Abnormal expression of ABCD3 is an independent prognostic factor for colorectal cancer

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Abstract. ATP binding cassette subfamily D member 3 (ABCD3) is a member of the superfamily of ATP-binding cassette (ABC) transporters, which serve crucial roles in the process of tumor cell resistance to chemotherapy. The present study investigated the diagnostic and prognostic capabilities of ABCD3 in colorectal cancer (CRC) by bioinformatics analysis. Gene expression data and corresponding clinical information of patients with CRC were collected from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. The results demonstrated that ABCD3 mRNA level was decreased in CRC tissues compared with normal tissues following Wilcoxon test analysis. Furthermore, ABCD3 protein expression was significantly higher in normal colon tissues compared with colon adenocarcinoma tissues according to the Human Protein Atlas. In addition, the area under the Receiver Operating Characteristic curve based on comparison between the tumor and normal groups derived from TCGA and GEO databases demonstrated that the use of ABCD3 mRNA level may be used for the diagnosis of CRC. ABCD3 expression was significantly associated with clinical stage, T stage, and lymph node status following Kruskal-Wallis test or Wilcoxon rank sum test, logistic regression and χ^2 test. Furthermore, the results from Kaplan-Meier survival analysis indicated that low ABCD3 mRNA expression had a poorer prognosis value compared with ABCD3 high expression in patients with CRC. In addition, results from univariate Cox regression analysis indicated that ABCD3 mRNA expression was associated with overall survival (OS), and results from multivariate Cox analysis indicated that ABCD3 mRNA expression may be considered an independent prognostic factor from other clinical factors, such as clinical stage, sex and age. The results from Gene Set Enrichment Analysis demonstrated that the ABCD3 high-expression phenotype was differentially enriched in five biological processes, including apoptosis, cell cycle, renal cell carcinoma, thyroid cancer and colorectal cancer. The findings from this study demonstrated that ABCD3 mRNA expression may be considered as a potential diagnostic and prognostic biomarker in patients with CRC. ABCD3 expression levels may participate in the regulation of cell apoptosis and cell cycle. In addition, GSEA analysis identified Kyoto Encyclopaedia of Genes and Genomes pathways for renal cell carcinoma, thyroid cancer and CRC involving ABCD3.

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Abbreviations: ABCD3, ATP binding cassette subfamily D member 3; CI, confidence interval; CRC, colorectal cancer; GEO, Gene Expression Omnibus; GSEA, Gene Set Enrichment Analysis; HR, hazard ratio; OR, odds ratio; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas

Key words: ATP binding cassette subfamily D member 3, The Cancer Genome Atlas, Gene Expression Omnibus, bioinformatics analysis, colorectal carcinoma, prognosis, diagnosis

Introduction

Colorectal cancer (CRC) is one of the most common malignancies globally at present. It was estimated that >1.8 million new CRC cases and 881,000 CRC-associated mortality cases occurred in 2018, accounting for ~1/10 cancer cases. CRC ranks therefore third in incidence and second in mortality (1). Patients with CRC commonly present with a survival rate <5 years due to the early development of metastasis (2). Although numerous treatments, including surgery, radiotherapy, chemotherapy and targeted therapy, can be used in CRC, the recurrence remains high (54.5%) (3), the mortality of CRC accounts for 9.5% of various cancers (1) and the prognosis of patients is poor (4). At present, certain biomarkers have been associated with CRC occurrence and progression and prognosis of patients with CRC, for example carcinoembryonic antigen, CA19-9 and CA72-4 (5), were used to detect whether the patient had recurrence and progression (6-8). In addition, microsatellite instability and mutations in the *p53* or *KRAS* genes have been used as prognostic factors (9,10). However, the reliability of these aforementioned biomarkers remains controversial. It is therefore crucial vital to identify novel diagnostic and prognostic biomarkers and therapeutic targets for CRC.

ATP binding cassette subfamily D member 3 (ABCD3), also known as ZWS2, ABC43, CBAS5, PMP70 and PXMP1, is a member of the superfamily of ATP-binding cassette (ABC) transporters associated with the peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle (11). ABC transporters serve crucial roles in the establishment of chemoresistance by regulating the flow of anticancer agents into the cancer cells (12). Inhibition of fatty acid oxidation has been reported to induce apoptosis in colorectal cancer cells (13). Furthermore, the use of gene co-expression network analysis in CRC demonstrated that ABCD3 is involved in the regulation of ABC transporters, transmembrane transport, fatty acid β-oxidation and ATP synthesis following nutrient catabolism (14). Seborova *et al* (15) demonstrated that ABCD3 downregulation is associated with a better sensitivity to chemotherapy and time to progression in patients with ovarian cancer. In addition, Reams *et al* (16) reported that high expression of ABCD3 mRNA is associated with the Gleason Score in Caucasian American men with prostate tumors. Elsnerova *et al* (17) demonstrated that ABCD3 mRNA expression was higher in high-grade serous ovarian carcinoma compared with other subtypes of epithelial ovarian cancer, and may therefore be considered as a progression biomarker for ovarian carcinoma. Although ABCD3 was demonstrated to be less expressed in colorectal cancer tissues compared with normal tissues (18), the diagnostic and prognostic abilities of ABCD3 mRNA expression in CRC have rarely been reported.

The current study demonstrated that decreased ABCD3 mRNA expression was associated with poor survival in patients with CRC, and that ABCD3 had a good diagnostic value with high sensitivity and specificity in patients with CRC, according to analysis of datasets from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO). To identify the biological pathways in which ABCD3 may be involved in patients with CRC, Gene Set Enrichment Analysis (GSEA) was used. The results demonstrated that the ABCD3 high-expression phenotype was differentially enriched in five biological pathways in CRC, including apoptosis, cell cycle, renal cell carcinoma, thyroid cancer and CRC.

