

Identification of key genes for predicting colorectal cancer prognosis by integrated bioinformatics analysis

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Abstract. Colorectal cancer (CRC) is a life-threatening disease with a poor prognosis. Therefore, it is crucial to identify molecular prognostic biomarkers for CRC. The present study aimed to identify potential key genes that could be used to predict the prognosis of patients with CRC. Three CRC microarray datasets (GSE20916, GSE73360 and GSE44861) were downloaded from the Gene Expression Omnibus (GEO) database, and one dataset was obtained from The Cancer Genome Atlas (TCGA) database. The three GEO datasets were analyzed to detect differentially expressed genes (DEGs) using the BRB-ArrayTools software. Functional and pathway enrichment analyses of these DEGs were performed using the Database for Annotation, Visualization and Integrated Discovery tool. A protein-protein interaction (PPI) network of DEGs was constructed, hub genes were extracted, and modules of the PPI network were analyzed. To investigate the prognostic values of the hub genes in CRC, data from the CRC datasets of TCGA were used to perform the survival analyses based on the sample splitting method and Cox regression model. Correlation among the hub genes was evaluated using Spearman's correlation analysis. In the three GEO datasets, a total of 105 common DEGs were identified, including 51 down- and 54 up-regulated genes in CRC compared with normal colorectal tissues. A PPI network consisting of 100 DEGs and 551 edges was constructed, and 44 nodes were identified as hub genes. Among these 44 genes, the four hub

genes TIMP metalloproteinase inhibitor 1 (TIMP1), solute carrier family 4 member 4 (SLC4A4), aldo-keto reductase family 1 member B10 (AKR1B10) and ATP binding cassette subfamily E member 1 (ABCE1) were associated with overall survival (OS) in patients with CRC. Three significant modules were extracted from the PPI network. The hub gene TIMP1 was present in Module 1, ABCE1 was involved in Module 2 and SLC4A4 was identified in Module 3. Univariate analysis revealed that TIMP1, SLC4A4, AKR1B10 and ABCE1 were associated with the OS of patients with CRC. Multivariate analysis demonstrated that SLC4A4 may be an independent prognostic factor associated with OS. Furthermore, the results from correlation analysis revealed that there was no correlation between TIMP1, SLC4A4 and ABCE1, whereas AKR1B10 was positively correlated with SLC4A4. In conclusion, the four key genes TIMP1, SLC4A4, AKR1B10 and ABCE1 associated with the OS of patients with CRC were identified by integrated bioinformatics analysis. These key genes may be used as prognostic biomarkers to predict the survival of patients with CRC, and may therefore represent novel therapeutic targets for CRC.

Introduction

Colorectal cancer (CRC) is the third most common type of cancer, and the fourth leading cause of cancer associated-mortality in the world, with 1.8 million new cases diagnosed and 881,000 deaths in 2018 (1,2). Since surgical techniques and chemotherapy regimen have been improved, and molecular targets have been determined, attention has focused on early detection of CRC, which led to an increase in survival time of patients with CRC (3,4). However, the 5-year overall survival (OS) rate of patients with CRC is still unsatisfactory, particularly for patients with advanced CRC (5). In China, CRC is the third most frequent type of cancer and the fourth leading cause of cancer-associated mortality (6). In order to precisely predict the prognosis of patients with CRC, it is crucial to identify novel molecular prognostic biomarkers.

Many lifestyle factors have been investigated, with imbalanced diet, tobacco use, alcohol consumption, lack of physical activity, obesity and sleep deprivation being considered as the risk factors in the progression of CRC (7). Heredity, epigenetic, somatic cell and endocrine aberration seem to play significant roles in CRC (8). Increasing evidence has suggested that multiple genes and cellular signaling pathways serve

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important roles in the pathogenesis of CRC (9,10). Diagnostic and prognostic signatures, for example BRAF, RAS and MSI, can monitor the response to therapy in patients with CRC (11). Therefore, it is critical to identify novel biomarkers with high sensitivity and specificity in order to allow the early detection of CRC and to choose the best treatment option for patients with CRC. Microarray analysis has been considered as a promising tool in cancer research and may have important clinical applications, including in diagnosis, cancer classification, prognosis prediction and detection of therapeutic targets (12-14). In the last decade, microarray technology has been used to study the gene expression profiles of CRC, which has led to the identification of thousands of differentially expressed genes (DEGs) (15). However, DEGs identified in one study might not be detected in another study. In addition, interactions among DEGs and their prognostic values remain unknown.

The present study aimed to determine potential novel prognosis biomarkers for CRC. Three microarray datasets obtained from the Gene Expression Omnibus (GEO) database were analyzed in order to further identify the DEGs in CRC, by comparing their expression levels between CRC and normal samples. Subsequently, a protein-protein interaction (PPI) network was constructed, from which modules were extracted, and functional and pathway analyses were performed to further analyze the roles of the CRC-associated DEGs. To verify the prognostic roles of DEGs in CRC, the CRC dataset from The Cancer Genome Atlas (TCGA) was used to perform survival analysis based on the sample splitting method and Cox regression model. The key genes that were identified may be used to characterize the survival of CRC, and may serve as potential therapeutic targets and prognostic biomarkers.

