval (CI). A relatively high expression of the AEBP1 gene was significantly correlated with poor OS (HR = 1.20, 95% CI: 1.10–1.30, *P* < 0.0001; Figure. 4G), indicating that AEBP1 may be a predictor of poor OS.

According to the ROC curve analysis and measurement based on the predictive power and accuracy of the risk characteristics expressed by AEBP1, the results show that the 1-year, 3-year, and 5-year predicted AUC are 0.540, 0.608, and 0.759, respectively (Figure. 4F). And accuracy, and check the forecasting ability and accuracy for 1, 3, and 5 years.

Multivariate Cox analysis and univariate Cox analysis are used to check whether AEBP1 can be used as an independent prognostic factor (Figure. 5). The results show that the p value of AEBP1 is less than 0.05, indicating that AEBP1 can be used as an independent prognostic factor. The hazard ratio is greater than 1, which is greater than the hazard ratio of the most common clinical features, indicating that the expression of base AEBP1 is highly predictive of risk, and the expression of base AEBP1 is more closely related to prognosis than common clinical features(Figure. 5A,B).

Correlation between AEBP1 expression and clinical characteristics

Analysis of the association between AEBP1 mRNA expression and clinicopathological parameters in GC patients showed that AEBP1 expression decreased with age (*P* = 0.043) (Figure. 6A). high expression of AEBP1 was associated with gastric cancer progression from G2 to G3 (*P* <0.0015) (Figure. 6A). In terms of tumor size, high expression of AEBP1 was associated with gastric cancer progression from T1 to T2, T1 to T3 and T1 to T4 has an important influence (*P* = 8.6e-07, 2.7e-07, 1.9e-07) (Figure. 6A). Regarding tumor stage, t high expression of AEBP1 was associated with gastric cancer progression from stage I to stage II, stage I to stage III, stage I to stage IV and stage II to stage III has an important influence (*P* = 5.5e-05, 0.0026, 0.023, 0.083) (Figure. 6A). The level of AEBP1 gene methylation was associated with T2 to T3, T2 to T4 (P=0.0064, 0.042). (Figure. 6B).

DNA methylation analysis

The degree of methylation of cg06852744 was the highest, followed from high to low by cg10480062, cg08495088, cg00009293, cg12955216, cg10873171, cg14249876, cg12978582, cg02126753, cg27493928, cg25289803, cg06128448, cg08739576 and cg01399219 (Figure. 7A). Among them, methylation sites such as cg00009293, cg06852744, cg08495088, cg10480062, cg12955216 and cg12978582 were positively correlated with high expression of AEBP1(Figure. 7C). The sites cg25289803, cg14249876, cg08739576, cg06128448, cg02126753, cg01399219, cg27493928, cg07476508, and cg10873171 methylation sites were negatively correlated with high expression of AEBP1 (Figure. 7B). The methylation level of AEBP1 was lower in GC than in the normal control group, indicating that changes in methylation are related to the abnormal expression of AEBP1. In addition, DNA methylation may be related to the molecular mechanism underlying the high expression level of AEBP1 in tumor tissues and the pathogenesis of GC.

Correlation analysis between infiltrating immune cells and AEBP1 expression

Tumor infiltrating lymphocytes affect the survival of various cancer patients. Therefore, we analyzed the correlation between AEBP1 expression and four infiltrating immune cells (neutrophils, CD8 + T cells, and CD4 + T cells) and three immune-related genes (CD2, CD3D, and CD3E). The results showed that the expression level of AEBP1 is comparable to neutrophils (r = 0.27, P = 8.76e-08), CD4+ T cells (r = 0.275, P = 5.34e-08), CD8 + T cells (r = 0.382, P = 1.18e-04), CD2 (r = 0.235, P = 1.25e-06), CD3D (r = 0.177, P = 2.94e-04) and CD3E (r = 0.227, P = 3.08e-06) (2021.8.17) infiltration levels are significantly positively correlated. P <0.05 was considered as the significance level (Figure. 8). These results indicate that high AEBP1 expression was related to the immunosuppressive microenvironment.

GO and KEGG enrichment analyses

GO enrichment analysis and KEGG pathway analysis were performed. Figure 9A shows the top 30 significantly enriched upregulated pathways. GO pathway analysis showed that relatively high expression of AEBP1 is associated with extracellular matrix organization (GO:0030198, P = 4.01e-60), extracellular structure organization (GO:0043062, P = 4.8e-60), collagen fibril organization (GO:0030199, P = 1.47e-27), and ossification (GO:0001503, P = 1.77e-20) (Figure. 9A). The KEGG pathway analysis identified many enriched pathways, including extracellular matrix organization, extracellular structure organization, collagen fibril organization, ossification and cell-substrate adhesion, and AEBP1 genes were strongly linked (Figure. 9B)

Discussion

Adipocyte enhancer binding protein 1 (AEBP1) was first found in adipocytes and has been reported to be involved in multiple biological processes, including cholesterol homeostasis and inflammation[29], adipogenesis[30], cell differentiation[31]. It is found that AEBP1 can promote the occurrence and

development of tumors[32–34]. We found that AEBP1 is upregulated in CRC, which is the same as the results of previous studies[34, 35].