Materials and methods

Data collection and processing. The Level 3 HTSeq-FPKM files, comprising 612 Transcriptome Profiling RNA-Seqs of 544 cases, were collected from a TCGA dataset (portal.gdc.cancer.gov/) that included information on 452 and 96 patients with colon and rectal cancer, respectively, on March 2019. Clinicopathological data was available for 548 patients but only 544 of these patients also had RNA-Seq data. The 612 transcripts included 568 tumor samples (some patients provided multiple tumor samples) and 44 normal samples. Patients had not received neoadjuvant treatment before tumor excision. The survival time (follow-up ≥30 days) and survival status information were available for 506 patients with cancer. The clinicopathological characteristics, including sex, age, clinical stage, T stage, M stage and lymph node status, were available for 448 samples obtained from 548 cases and the 100 samples with missing clinical information were removed. The ABCD3 mRNA expression was collected for colorectal adenocarcinoma tissues and adjacent normal tissues. Since the association between ABCD3 mRNA expression and the clinicopathological characteristics of patients was independent of the follow-up days, the RNA transcript data for 448 cancer samples were used for further association analysis (100/548 were excluded from this analysis due to incomplete clinical data). Meanwhile, only 506 patients had a survival time ≥30 days and were used for survival analysis. Furthermore, comparison of ABCD3 expression between tumor and normal samples from the GEO database was performed. The four gene expression datasets (series_matrix) GSE21510 (19), GSE25071 (20), GSE41258 (21) and GSE39582 (22) associated with CRC were downloaded from GEO and included 19, 46, 186 and 566 tumor samples, respectively and 25, 4, 54 and 19 normal samples, respectively. In addition, GSE39582 dataset included the complete clinical information, including survival time, survival status, sex, age, clinical stage, T stage, M stage, and lymph node status; however, the three other datasets didn't have complete clinical information. Furthermore, the protein expression of ABCD3 was determined by using the Human Protein Atlas (http://www.proteinatlas.org/).

GSEA of ABCD3 in CRC. GSEA is a computing tool used to identify classes of genes or proteins that are over-represented in a large set of genes or proteins, and which may be associated with certain disease phenotypes (gsea-msigdb.org/gsea/) (23). In the present study, all genes associated with ABCD3 expression were sequenced by this method in TCGA dataset using gsea-3.0.jar. Each analysis ran 1,000 genome sequences. ABCD3 mRNA was treated as a phenotypic marker and samples were divided into high and low expression groups based on the median expression value. The signaling pathways enriched in each phenotype were based on nominal (NOM) P-value <0.05 and false discovery rate (FDR)<0.25.

Statistical analysis. All statistical analyses were performed using R language (version 3.5.1) (mirrors.tuna.tsinghua.edu.cn/CRAN/). The comparison between ABCD3 mRNA expression in paired CRC and normal tissues from the TCGA and GEO databases was performed using Wilcoxon rank sum tests, some patients in the TCGA database has both tumor samples and normal samples, which was analyzed by Wilcoxon signed-rank test. The diagnostic value of ABCD3 mRNA expression was evaluated by the receiver operating characteristic (ROC) curve using pROC package (24). The association between clinicopathological characteristics and ABCD3 mRNA expression levels was determined using Kruskal-Wallis test, Bonferroni's test (when >2 groups were compared) or Wilcoxon rank sum test (when 2 non-parametric groups were compared), logistic regression and χ^2 test. The association between clinicopathological characteristics, including sex, age, clinical stage, T stage, M stage and lymph node status and ABCD3 mRNA expression and patients' overall survival (OS), was evaluated using univariate Cox regression analysis and Kaplan-Meier method with the Survival package in R and P-values were calculated using log-rank test. Multivariate Cox regression analysis was used to identify whether ABCD3 mRNA expression could be considered as an independent prognostic factor in CRC. All P<0.05 was considered to indicate a statistically significant difference.

Results

Clinicopathological characteristics of patients. The clinical data of the 548 patients collected from the TCGA database, including sex, age, ethnicity, clinical stage, T stage, distant metastasis, and lymph node status and cancer type are presented in Table I.

ABCD3 mRNA expression is decreased in CRC tissues. The results demonstrated that ABCD3 mRNA expression was significantly decreased in CRC tissues compared with normal tissues (P<0.001; Fig. 1A). Furthermore, analysis of ABCD3 mRNA expression in 44 matched tumor tissues and normal tissues demonstrated that ABCD3 mRNA expression was significantly decreased in tumor tissues compared with normal tissues (P<0.001; Fig. 1B). In datasets GSE21510, GSE25071, GSE41258 and GSE39582 from the GEO database; same analyses were performed, and the results demonstrated that ABCD3 mRNA expression was decreased in the tumor group compared with the normal group (P<0.001; Fig. 2A-D). In addition, representative images from the Human Protein Atlas demonstrated that ABCD3 protein expression determined by using three different antibodies was higher in normal colon tissues compared with colon adenocarcinoma (Fig. 3).

Diagnostic capability of ABCD3 mRNA expression in CRC. To evaluate the diagnostic value of ABCD3 mRNA expression, a ROC curve was designed based on ABCD3 mRNA expression data in CRC and normal tissues from TCGA database. The area under the ROC curve (AUC) was 0.923 with the sensitivity and specificity of 0.909 and 0.847, respectively, which indicated a modest diagnostic value of ABCD3 mRNA expression (Fig. 4A). Similar analysis was performed in the GSE21510, GSE25071, GSE39582 and GSE41258 datasets from GEO database. The AUC in GSE21510 dataset was 0.874, with sensitivity and specificity of 0.800 and 0.947, respectively (Fig. 4B). The AUC in GSE225071 dataset was 0.951, with sensitivity and specificity of 1.000 and 0.848, respectively (Fig. 4C). The AUC in GSE9582 dataset was 0.968, with sensitivity and specificity of 0.895 and 0.961 (Fig. 4D). The AUC in GSE41258dataset was 0.905, with sensitivity and specificity of 0.852 and 0.892 (Fig. 4E). These results suggested that ABCD3 mRNA expression may have a diagnostic value in CRC.