Materials and methods

Microarray datasets. The GEO database (<http://www.ncbi.nlm.nih.gov/geo>) is a public functional genomics data repository that contains multifaceted data, including data derived from microarray and next-generation sequencing. GEO database was searched using the following key words: ('colorectal neoplasms'[MeSH Terms] OR colorectal cancer [All Fields]) AND 'Homo sapiens'[porgn] AND ('gse'[Filter] AND 'Expression profiling by array'[Filter] AND 'attribute name tissue'[Filter] AND ('60'[n_samples] : '3000'[n_samples])) AND ('gse'[Filter] AND 'Expression profiling by array'[Filter] AND 'attribute name tissue'[Filter] AND ('60'[n_samples] : '3000'[n_samples])). Following systematic review based on the key words, a total of 77 datasets were identified for further analysis. The selection criteria for these datasets were as follows: i) Included datasets must include paired CRC and normal control tissues; ii) sample size of each group must be >30; and iii) adequate clinical information must be available to perform the analysis. According to the selection criteria, three gene expression profiles were collected, including GSE20916 (16), GSE73360 (17) and GSE44861 (18). The microarray data of GSE20916, GSE73360 and GSE44861 were downloaded from the GEO database. GSE20916 was based on the Affymetrix GPL570 platform (Human Genome U133 Plus 2.0 Array), GSE73360 was based on the Affymetrix GPL17586 platform (Human Transcriptome Array 2.0) and GSE44861 was based on Affymetrix GPL3921 platform (Human

Genome U133A Array). The GSE20916 dataset included 101 CRC tissue samples and 44 normal samples. Expression data from the GSE73360 dataset included 55 CRC samples and 37 normal samples. Microarray data from the GSE44861 dataset included 56 CRC samples and 55 normal samples.

Data pre-processing and identification of DEGs in CRC. The Series Matrix File(s) of GSE20916, GSE73360 and GSE44861 were downloaded from the GEO database. Prior to analysis, the probes in each dataset were transformed into standard gene symbols. Normalization of the three datasets was implemented based on robust multi-array average in the R software, version 2.6.0 (www.R-project.org/) (19), and normalization was separately conducted in each gene expression dataset.

The BRB-ArrayTools package (v4.6.0 Beta 1; <https://brb.nci.nih.gov/BRB-ArrayTools/download.html>) is an integrated software for the visualization and statistical analysis of microarray gene-expression data, gene-methylation data and RNA-sequencing data (20). In the present study, DEGs from each dataset were identified using BRB-ArrayTools software. A P-value <0.01 and \log_2 fold change >1 were set as the cut-off criteria. A heatmap and Volcano Plot of the DEGs from each dataset were subsequently generated using BRB-ArrayTools. The Venn diagram illustrating the intersection of these DEGs among the three microarray profiles was visualized using FunRich_V3.1.3 software (<http://www.funrich.org>).

PPI network construction and module analysis. The Search Tool for the Retrieval of Interacting Genes (STRING; <https://string-db.org>) database is an online software that is used to analyze PPI information, and to present the interactions with a combine-score (21). In the present study, DEGs with a confidence score >0.4 were extracted, and the PPI network of these DEGs was subsequently constructed and visualized using Cytoscape software (version 3.5.1; <http://www.cytoscape.org>) (22). Edge width was determined according to the combined score of the PPI relationship. Since the networks were scale-free, the biological importance of genes associated with degree centrality was described to improve the understanding of the functionality of these complicated networks. The degree is determined as the number of links between a node and its adjacent nodes (23). In the current study, hub genes were defined as the nodes with a degree >10, and were extracted.

The analysis of modules was conducted on the PPI network by using MCODE version 2.2 (<https://dSPACE.mit.edu/handle/1721.1/75242>) according to the following parameters: Node score cut-off=0.2, k-core=2 and max. Depth=100 (24).

Functional and pathway enrichment analysis. Gene Ontology (GO) (25) analysis has become a common method used for functional studies of large-scale transcriptomic data and analysis of genomic data. Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/pathway>) is a reference database for systematic analysis of gene functions (26). The Database for Annotation Visualization and Integrated Discovery (DAVID; <https://david.ncifcrf.gov>) is a tool used to systematically detect biological significance in large lists of genes or proteins (27). In the present study,

the GO function and KEGG pathway enrichment analysis of identified DEGs and the genes in the significant modules were performed using DAVID. Terms with a P-value <0.001 were considered statistically significant.

Pathway crosstalk analysis. Enrichment map is a network-based method for gene-set enrichment visualization and interpretation (28). In order to extract the interactions between significantly enriched signaling pathways, pathway crosstalk analysis was performed using the EnrichmentMap tool (<http://www.baderlab.org/Software/EnrichmentMap>). Benjamini-Hochberg adjusted P-value <0.05 and the Jaccard Coefficient (50%) + Overlap Coefficient (50%) >0.5 were considered as the thresholds. The Jaccard Coefficient, and Overlap Coefficient are two indexes to examine the similarity between sample sets.