We identified AEBP1 as a new potential therapeutic target and predictive biomarker for GC. AEBP1 can be used as a prognostic indicator of the immune status. Relatively high expression and methylation of AEBP1 in tumor tissues are related to the infiltration of immune cells. In addition, we found that the methylation level of AEBP1 was related to tumor size. AEBP1 was related to age, grade, stage, tumor size.

KEGG pathway analysis and GO functional enrichment analysis showed that AEBP1 was significantly associated with tumorigenesis and tumor development pathways, including extracellular matrix organization[36], cell-substrate adhesion [37]. Thegastric ECM is mainly composed of collagen, which is essential for regulating cell division, differentiation, proliferation, growth, migration and apoptosis. This indicates that it plays a vital part in the development and progression of cancer[38, 39]. We used the TIMER database to reveal, for the first time, that the expression of AEBP1 in CRC is associated with the infiltration of a variety of immune cells. Tumor infiltrating lymphocytes, such as tumor correlated macrophages and cancer infiltrating neutrophils, may affect the prognosis and efficacy of chemotherapy and immunotherapy[39]. However, there are no studies analyzing the relationship between AEBP1 expression and immune cell infiltration. We evaluated the relation between AEBP1 expression and the immune infiltration level of CRC using the TIMER website. CD2, CD3D, and CD3E genes were positively related to the infiltration of neutrophils, macrophages, CD8 + T cells, CD4 + T cells, CD2, CD3D, and CD3E genes. Our findings confirm that the infiltration level of immune cells is crucial for the progression of CRC, and AEBP1 expression is a predictor of the increase of immune cell infiltration. These results provide ideas for further research on the relationship between AEBP1 and CRC immune infiltration.

The present study had several limitations. According to the requirements regarding data type, including mRNA expression and methylated expression, we only obtained data from TCGA, which may lead to data deviation in this study. A larger number of tumor specimens and further experimental verification are needed to evaluate the biological role of AEBP1 in CRC. In addition, further experimental validation is needed to determine the value of AEBP1 for predicting the prognosis of CRC and the formulation of treatment strategies.

Conclusion

The present study provides important evidence supporting the importance of the AEBP1 in the prognosis of human GC. We identified AEBP1 as a novel biomarker and clarified its prognostic potential in GC through analysis of online public databases. Its biological function and role in immune infiltration were examined to elucidate the mechanism underlying its relatively high expression, which is the basis of GC. Our results indicate that AEBP1 can be used as a potential tumor suppressor gene in GC. AEBP1 may be a new potential therapeutic target and predictive biomarker for GC. Our experimental data provides insight for further research and the development of appropriate treatment strategies. In addition, we are committed to studying cancer cell lines and mouse models of GC to validate the present findings and develop effective treatment strategies by targeting the AEBP1 gene.