Association between ABCD3 mRNA expression and clinicopathological characteristics of patients with CRC. The results demonstrated that the ABCD3 mRNA levels in tumor tissues were significantly different in different clinical stages (P=0.013), T stages (P=0.061), lymph node metastasis statuses (P=0.003) by using Kruskal-Wallis test, and in distant metastasis statuses (P=0.017) by using Wilcoxon rank sum test. Furthermore, the use of Bonferroni test demonstrated that ABCD3 mRNA expression in Stage IV vs. Stage I (P=0.022) and N2 vs. N0 (P=0.002) was statistically significant (Fig. 5A-D).

Furthermore, in the GSE39582 dataset, the ABCD3 mRNA levels in tumor tissues were only significantly different between T1 and T4 stages (P=0.015; Fig. 5F).

Logistic regression analysis demonstrated that ABCD3 mRNA expression in CRC tissues was significantly associated with stage (OR=0.51 for stage IV vs. stage I; P=0.031); T stage (OR=0.50 for T4 vs. T2; P=0.035), lymph node status (OR=0.6 for N1+2 vs. N0; P=0.036). However, it was not significantly associated with distant metastasis status (OR=0.64 for M1 vs. M0; P=0.081; Table II). The results from χ^2 test demonstrated that only stage (P=0.040) and lymph-node status (P=0.049) were associated with ABCD3 mRNA expression (Table III). These findings suggested that ABCD3 mRNA expression may serve a tumor suppressor role in CRC.

Role of ABCD3 mRNA expression in OS. Kaplan-Meier survival analysis demonstrated that patients from the TCGA database with

Table I. Clinicopathological characteristics of patients with colorectal cancer from The Cancer Genome Atlas.

Variables	Number	Percentage
Sex		
Male	292	53.28
Female	256	46.72
Age, years		
Range	31-90	
Median	67.5	
Ethnicity		
American Indian	1	0.18
Asian	12	2.19
Black	59	10.77
White	252	45.99
Unidentified	224	40.88
Stage		
I	96	17.52
II	210	38.32
III	149	27.19
IV	78	14.23
Unidentified	15	2.74
T stage		
T1+Tis	16	2.92
T2	96	17.52
T3	373	68.07
T4	63	11.50
Lymph node status		
N0	323	58.94
N1	130	23.72
N2	94	17.15
Nx	1	0.18
Metastatic		
M0	408	74.45
M1	77	14.05
Mx	55	10.04
Unidentified	8	1.46
Cancer type		
Colon adenocarcinoma	452	82.48
Rectal adenocarcinoma	96	17.52

N, node; Nx, unclear N stage; M, metastasis; Mx, unclear M stage.

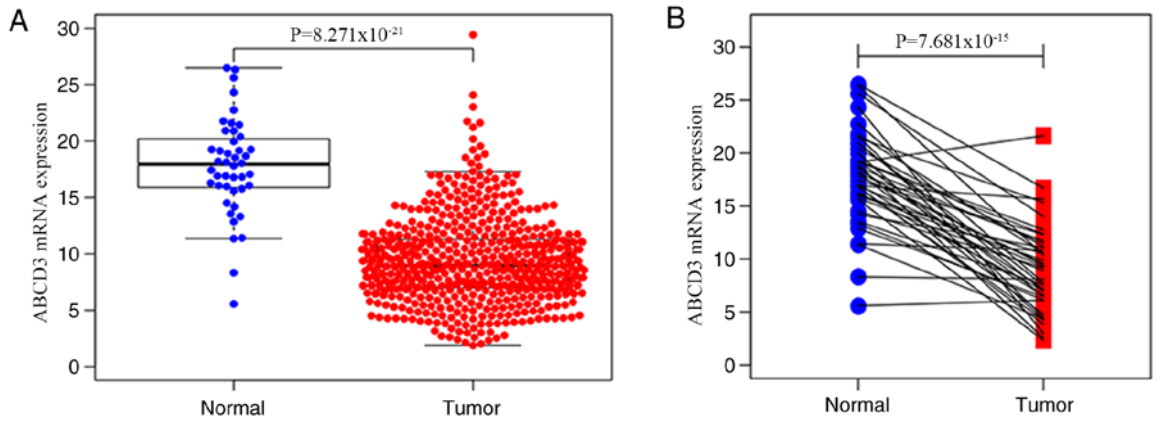

Figure 1. ABCD3 mRNA expression was decreased in CRC tissues compared to normal or adjacent normal tissues in The Cancer Genome Atlas. (A) ABCD3 mRNA expression was significantly lower in cancer tissues compared with normal tissues (P<0.001). (B) ABCD3 mRNA expression was downregulated in CRC tissues (P<0.001) compared with 44 paired non-cancerous adjacent tissues using Wilcoxon signed-rank tests. ABCD3, ATP binding cassette subfamily D member 3; CRC, colorectal cancer.