Survival analysis of hub genes. The Human Protein Atlas (<http://www.proteinatlas.org>) is an online database that contains clinical data of 597 patients with CRC, which were from the TCGA database (<https://tcga-data.nci.nih.gov/tcga/>). The present study aimed to investigate the impact of the expression levels of the identified hub genes on the OS of patients with CRC. Kaplan-Meier survival analysis was carried out by using the Human Protein Atlas database. The survival rates of patients with CRC according to the expression level of each hub gene were compared. Briefly, according to the Fragments Per Kilobase Million (FPKM) value of each hub gene, patients with CRC were classified into a high or low expression group based on the best expression cut-off value, which is the FPKM value that yields maximal difference by survival analysis between the two groups at the smallest log-rank P-value. Kaplan-Meier survival curves were plotted, and the log-rank test was applied to compare the survival rates between the high and low expression groups. $P<0.001$ was set as the cut-off criterion. Univariate and multivariate Cox models were subsequently used to investigate the prognostic value of OS-associated hub genes. $P<0.01$ was considered to indicate statistical significance.

Correlation between hub genes. The correlation between the hub genes that were associated with CRC was evaluated using Spearman's correlation analysis. Spearman's correlation coefficients range between -1 and 1, with -1, 0 and 1 indicating negative, no and positive correlation, respectively. In addition, the threshold for gene correlation was set as Spearman's correlation coefficient ($|r|<0.3$) and $P<0.05$.

Results

Identification of DEGs. A total of 2,836, 2,942 and 420 DEGs were identified from the GSE20916, GSE73360 and GSE44861 datasets, respectively. Volcano plots showing the distribution of these DEGs in the three datasets were created (Fig. 1A). The results from cluster heat maps (Fig. 1B) identified distinctive patterns in CRC and normal samples. Subsequently, the intersection of these DEGs from the three microarray profiles was identified using FunRich_V3 software. A total of 105 mutual DEGs were identified in CRC tissues among the three datasets compared with normal colorectal tissues

(Fig. 1C), including 51 down-regulated and 54 up-regulated genes. Fig. 1D shows the expression pattern of the common up-regulated and down-regulated DEGs from the expression data of the GSE20916, GSE44861 and GSE73360 datasets.

Functional and pathway enrichment analysis. In order to comprehensively understand the biological roles of these DEGs in CRC, DAVID was used to determine the GO functions and pathways in which they were involved (Table I). The down-regulated DEGs were significantly enriched in the biological processes 'bicarbonate transport', and 'regulation of intracellular pH', and in the cellular components 'extracellular exosome' and 'integral component of plasma membrane'. Furthermore, down-regulated DEGs were enriched in the molecular functions 'chloride channel activity' and 'carbonate dehydratase activity'. The up-regulated DEGs were significantly enriched in the biological processes 'cell proliferation', 'collagen catabolic process', 'extracellular matrix organization', 'extracellular matrix disassembly', and 'leukocyte migration' in certain cellular components, including 'extracellular space', 'extracellular region', 'proteinaceous extracellular matrix', 'collagen trimer' and 'cell surface', and in the molecular functions 'CXCR chemokine receptor binding', 'platelet-derived growth factor binding' and 'endopeptidase activity'.

The 20 significant pathways which the up- and down-regulated DEGs were involved in are presented in Table II. The results demonstrated that down-regulated DEGs were significantly enriched in the pathways 'proximal tubule bicarbonate reclamation' and 'nitrogen metabolism', whereas up-regulated DEGs were enriched in 'ECM-receptor interaction', 'focal adhesion', 'PI3K-Akt signaling pathway' and 'TNF signaling pathway'.

PPI network construction and module analysis. The PPIs of the DEGs were obtained using STRING with a confidence score >0.4 , and the PPI network of these DEGs was visualized using Cytoscape. The results demonstrated that the PPI network covered 100 nodes and 551 edges, including 49 down-regulated genes and 51 up-regulated genes (Fig. 2A). Subsequently, hub nodes in the PPI network with a connectivity degree >10 were selected. A total of 44 genes were identified as hub genes. Among these hub genes, 17 were down-regulated [transmembrane serine protease 2, sorcin, solute carrier family 4 member 4 (SLC4A4), solute carrier family 26 member 3, nuclear receptor subfamily 3 group C member 2, myosin heavy chain 11, keratin 20, 15-hydroxyprostaglandin dehydrogenase, guanylate cyclase activator 2A, fatty acid binding protein 1, CEA cell adhesion molecule 7, carbonic anhydrase 4, carbonic anhydrase 2, carbonic anhydrase 1, ADP ribosylation factor like GTPase 14, alanyl aminopeptidase, membrane and aldo-keto reductase family 1 member B10 (AKR1B10)] and 27 were up-regulated [TIMP metalloproteinase inhibitor 1 (TIMP1), thrombospondin 2, transforming growth factor β induced, stanniocalcin 1, secreted phosphoprotein 1, secreted protein acidic and cysteine rich, serpin family B member 5, stearoyl-CoA desaturase, receptor interacting serine/threonine kinase 2, regenerating family member 3 α , prostaglandin-endoperoxide synthase 2, MYC proto-oncogene, bHLH transcription factor, matrix metalloproteinase 3, matrix metalloproteinase 1, MET proto-oncogene, receptor tyrosine kinase, leucine rich repeat containing G