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Figures

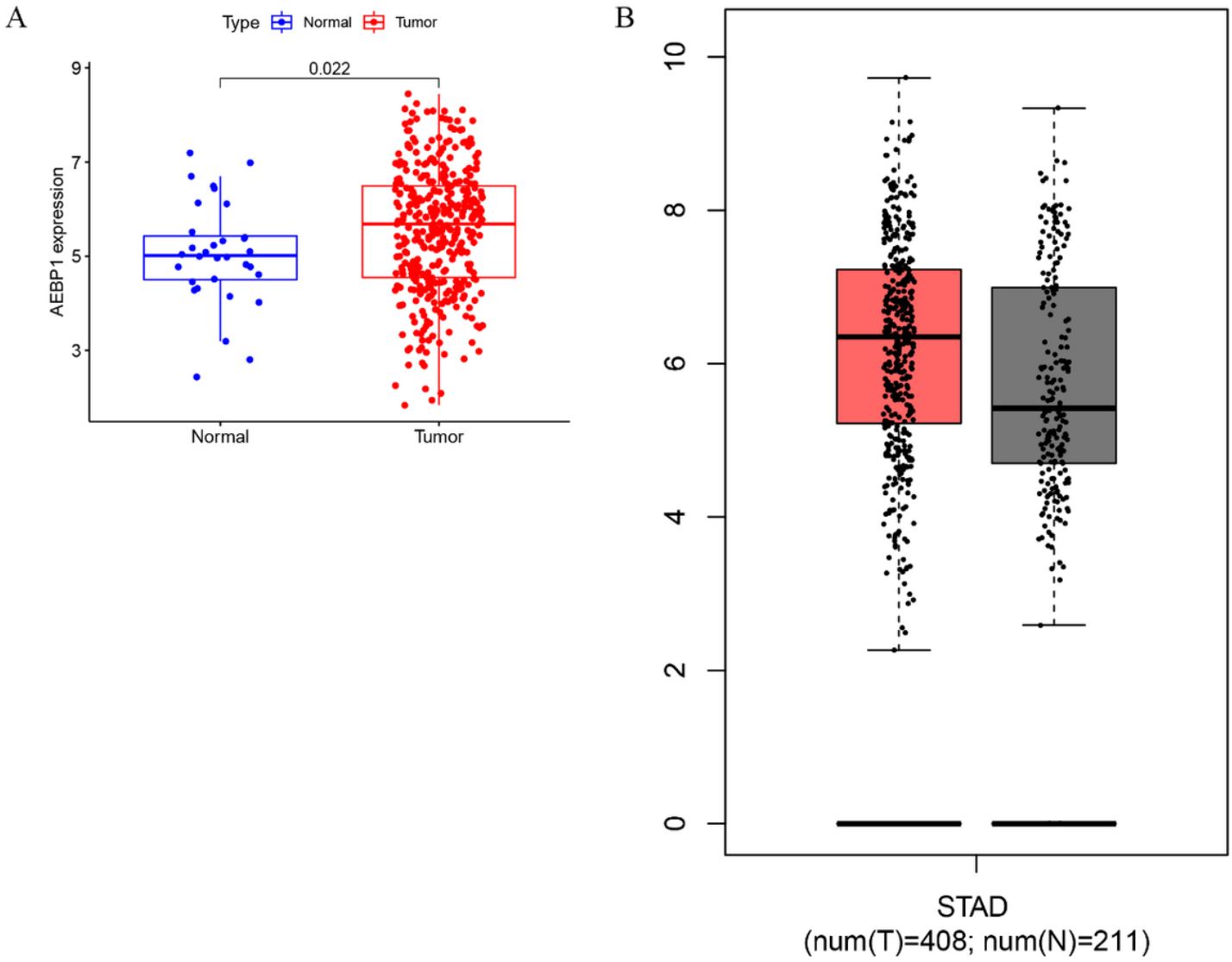

Figure 1

Differential expression of the CRYAB gene. (A-B) Human CRYAB expression levels in gastric cancer tissues and corresponding normal tissues. ROC, receiver operating characteristics.