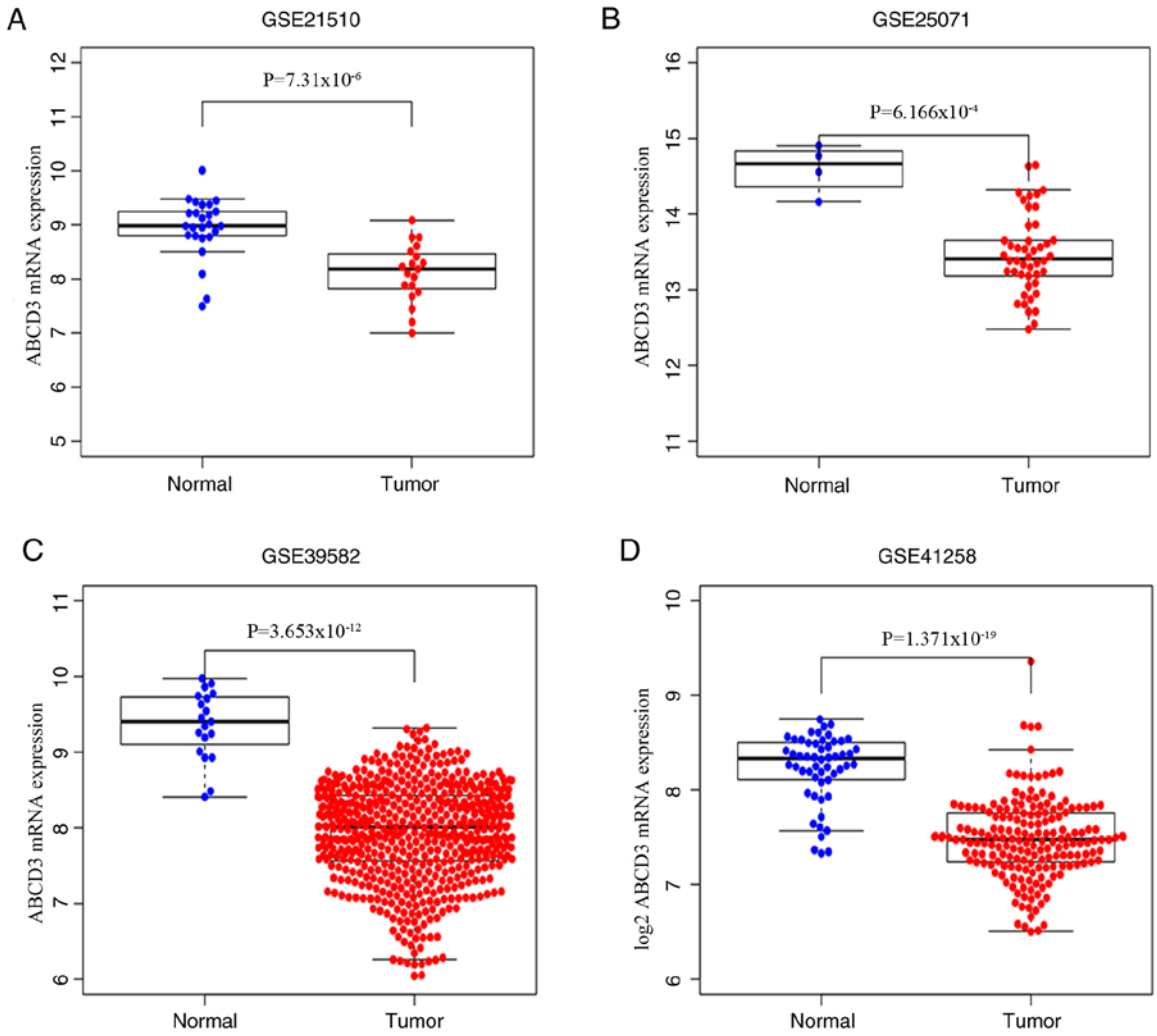

Figure 2. ABCD3 mRNA expression is significantly downregulated in colorectal cancer tissues compared with normal tissues form the Gene Expression Omnibus datasets (A) GSE21510 (P<0.001), (B) GSE25071 (P<0.001), (C) GSE39582 (P<0.001) and (D) GSE41258 (P<0.001). ABCD3, ATP binding cassette subfamily D member 3.

low ABCD3 mRNA expression had a poorer OS compared with patients with high ABCD3 mRNA expression (P=0.013; Fig. 6A). Furthermore, similar analysis in patients from the GSE39582 dataset demonstrated that OS was significantly decreased in patients with low ABCD3 mRNA expression compared with those with high ABCD3 mRNA expression (P=0.032; Fig. 6B).