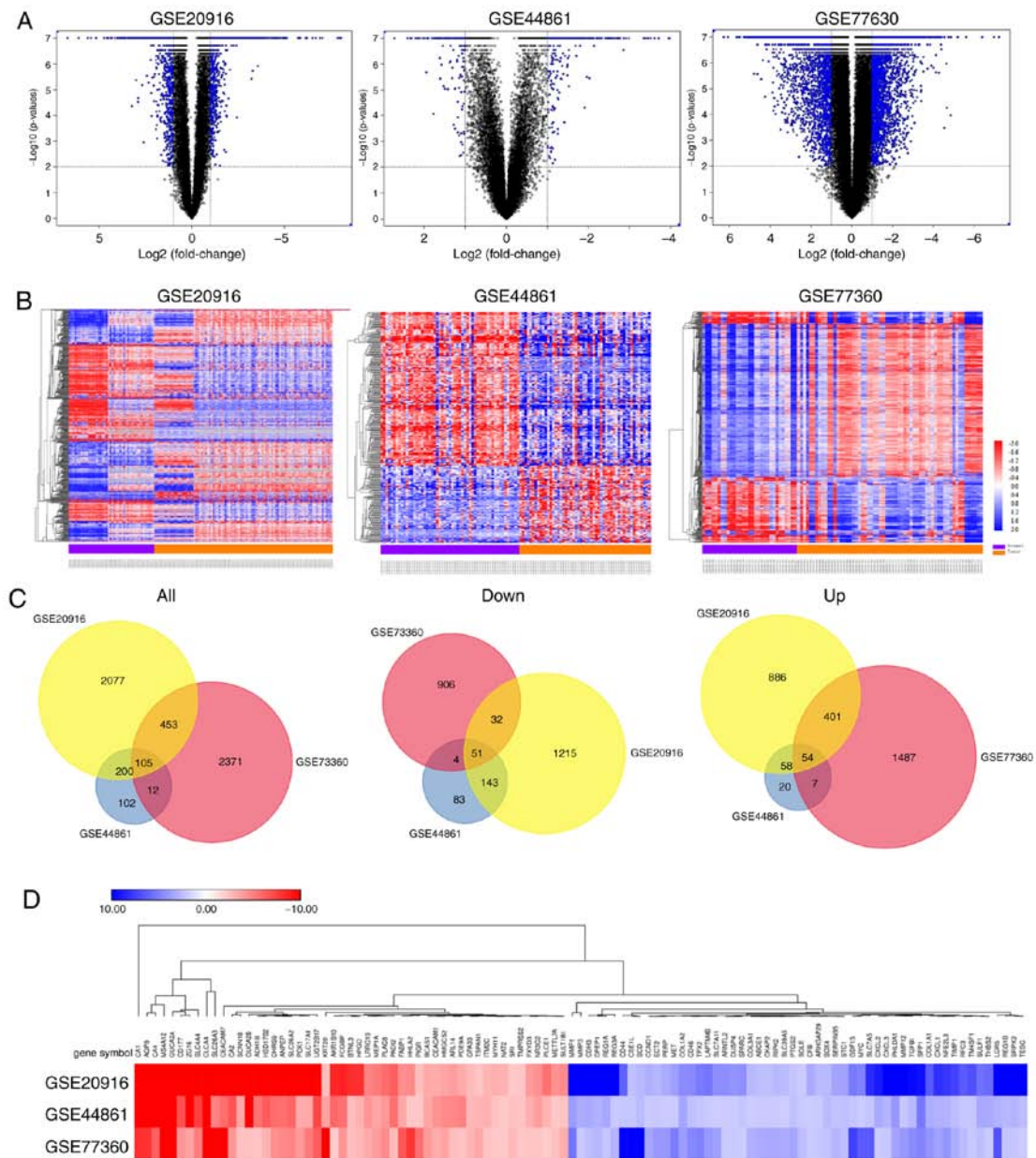


Figure 1. Expression pattern of genes between CRC and normal samples. (A) Volcano plots exhibiting expression data of CRC and normal tissues in the microarray profiles of GSE20916, GSE44861 and GSE73360. The x-axis presents the mean differences between CRC and normal samples. The y-axis presents the log transformed P-values. DEGs are shown in blue. (B) Hierarchical clustering analysis of DEGs between CRC and normal samples in GSE20916, GSE44861 and GSE77360. Each row represents a DEG and each column represents a different sample. (C) Intersection of all DEGs (n=105), up-regulated DEGs (n=54) and down-regulated DEGs (n=51) among the expression data of GSE20916, GSE44861 and GSE73360. (D) Hierarchical clustering analysis of the mutual up-regulated and down-regulated DEGs in the expression data of GSE20916, GSE44861 and GSE73360. Red represents down-regulated genes, whereas blue represents up-regulated genes. CRC, colorectal cancer; DEGs, differentially expressed genes.

protein-coupled receptor 5, growth differentiation factor 15, dual specificity phosphatase 4, C-X-C motif chemokine ligand 2, C-X-C motif chemokine ligand 1, collagen type III α 1 chain, collagen type I α 2 chain, collagen type I α 1 chain, complement factor B, CD44 molecule (Indian blood group), cyclin D1 and ATP binding cassette subfamily E member 1 (ABCE1)].

Three significant modules with a node score cut-off=0.2, k-core=2, and max. Depth=100 were extracted from the PPI network. A total of 18 nodes and 136 edges were included in Module 1 (Fig. 2B). There were six nodes and 12 edges in Module 2 (Fig. 2C), and Module 3 contained six nodes and 10 edges (Fig. 2D). The up-regulated hub gene TIMP1 was

included in Module 1, the up-regulated ABCE1 gene was involved in Module 2 and the down-regulated SLC4A4 was present in Module 3.