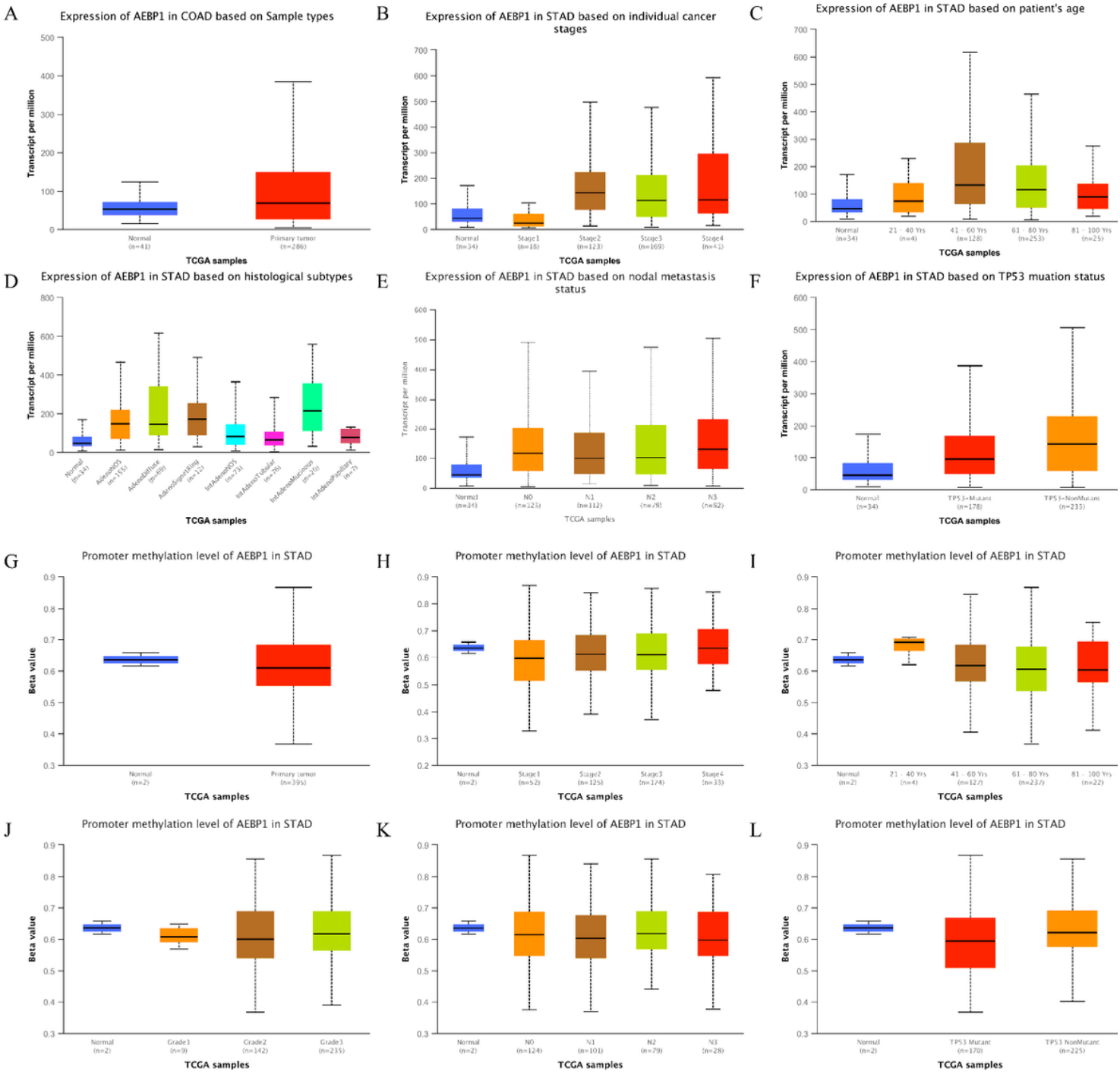

Figure 2

Expression and promoter methylation of the AEBP1 gene in STAD for different clinicopathological parameters. (A–F) Box-plot showing relative expression of AEBP1 mRNA in panel (A). cancer tissues and normal tissues, (B) individual cancer stage, (C) patient's age, (D) histological subtypes, (E) nodal metastasis status, (F) TP53 mutation status. (G–L) Box-plot showing promoter methylation of AEBP1 mRNA in, (G) cancer tissues and normal tissues, (H) individual cancer stage, (I) patient's age, (J) individual cancer grades, (K) nodal metastasis status, (L) TP53 mutation status.