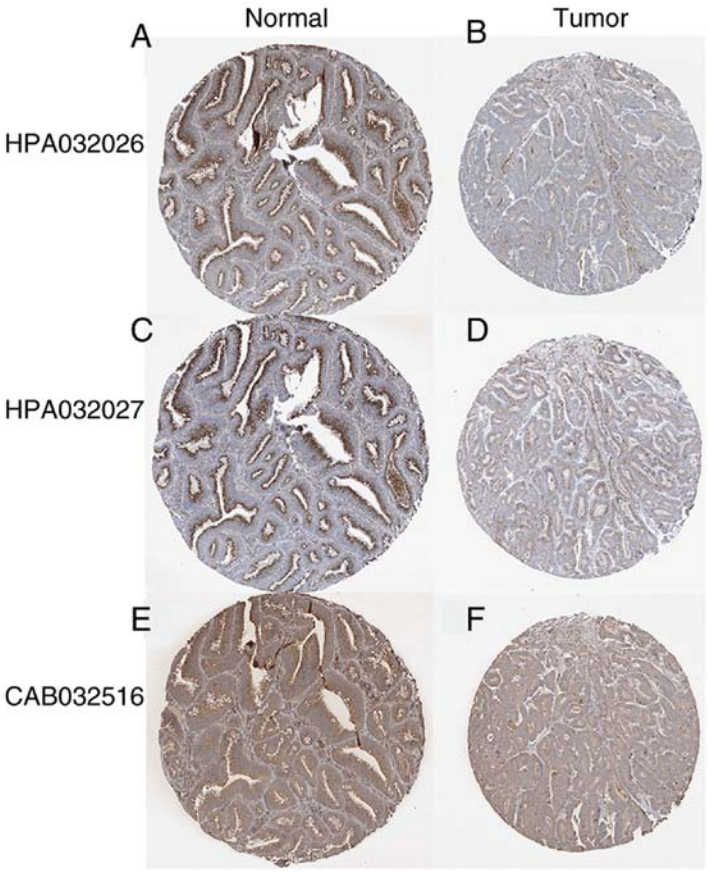

Figure 3. ATP binding cassette subfamily D member 3 protein expression was downregulated in colon cancer tissues compared with normal tissues based on the Human Protein Atlas database. (A and B) Antibody HPA032026. (A) Normal colon tissue was from a woman aged 82 years (patient ID: 2960; staining: high; intensity: strong; quantity: >75%). (B) Colon adenocarcinoma was from a man aged 83 years (patient ID: 2947; staining: low; intensity: weak; quantity: 75-25%). (D and D) Antibody HPA032027. (C) Normal colon tissue was from a woman aged 82 years (patient ID: 2960; staining: high; intensity: strong; quantity: >75%). (D) Colon adenocarcinoma was from a man aged 83 years (patient ID: 2947; staining: medium; intensity: moderate; quantity: 75-25%). (E and F) Antibody CAB032516 (E) Normal colon tissue was from a woman aged 82 years (patient ID: 2960; staining: medium; intensity: moderate; quantity: >75%). (F) Colon adenocarcinoma was from a man aged 83 years (patient ID: 2947; staining: low; intensity: weak; quantity: >75%).

Univariate analysis of clinicopathological characteristics demonstrated that ABCD3 mRNA level was a predictor of OS [P=0.0028; HR=0.89; 95% CI (0.84-0.96)], which was also the case for age(P=0.008; HR=1.03; 95% CI, 1.01-1.05), clinical stage (P<0.001; HR=2.59; 95% CI, 1.99-3.36), T stage (P<0.001; HR=3.27; 95% CI, (2.09-5.12), lymph nodes status (P<0.001; HR=2.24; 95% CI, 1.72-2.93) and distant metastasis status (P<0.001; HR=5.27; 95% CI, 3.33-8.34) (Table IV). Following multivariate analysis, ABCD3 mRNA expression remained independently associated with OS [P=0.016; HR=0.92; 95% CI (0.86-0.92)], as well as age (P<0.001; HR=1.04; 95% CI, 1.02-1.07) and T stage (P=0.007; HR=2.01; 95% CI, 1.21-3.32) (Table IV). These findings suggested that ABCD3 mRNA expression may be considered as an independent prognostic factor for patients with CRC, and that decreased ABCD3 mRNA expression may be associated with a poorer OS.

Screening of signaling pathways associated with ABCD3. GSEA method was used to determine the signaling pathways in which high and low ABCD3 expression levels are identified in the enrichment of MSigDB Collection (c2.cp.v6.2.symbols.gmt). The results demonstrated that five signaling pathways, including cell apoptosis, cell cycle, renal cell carcinoma, thyroid cancer, and CRC were significantly enriched in ABCD3 high mRNA expression (FDR values, 0.216, 0.178, 0.214, 0.214 and 0.188, respectively; NOM P-values, 0.032, 0.037, 0.040, 0.025 and 0.006, respectively; and NES, 1.586, 1.696, 1.563, 1.595 and 1.699, respectively; Fig. 7A-E). These findings suggested that ABCD3 may be involved in the progression of CRC.

Discussion

At present, there are only a few studies on the role and underlying mechanism of ABCD3 in tumors, such as prostate tumor (16), ovarian cancer (15,17), colon cancer (14), chronic myeloid leukemia (25), melanoma (26) and retinoblastoma (27). However, the aforementioned studies only investigated the difference of ABCD3 mRNA or protein expression in tumors. For instance, ABCD3 protein expression is significantly decreased in colon adenocarcinoma tissues compared with adjacent normal colon tissues (28). In addition, Yasui *et al* (29) demonstrated that ABCD3 is amplified in 19 resistant cancer cell lines. To the best of our knowledge, no parameters of CRC have been used to evaluate the correlation between ABCD3 mRNA expression and CRC before. The present study investigated the difference in ABCD3 mRNA expression between CRC and normal tissues. In addition, to the best of our knowledge, this study was

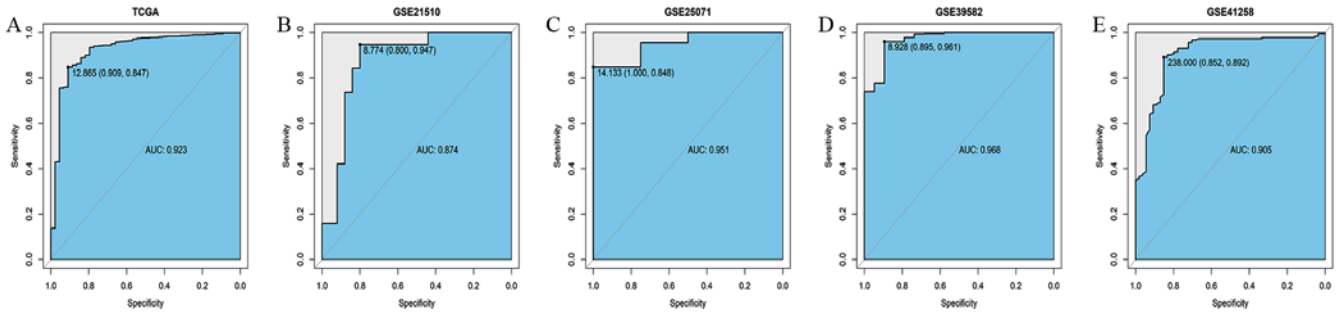

Figure 4. Receiver operating characteristic curves using ATP binding cassette subfamily D member 3 mRNA expression data of patients with colorectal cancer and healthy individuals from the (A) TCGA and (B) GSE39582 datasets. (A) TCGA. (B) GSE21510. (C) GSE25071. (D) GSE39582. (E) GSE41258. AUC, area under the curve; TCGA, The Cancer Genome Atlas.