Module 1 was enriched in eight GO terms, including 'extracellular matrix organization', 'extracellular space' and 'platelet-derived growth factor binding'. Module 2 was enriched in two GO terms; 'ATPase activity' and 'protein binding'. Module 3 was enriched in the four terms 'regulation of intracellular pH', 'bicarbonate transport', 'chloride transmembrane transport' and 'chloride channel activity' (Table III).

In addition, the results from KEGG pathway enrichment analysis demonstrated that genes in Module 1 were significantly

Table I. GO analysis of differentially expressed genes in colorectal cancer tissues.

A, Downregulated				
Category	Term	Count, n	Percentage	P-value
GOTERM_BP_DIRECT	GO:0015701 'bicarbonate transport'	6	9.17	1.10x10 ⁻⁷
	GO:0051453 'regulation of intracellular pH'	4	6.11	1.21x10 ⁻⁴
GOTERM_CC_DIRECT	GO:0070062 'extracellular exosome'	29	44.32	2.28x10 ⁻¹¹
	GO:0005887 'integral component of plasma membrane'	15	22.95	1.38x10 ⁻⁵
GOTERM_MF_DIRECT	GO:0005254 'chloride channel activity'	4	6.11	4.54x10 ⁻⁴
	GO:0004089 'carbonate dehydratase activity'	3	4.59	6.76x10 ⁻⁴
B, Upregulated				
Category	Term	Count, n	Percentage	P-value
GOTERM_BP_DIRECT	GO:0030574 'collagen catabolic process'	6	7.70	1.55x10 ⁻⁶
	GO:0030198 'extracellular matrix organization'	8	10.26	2.27x10 ⁻⁶
	GO:0022617 'extracellular matrix disassembly'	6	7.70	3.66x10 ⁻⁶
	GO:0050900 'leukocyte migration'	6	7.70	3.69x10 ⁻⁵
	GO:0008283 'cell proliferation'	8	10.26	1.27x10 ⁻⁴
GOTERM_CC_DIRECT	GO:0005615 'extracellular space'	19	24.38	2.07x10 ⁻⁸
	GO:0005576 'extracellular region'	17	21.81	8.07x10 ⁻⁶
	GO:0005578 'proteinaceous extracellular matrix'	7	9.00	1.23x10 ⁻⁴
	GO:0005581 'collagen trimer'	5	6.42	1.47x10 ⁻⁴
	GO:0009986 'cell surface'	8	10.26	9.33x10 ⁻⁴
GOTERM_MF_DIRECT	GO:0045236 'CXCR chemokine receptor binding'	3	3.85	3.05x10 ⁻⁴
	GO:0048407 'platelet-derived growth factor binding'	3	3.85	4.65x10 ⁻⁴
	GO:0004175 'endopeptidase activity'	4	5.13	5.45x10 ⁻⁴

BP, biological process; CC, cellular component; GO, Gene Ontology; MF, molecular function.

involved in the four pathways 'ECM-receptor interaction', 'PI3K-Akt signaling pathway', 'focal adhesion' and 'miRNAs in cancer'. The genes in Module 3 were significantly enriched in the 'pancreatic secretion' pathway (Table IV).

Pathway crosstalk analysis. To determine how the 20 significant pathways interacted with each other, a pathway crosstalk analysis was conducted. The results demonstrated that a cross talk existed among 'PI3K-Akt signaling pathway', 'focal adhesion' and 'ECM-receptor interaction'. In addition, a separate crosstalk existed between 'proximal tubule bicarbonate reclamation' and 'nitrogen metabolism'. No interaction was observed between 'TNF signaling pathway' and the other pathways (Fig. 3).

Survival analysis of hub genes. The prognostic value of the 44 hub genes from the PPI network was investigated using The Human Protein Atlas database. The results demonstrated that four hub genes were associated with the OS of patients with CRC, including TIMP1, SLC4A4, AKR1B10 and ABCE1 (Fig. 4). The results of the survival analysis suggested that TIMP1 may be considered as an oncogene, whereas SLC4A4, AKR1B10 and ABCE1 may be tumor suppressor genes.

The results from univariate analysis revealed that the Union for International Cancer Control (UICC) stage (29) and TIMP1, SLC4A4, AKR1B10 and ABCE1 expression levels were significantly associated with the OS of patients with CRC (all P<0.01). However, there was no significant association between age/sex and OS in the univariate analysis (P>0.01). The results of multivariate analysis demonstrated that age, UICC stage and SLC4A4 expression were independent prognostic factors associated with the OS of patients with CRC (Table V; all P<0.01).

Correlation between hub genes. Correlation analysis between the expression levels of the four hub genes (TIMP1, SLC4A4, ABCE1 and AKR1B10) was performed using Spearman's correlation analysis. The results demonstrated that there was no correlation between TIMP1, SLC4A4 and ABCE1 expression (Fig. 5); however, the expression levels of AKR1B10 and SLC4A4 were positively correlated (R=0.39; P<0.05; Fig. 5).