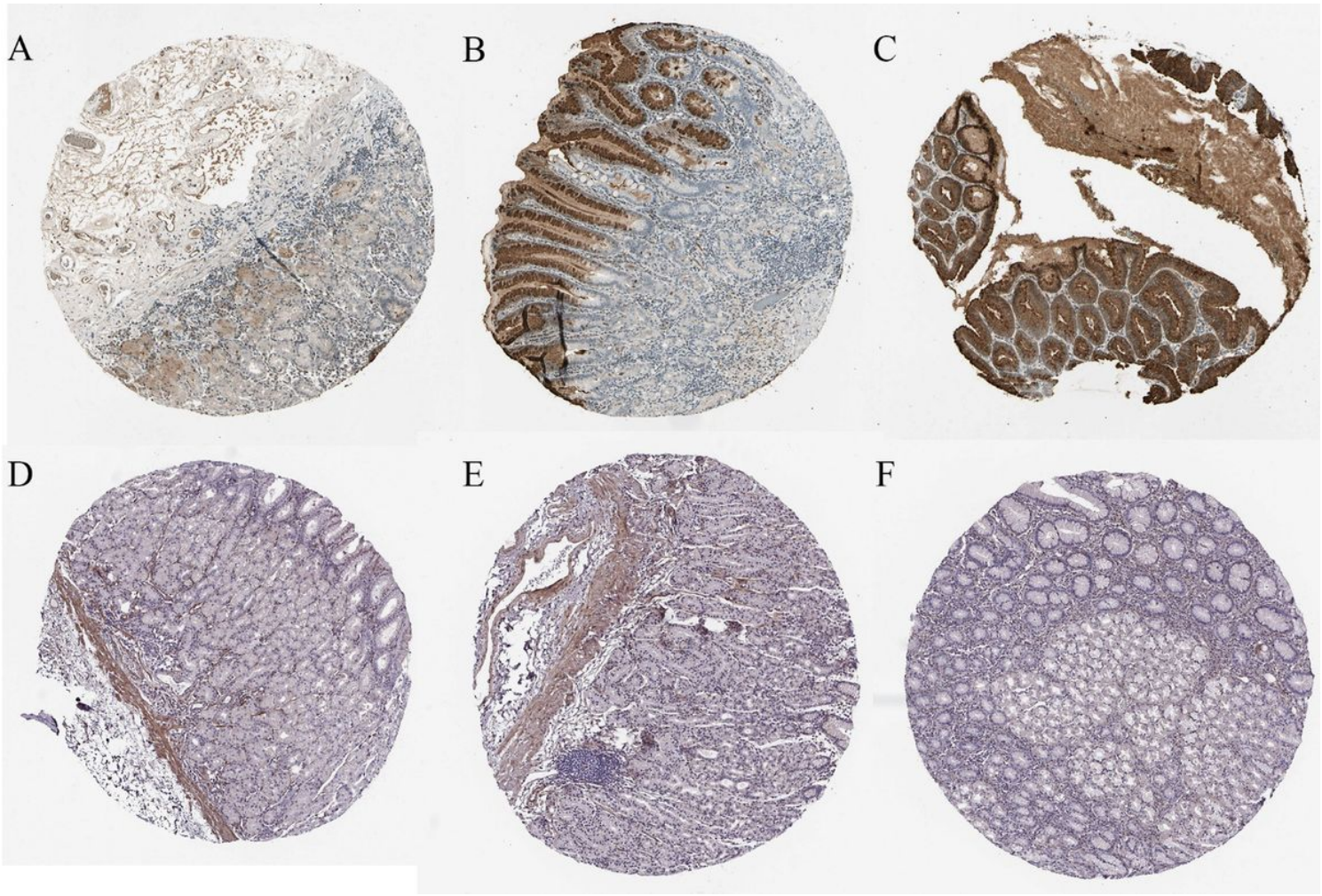

Figure 3

Representative IHC staining of CRYAB expression in GC tissues and normal gastric tissues. Representative histopathological sections of (A-C) gastric cancer tissues and (D-F) normal gastric tissues stained with IHC. The colon section was incubated with HRP-labeled goat anti-pika universal antibody and stained with DAB (brown). IHC: immunohistochemistry.

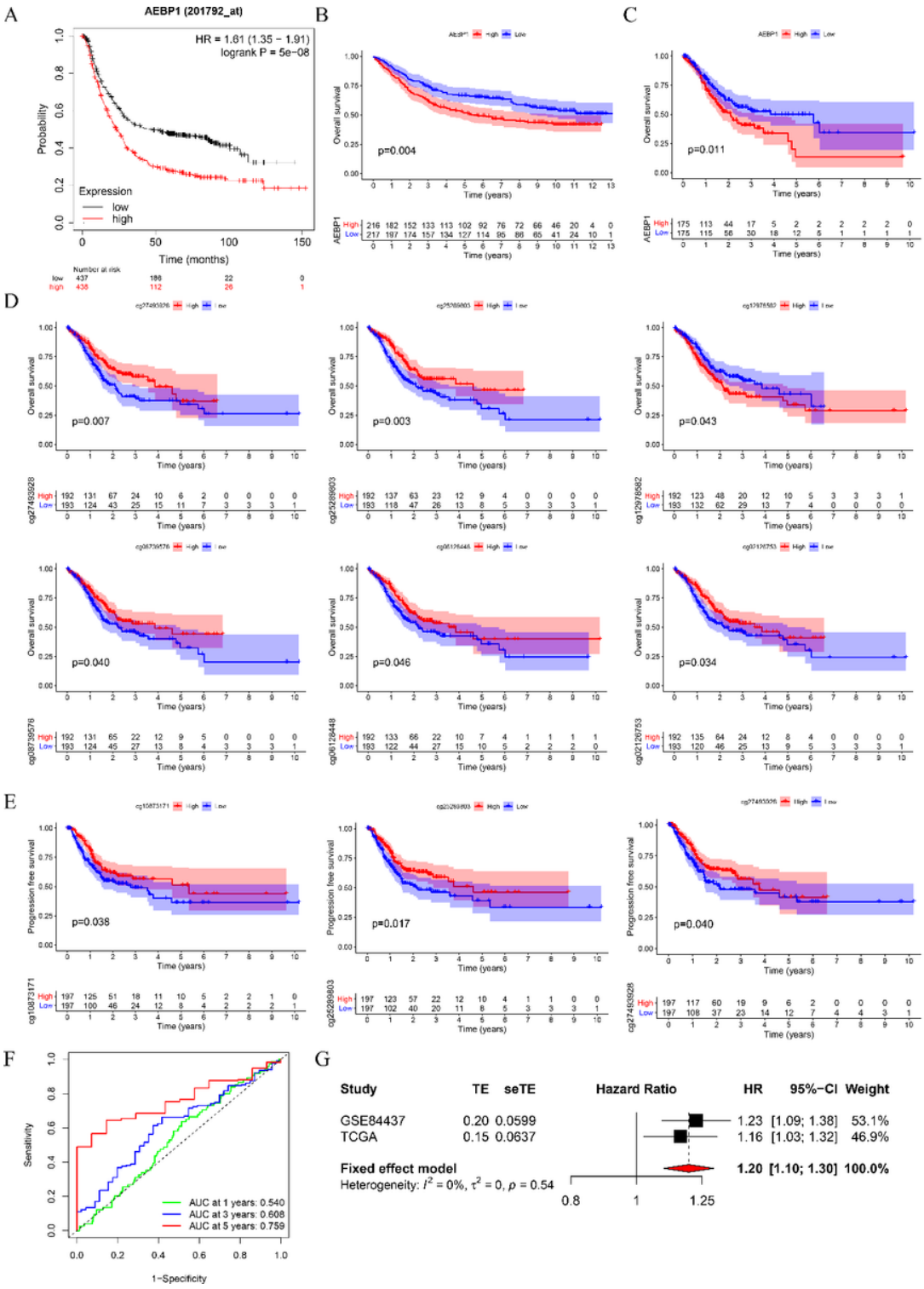

Figure 4

The Kaplan-Meier survival curves of STAD patients with high and low AEBP1 expression level. (AC) (D) Overall survival (OS) of each methylation site of STAD patients with high and low AEBP1 expression level. (E) Profession-free survival (PFS) of each methylation site of STAD patients with high and low expression of AEBP1. (F) ROC curve of the survival rate of patients in the target cohort. Shows the ROC curve and AUC for 1, 3 and 5 years forecast. ROC, receiver operating characteristics. AUC, area under the curve. (G) A meta-

analysis with two data sets. High expression of CRYAB is significantly associated with poor OS . TE: estimated treatment effect; seTE: standard error of treatment estimate.