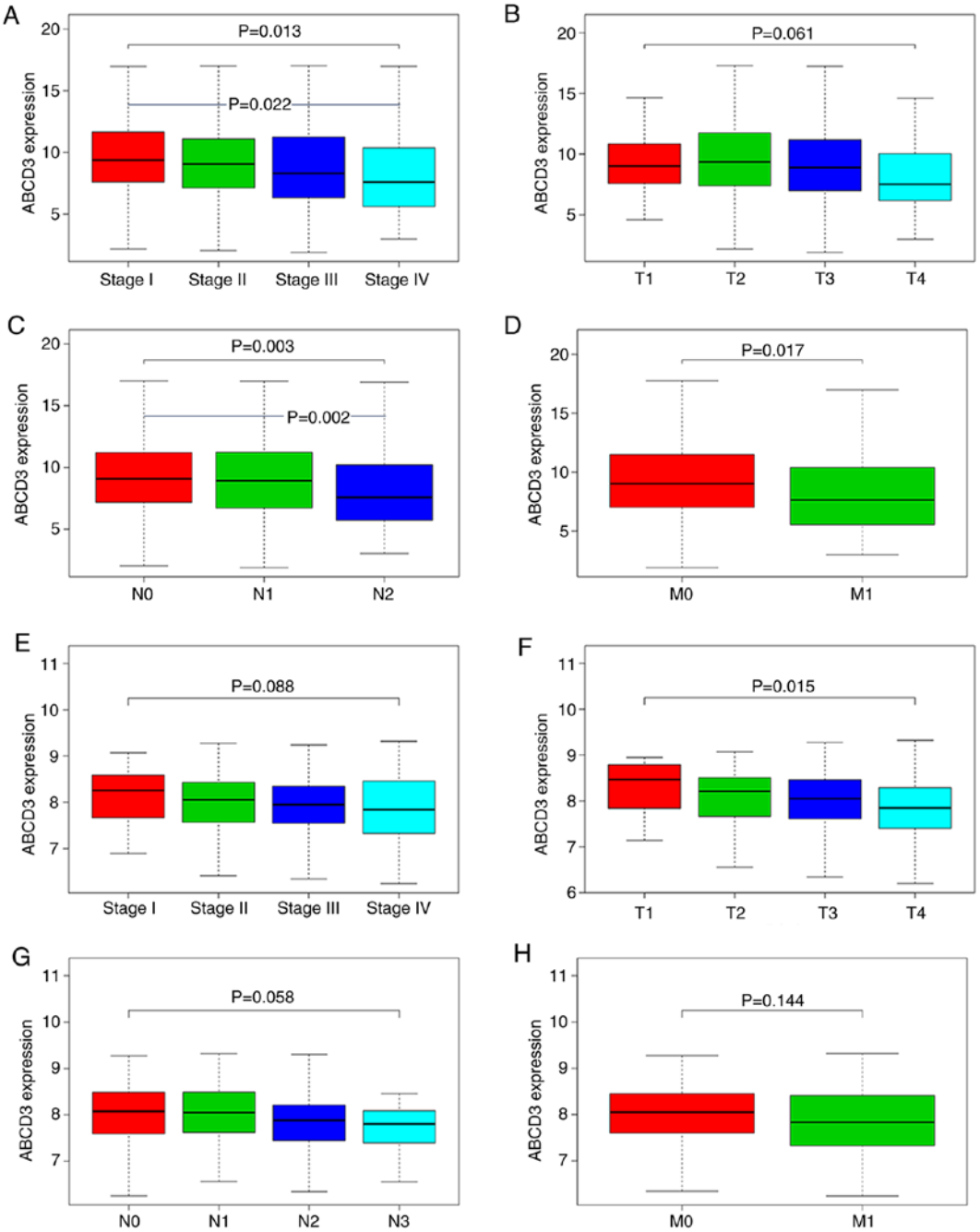

Figure 5. Association between ABCD3 mRNA expression and clinicopathologic characteristics of patients with colorectal cancer obtained from (A-D) The Cancer Genome Atlas and (E-H) GSE39582 (E-H). (A) Clinical stage. (B) T stage. (C) N stage. (D) M stage. (E) Clinical stage. (F) T stage. (G) N stage. (H) M stage. ABCD3, ATP binding cassette subfamily D member 3; T, topography distribution; N, lymph node status; M, distant metastasis status.

Table II. Association between ABCD3 mRNA expression and the clinicopathological characteristics of patients with colorectal cancer from The Cancer Genome Atlas (logistic regression).

Clinical characteristics	Total, n	OR in ABCD3 expression (range)	P-value
Stage (IV vs. I)	170	0.51 (0.27-0.94)	0.031
T stage (T4 vs. T2)	156	0.50 (0.26-0.95)	0.035
Lymph node status (N1+2 vs. N0)	540	0.69 (0.49-0.98)	0.036
Distant metastasis (M1 vs. M0)	478	0.64 (0.39-1.05)	0.081

ABCD3, ATP binding cassette subfamily D member 3; OR, odds ratio.

Table III. Association between ABCD3 mRNA expression and the clinicopathological characteristics of patients with colorectal cancer from The Cancer Genome Atlas.

		ABCD3			
Variables	Number	Low expression	High expression	χ^2 value	P-value
Sex					
Female	243	120	123	0.054	0.816
Male	204	103	101		
Age at diagnosis, years					
>60	307	148	159	1.106	0.293
≤60	140	75	65		
T stage					
T1+2	93	42	51	1.05	0.306
T3+4	354	181	173		
Metastasis					
M0	375	180	195	3.32	0.068
M1	72	43	29		
Lymph node status					
N0	267	123	144	3.872	0.049
N1-2	180	100	80		
Clinical stage					
I+II	118	140	258	4.207	0.040
III+IV	105	84	189		

ABCD3, ATP binding cassette subfamily D member 3.

the first to evaluate the diagnostic and prognostic values of ABDC3 mRNA expression.