Discussion

Despite advances in surgical and medical therapies for the treatment of CRC, the incidence and mortality rates remain

Table II. Kyoto Encyclopedia of Genes and Genomes pathway analysis of differentially expressed genes in colorectal cancer tissues.

A, Downregulated				
Term	Count, n	Percentage	P-value	Genes
hsa04964: Proximal tubule bicarbonate reclamation	4	6.11	7.06x10 ⁻⁵	CA4, CA2, SLC4A4, PCK1
hsa00910: Nitrogen metabolism	3	8.25	5.12x10 ⁻⁴	CA2, CLCA4, SLC4A4
B, Upregulated				
Term	Count, n	Percentage	P-value	Genes
hsa04512: ECM-receptor interaction	6	7.70	1.83x10 ⁻⁵	CD44, COL3A1, COL1A2, COL1A1, THBS2, SPP1
hsa04510: Focal adhesion	7	9.00	1.14x10 ⁻⁴	CCND1, COL3A1, MET, COL1A2, COL1A1, THBS2, SPP1
hsa04151: PI3K-Akt signaling pathway	8	10.26	2.71x10 ⁻⁴	CCND1, COL3A1, MET, COL1A2, COL1A1, THBS2, MYC, SPP1
hsa04668: TNF signaling pathway	5	6.42	7.00x10 ⁻⁴	CXCL1, PTGS2, CXCL3, CXCL2, MMP3

ECM, extracellular matrix; TNF, tumor necrosis factor.

high (1,2). Successful screening techniques and reducing the risk of CRC are essential to help decrease the incidence of CRC. Understanding the etiology and mechanisms of CRC progression is crucial to improve the survival rate of patients with CRC and prevent the disease occurrence. Recently, microarray technology, which has rapidly developed, has been widely used to compare the expression levels of genes, and has been used to predict disease progression, to make an accurate diagnosis and evaluate prognosis (30-32). In the present study, a meta-analysis method was used to analyze three microarray datasets (GSE20916, GSE73360 and GSE44861) in order to identify DEGs in CRC tissue samples. A total of 105 mutual DEGs were identified in the three datasets, including 51 down-regulated genes and 54 up-regulated genes. A total of 44 DEGs were subsequently selected from the PPI network and were identified as hub genes. Furthermore, three significant modules were identified in the PPI network. In addition, survival analysis of the hub genes demonstrated that two down-regulated genes (SLC4A4 and AKR1B10) and two up-regulated genes (ABCE1 and TIMP1) were significantly associated with the OS of patients with CRC. These four genes may be the most reliable genes that could be applied in clinical settings for the following reasons: i) These four genes were simultaneously confirmed using three gene expression profile datasets; and ii) these genes have been demonstrated to be associated with cancer.

SLC4A4, which is a member of the SLC4 family, is an electrically induced transmembrane transporter, which is mostly involved in sodium and bicarbonate transport to the epithelial cell membrane (33). A previous study reported that the SLC4 family is mainly involved in CO₂ transport by red blood cells, the

absorption or secretion of H⁺ or HCO₃⁻ by epithelial cells and regulation of cell volume and intracellular pH in the majority of cells (34). Furthermore, abnormal SLC4A4 expression has been reported in thyroid carcinoma, and this biomarker may be used for the diagnosis of thyroid cancer (35). However, to the best of our knowledge, only a few studies have investigated SLC4A4 expression in CRC. For example, Chen *et al* (36) demonstrated that the expression of SLC4A4 was decreased in CRC samples. Another study also reported that SLC4A4 was significantly down-regulated in the CRC group (37). In the present study, a PPI network was constructed to identify the hub genes for CRC, and SLC4A4 was identified to be down-regulated. The clinical data of 597 patients with CRC were then collected using TCGA database. The results from survival analysis demonstrated that patients with CRC in the SLC4A4 high expression group had a longer OS compared with patients with CRC in the SLC4A4 low expression group. These findings suggested that SLC4A4 may be used to evaluate the prognosis of patients with CRC.

ABCE1 is located on chromosome 4q31 and codes for 599 amino acids. Previous studies have reported that ABCE1 suppresses the interferon (IFN)-dependent 2-5A/RNase L system and serves key roles in cell proliferation and apoptosis (38,39). IFN is involved in cell immune defense by inducing transcription processes of a large number of genes, and has been demonstrated to be able to fight virus infection, inhibit tumor growth and control cell proliferation and differentiation (39). Zheng *et al* (40) have demonstrated that ABCE1 is up-regulated in lung cancer samples, and that decreased ABCE1 expression can impair cell proliferation and increase cell apoptosis. Hlavata *et al* (41) also reported that ABCE1 is up-regulated in CRC. Furthermore, it has been reported that ABCE1 knockdown can inhibit the