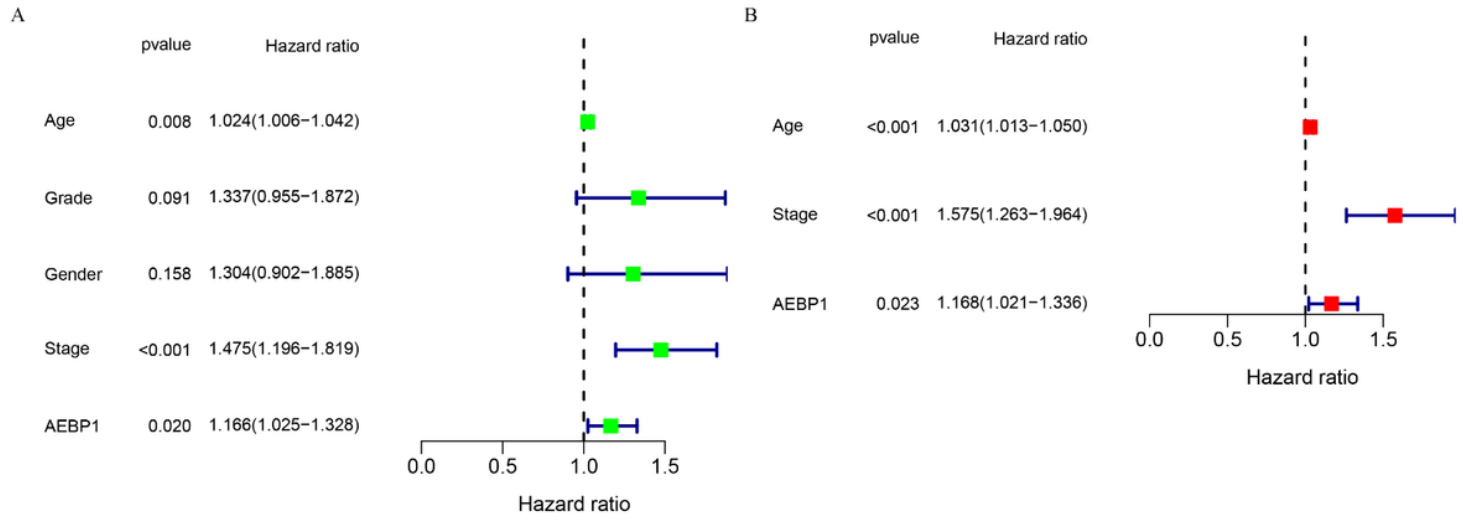

Figure 5

Univariable and multivariable Cox analyses were performed for common clinical characteristics. (A) Forest plot based on univariable Cox proportional hazard regression analysis shows AEBP1 and its hazard ratio. (B) The forest plot based on multivariate Cox proportional hazard regression analysis shows AEBP1 and its hazard ratio.

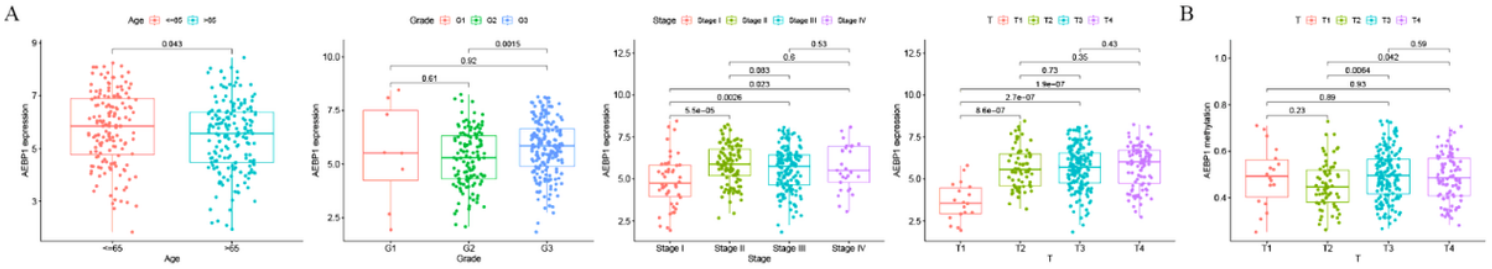

Figure 6

Correlation between CRYAB expression and immune infiltration in CRC. Correlations between CRYAB expression and different immune cells: (A) neutrophils, (B) CD4+T cells and (C) CD8+T cells. Correlations between CRYAB expression and different immune-related genes: (D) CD2, (E) CD2D, and (F) CD2E.