In the present study, ABCD3 mRNA data of patients with CRC from TCGA and GEO databases were collected, and ABCD3 protein expression was obtained from the Human Protein Atlas. The results demonstrated that the mRNA and protein expression of ABCD3 was downregulated in CRC tissues compared with normal tissues. Furthermore, the high ABCD3 mRNA expression in CRC tissues was negatively associated with clinical stage, T stage, lymph node status and distant metastasis, but was associated with better prognosis. In addition, results from GSEA demonstrate that high ABCD3 mRNA expression was enriched in cell apoptosis, cell cycle, renal cell carcinoma, thyroid cancer, and CRC, suggesting that ABCD3 mRNA may be considered as a new therapeutic target and diagnostic and prognostic biomarker in CRC.

ABCD3, which is a member of the superfamily of ABC transporters, participates in the peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle (11). Increasing evidence demonstrated that ABC transporters affect the progression of chemoresistance by regulating the efflux of anticancer agents outside cancer cells (26,30,31). This common feature could be due to a subpopulation of slow-cycling cancer stem cells, which show enhanced multidrug resistance and tumorigenic potential (32). Although the underlying mechanisms of ABCD3 remain unknown, other molecules from the ABC transporters family have been studied. For example, elevated expression of ABCF1 enhances drug efflux and

Table IV. Univariate cox regression and multivariate cox regression analyses in patients with colorectal cancer from The Cancer Genome Atlas.

	Univariate analysis			Multivariate analysis		
Clinicopathologic variable	HR	95% CI	P-value	HR	95% CI	P-value
Age (continuous variable)	1.03	1.01-1.05	0.008	1.04	1.02-1.07	<0.001
Sex	0.95	0.61-1.49	0.833			
Stage	2.59	1.99-3.36	<0.001	1.80	0.83-3.90	0.136
T stage	3.27	2.09-5.12	<0.001	2.01	1.21-3.32	0.007
Metastasis	5.27	3.33-8.34	<0.001	1.54	0.54-4.42	0.422
Lymph node status	2.24	1.72-2.93	<0.001	1.13	0.71-1.77	0.611
ABCD3 expression	0.90	0.84-0.96	0.003	0.92	0.86-0.98	0.016

HR, hazard ratio; CI, confidence interval.

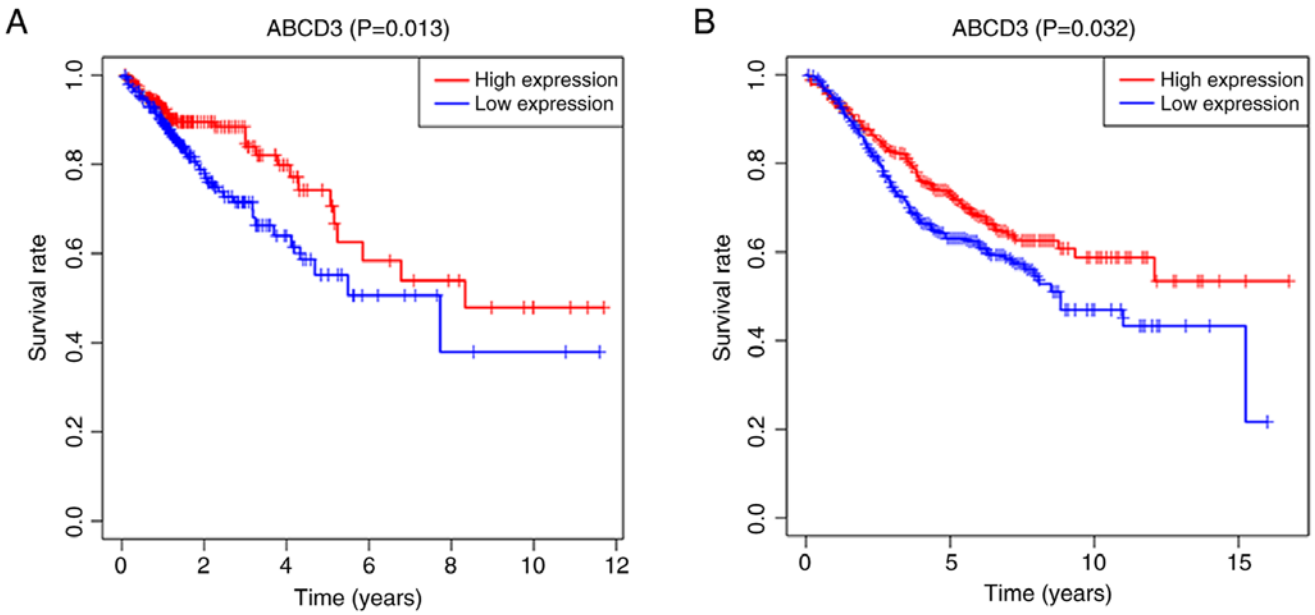

Figure 6. Kaplan-Meier curves showed that ABCD3 mRNA expression was associated with overall survival in patients with colorectal cancer from (A) The Cancer Genome Atlas and (B) GSE39582 dataset. (A) TCGA. (B) GSE39582. ABCD3, ATP binding cassette subfamily D member 3.

chemoresistance, and accelerates colony and spheroid formation, cell migration and epithelial-mesenchymal transition in hepatocellular carcinoma (33). In addition, the molecule Guajadial can suppress drug resistance that could be mediated by the repression of ABC transporter expression and by the PI3K/Akt pathway in drug-resistant breast cancer cells (34). A previous study similar to the present one demonstrated that ABCB9 mRNA expression is decreased in ovarian cancer tissues compared with normal ovarian tissues, and that decreased ABCB9 mRNA expression is associated with poor OS in patients. These results suggested that ABCB9 might be considered as an independent prognostic indicator in ovarian cancer (35). Similarly, the findings form the present study suggested that ABCD3 may serve a crucial role in drug resistance and may affect the OS of patients with CRC.

The present study demonstrated that ABCD3 was involved in cell apoptosis, cell cycle, renal cell carcinoma, thyroid cancer and CRC according to results from GSEA. However, these predictions must be further investigated and confirmed.