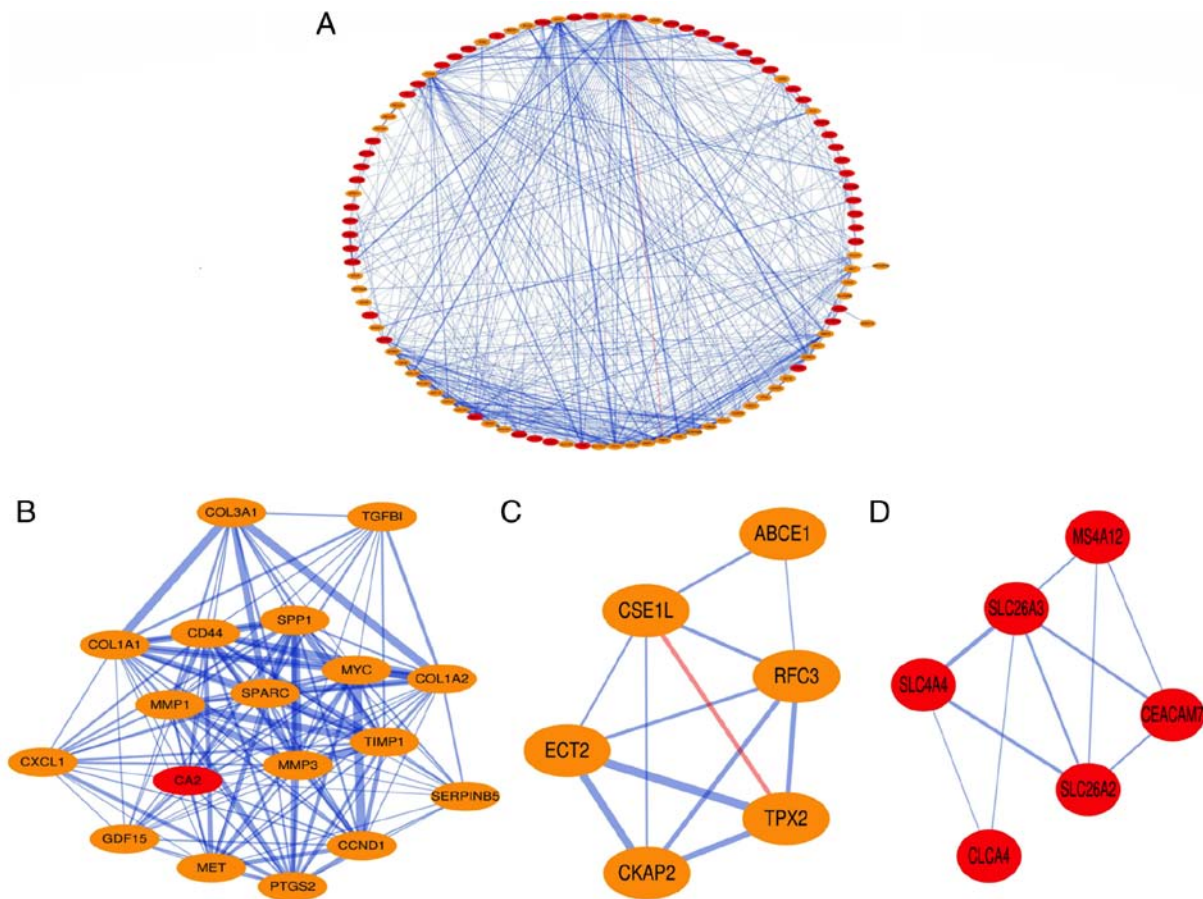


Figure 2. PPI network construction and module composition. (A) Construction of the PPI network covered 100 nodes and 551 edges, including 49 up-regulated genes and 51 down-regulated genes. The nodes represent the protein (gene). Red nodes represent down-regulated DEGs. Orange nodes represent up-regulated DEGs. Edge width was determined according to the combined score of the PPI relationship. (B) A total of 18 nodes and 136 edges were included in Module 1. (C) A total of six nodes and 12 edges were included in Module 2. (D) A total of six nodes and 10 edges were included in Module 2. DEGs, differentially expressed genes; PPI, protein-protein interaction.

proliferation and invasion of breast cancer cells (42) and small cell lung cancer cells (43). In addition, a previous study has demonstrated that ABCE1 participates in the immune response of colon cancer (44). Consistent with these results, the present study demonstrated that ABCE1 was an upregulated hub DEG, and that high ABCE1 expression was associated with a high OS in patients with CRC. These findings indicated that ABCE1 may be considered as a diagnostic and prognostic biomarker in CRC and may be used for targeted therapy of CRC.

TIMP1 is a soluble protein released from endometrium cells, fibroblasts and cancer cells, which has been demonstrated to be associated with prognosis in various types of carcinoma (45-47). Xiong *et al* (48) reported that TIMP1 is overexpressed in CRC. A recent study demonstrated that TIMP1 depletion can inhibit the proliferation, migration and invasion of colonic carcinoma cells, and inhibit the tumorigenesis and metastasis in CRC (49). In addition, TIMP1 overexpression is associated with focal adhesion kinase (FAK) activation. FAK is an upstream regulator of the PI3K-Akt signaling pathway, which is crucial in the cell survival pathway (50-52). Consistent with these studies, the present study reported that TIMP1 was up-regulated in CRC tissues samples compared with normal tissue samples, and that low TIMP1 expression was associated with a higher OS of patients with CRC. Therefore, TIMP1 may

be considered as a potential biomarker that could be used to predict the clinical outcome of patients with CRC.