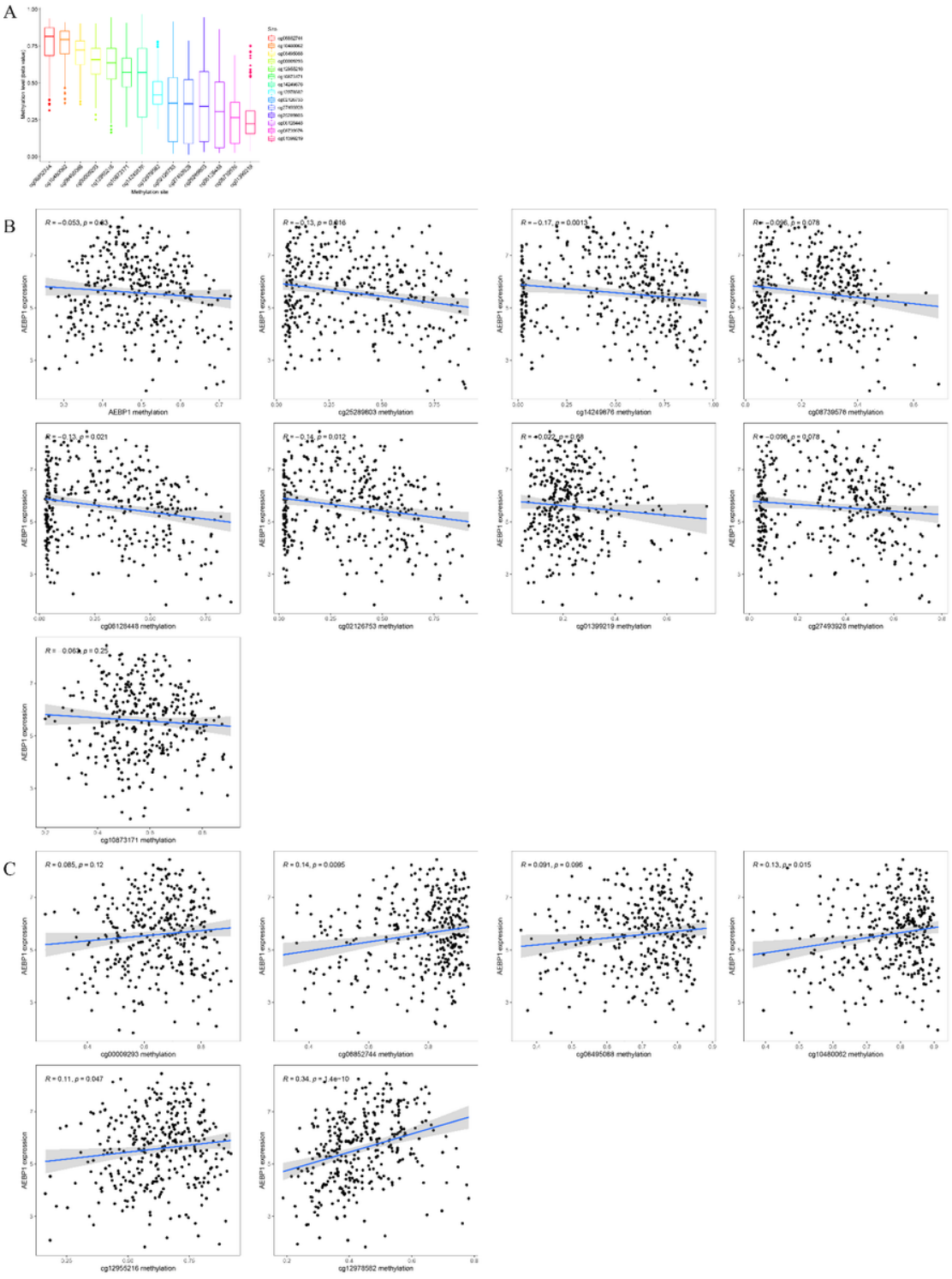

Figure 7

Relationship between AEBP1 methylation and AEBP1 gene expression in CRC (A) Methylation level of each methylation site (B-C) Correlation between AEBP1 gene methylation site and AEBP1 gene expression.