Although the present study suggested some associations between ABCD3 mRNA expression and CRC, it presented some limitations. Firstly, to fully elucidate the crucial role of ABCD3 in the progression of CRC, numerous clinical factors, including recurrence, histological grade and treatment situation, should be considered. Secondly, the number of tumor samples differed significantly from the number of normal samples, and further investigation using a higher sample size is therefore required. Thirdly, the GSE21510, GSE25071, GSE41258 and GSE39582 datasets were not analyzed by same group or individuals, therefore the testing standards may have differed between these datasets and so no systematic meta-analysis was performed. Fourthly, the results from the present study were only based on the bioinformatics analysis of multiple databases. Future study will

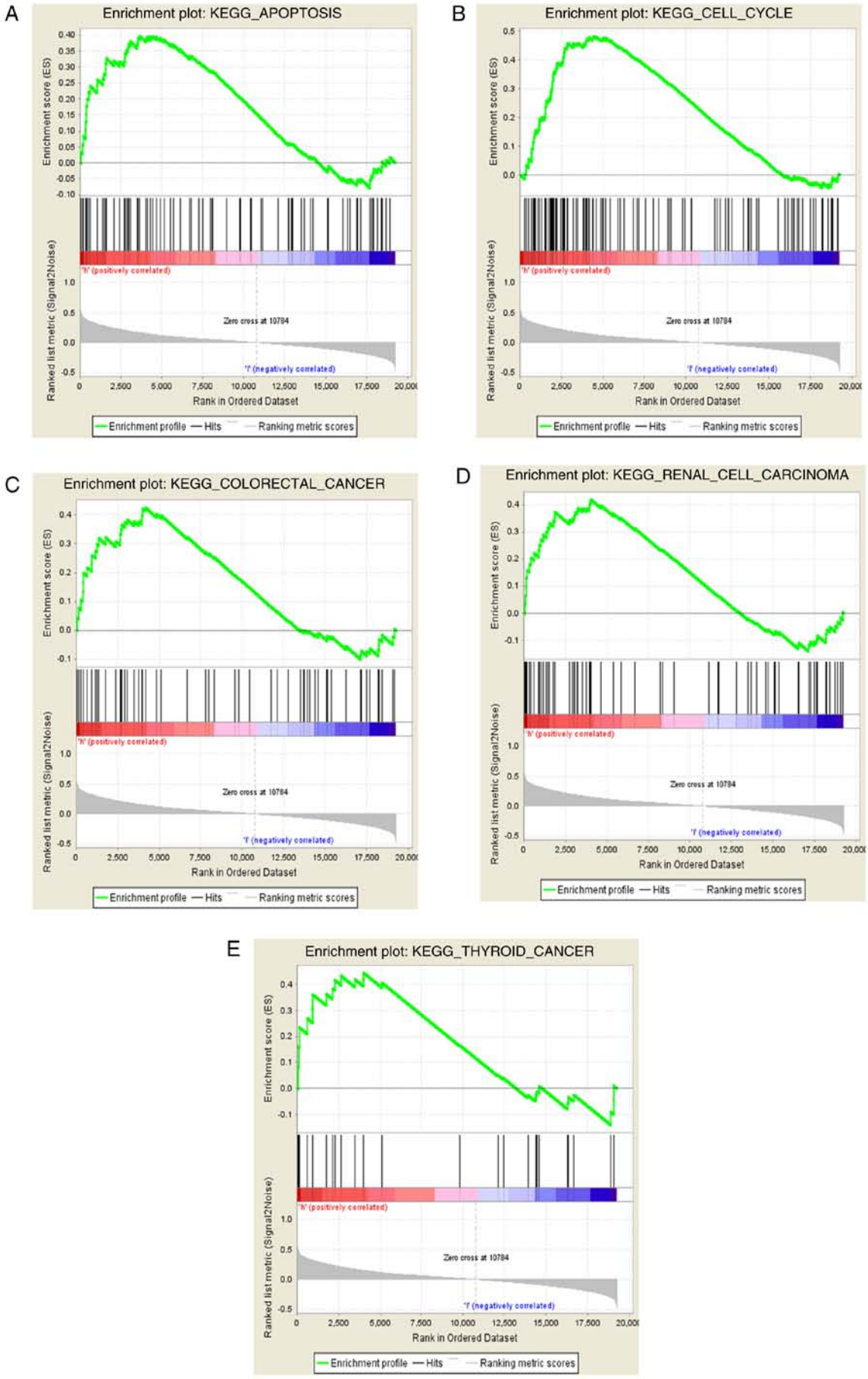

Figure 7. Kyoto Encyclopedia of Genes and Genomes pathway enrichment plots from Gene Set Enrichment Analysis. (A) Apoptosis. (B) Cell cycle. (C) Colorectal cancer. (D) Renal cell carcinoma. (E) Thyroid cancer. These results showed significantly differential enrichment in the mRNA ABCD3 high expression phenotype based on normalized enrichment score, normalized P-value and false discovery rate value.

therefore include experimental *in vitro* results. However, despite the limitations of the present study, the present bioinformatics analysis did provide novel insight into the function of ABCD3 in CRC, including target molecules screening, gene function analysis and identification of molecular signaling pathways.

In conclusion, the present study demonstrated that low ABCD3 mRNA expression in patients with CRC was associated with poor OS. In addition, ABCD3 was enriched in signaling pathways such as apoptosis, cell cycle, renal cell carcinoma, thyroid cancer, and CRC, therefore ABCD3 may function in CRC progression. The present study partially revealed the function of ABCD3 in CRC and demonstrated that it may be considered as a diagnostic and prognostic biomarker in patients with CRC.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the TCGA database (https://mirrors.tuna.tsinghua.edu.cn/CRAN/) and GEO database (https://www.ncbi.nlm.nih.gov/gds/).

Authors' contributions

GY and JY conceived and designed the study. GY performed the bioinformatics analysis. YuZ analyzed the data. GY and YaZ wrote the manuscript. JY reviewed the manuscript. JW participated in the collection of data and the bioinformatics analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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