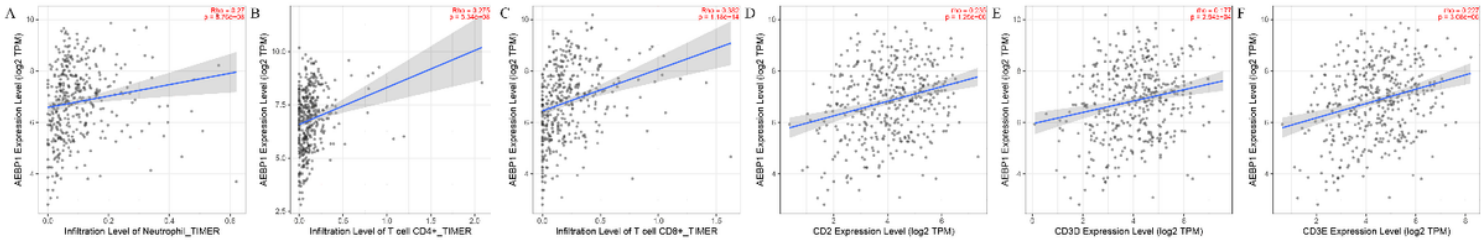

Figure 8

For diffffferent clinicopathological parameters, the expression of AEBP1 gene and AEBP1 gene methylation in colon cancer. (A) Box-plot showing the relation of AEBP1 mRNA with age, gender, stage, tumor size. Box-plot showing Methylation of AEBP1 mRNA in (B) tumor size.

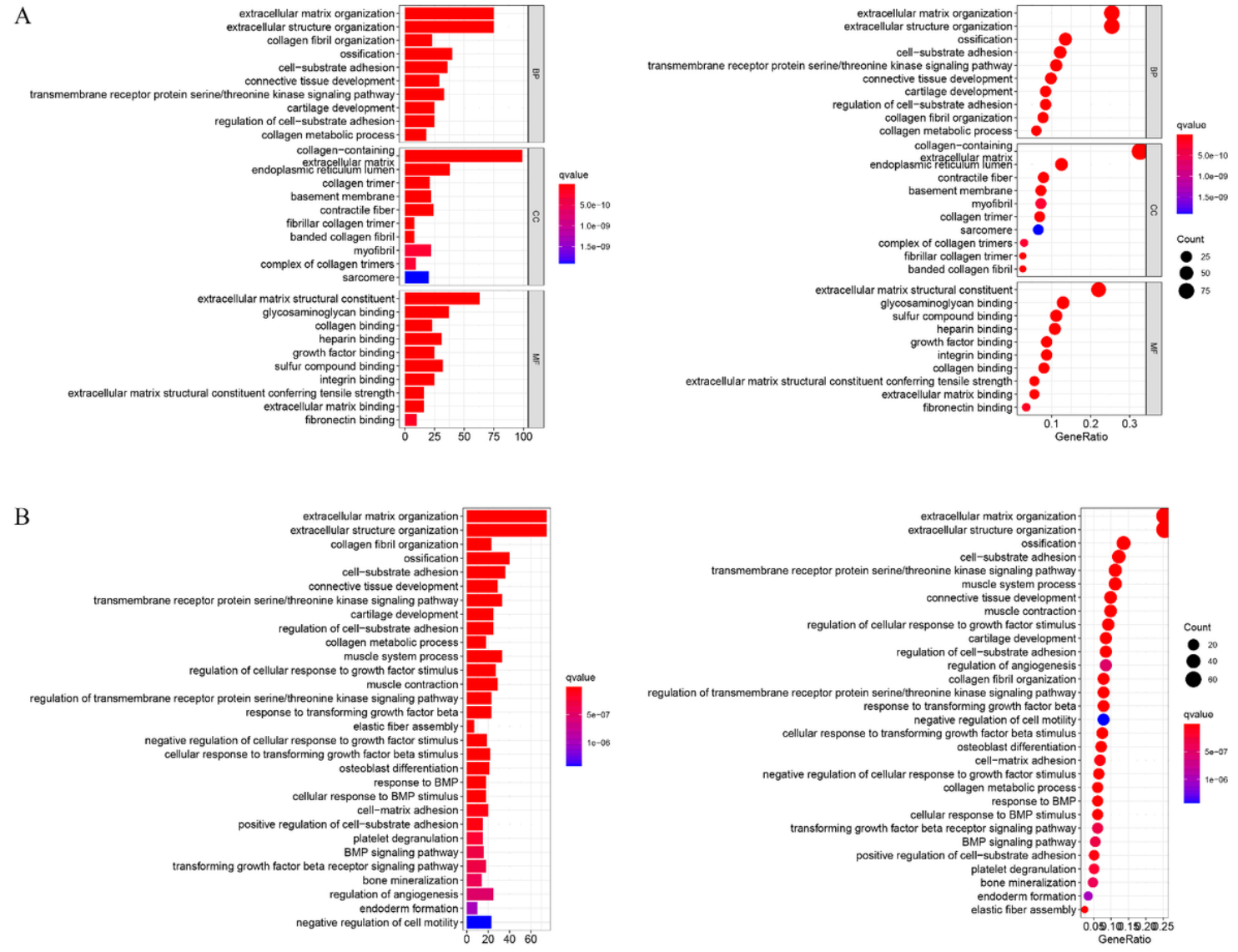

Figure 9

GO and KEGG enrichment analyses. (A) Gene enrichment in three different GO functions and (B) KEGG pathways were respectively ranked by p-value and gene enrichment count.