Abnormal AKR1B10 expression has been reported in numerous types of cancer, including breast cancer, endometrial cancer, oral squamous cell carcinoma and lung cancer (35,53,54). AKR1B10, which is one member of the AKR superfamily, is an essential regulatory gene involved in the physiological function of intestinal tissue, including cell apoptosis, proliferation and migration (55). Previous studies have demonstrated that AKR1B10 expression is lower in CRC tissues compared with in normal colorectal tissues (44,56). Furthermore, AKR1B10 is one of the direct transcriptional targets of the p53 gene (34,57). Significantly, p53 is activated in cases of DNA damage, oncogene activation, anoxia and excessive proliferation (38). A previous study reported that the p53 mutation rate in CRC is ~40% (56). In addition, survival analysis using the TCGA database has demonstrated that patients with CRC and high AKR1B10 expression have a significantly longer survival rate (58). Consistent with these findings, the present study demonstrated that AKR1B10 was down-regulated in CRC tissues compared with normal tissues, and that patients with CRC in the AKR1B10 high expression group had a higher OS compared with patients in the AKR1B10 low expression group. These results suggest

Table III. GO analysis of the genes of each module in colorectal cancer tissues.

A, Module 1				
Category	Term	Count, n	Percentage	P-value
GOTERM_BP_DIRECT	GO:0030198 'extracellular matrix organization'	8	44.44	4.67x10 ⁻¹⁰
	GO:0030574 'collagen catabolic process'	5	27.78	4.40x10 ⁻⁷
	GO:0022617 'extracellular matrix disassembly'	5	22.78	8.82x10 ⁻⁷
	GO:0032355 'response to estradiol'	4	22.22	9.91x10 ⁻⁵
GOTERM_CC_DIRECT	GO:0005615 'extracellular space'	12	66.67	2.28x10 ⁻¹¹
	GO:0005576 'extracellular region'	12	66.67	1.38x10 ⁻⁵
GOTERM_MF_DIRECT	GO:0048407 'platelet-derived growth factor binding'	3	16.67	5.22x10 ⁻⁵
	GO:0050840 'extracellular matrix binding'	3	16.67	3.06x10 ⁻⁴
B, Module 2				
Category	Term	Count, n	Percentage	P-value
GOTERM_MF_DIRECT	GO:0016887 'ATPase activity'	2	33.33	3.05x10 ⁻⁴
	GO:0005515 'protein binding'	5	83.33	4.65x10 ⁻⁴
C, Module 3				
Category	Term	Count, n	Percentage	P-value
GOTERM_BP_DIRECT	GO:0051453 'regulation of intracellular pH'	3	50	1.34x10 ⁻⁵
	GO:0015701 'bicarbonate transport'	3	50	2.01x10 ⁻⁵
	GO:1902476 'chloride transmembrane transport'	3	50	9.07x10 ⁻⁵
GOTERM_MF_DIRECT	GO:0005254 'chloride channel activity'	3	50	6.00x10 ⁻⁵

BP, biological process; CC, cellular component; GO, Gene Ontology; MF, molecular function.

Table IV. Kyoto Encyclopedia of Genes and Genomes pathway analysis of the genes of each module in colorectal cancer tissues.

A, Module 1				
Term	Count, n	Percentage	P-value	Genes
hsa04512: ECM-receptor interaction	5	27.78	2.13x10 ⁻⁵	CD44, COL3A1, COL1A2, COL1A1, SPP1
hsa04151: PI3K-Akt signaling pathway	7	38.89	3.17x10 ⁻⁵	CCND1, COL3A1, MET, COL1A2, COL1A1, MYC, SPP1
hsa04510: Focal adhesion	6	33.33	3.61x10 ⁻⁵	CCND1, COL3A1, MET, COL1A2, COL1A1, SPP1
hsa05206: MicroRNAs in cancer	3	33.33	1.70x10 ⁻⁴	CCND1, CD44, PTGS2, SERPINB5, MET, MYC
B, Module 3				
Term	Count, n	Percentage	P-value	Genes
hsa04972: Pancreatic secretion	3	50	1.79x10 ⁻⁴	SLC26A3, CLCA4, SLC4A4

ECM, extracellular matrix.

Table V. Predictive values of clinical characteristics of patients with colorectal cancer and the four hub genes.

Variable	Group	Number	Univariate analysis		Multivariate analysis	
			95% CI	P-value	95% CI	P-value
Age (years)	<60 vs. ≥60	173 vs. 424	1.043-2.483	0.030	1.335-3.414	0.002
Sex	Male vs. female	322 vs. 275	-	0.818	-	0.566
UICC stage	I and II vs. III and IV	338 vs. 259	2.094-4.591	<0.001	2.123-4.716	<0.001
TIMP1	FPKM <172.3 vs. ≥172.3	272 vs. 325	1.376-2.929	<0.001	1.019-2.302	0.040
SLC4A4	FPKM <2.5 vs. ≥2.5	477 vs. 120	0.195-0.673	0.001	0.189-0.709	0.003
AKR1B10	FPKM <2.5 vs. ≥2.5	207 vs. 390	0.365-0.739	<0.001	0.454-0.962	0.030
ABCE1	FPKM <16.2 vs. ≥16.2	331 vs. 266	0.373-0.784	0.001	0.414-0.930	0.021

ABCE1, ATP binding cassette subfamily E member 1; AKR1B10, aldo-keto reductase family 1 member B10; CI, confidence interval; FPKM, fragments per kilobase million; SLC4A4, solute carrier family 4 member 4; TIMP1, TIMP metalloproteinase inhibitor 1; UICC, Union for International Cancer Control.

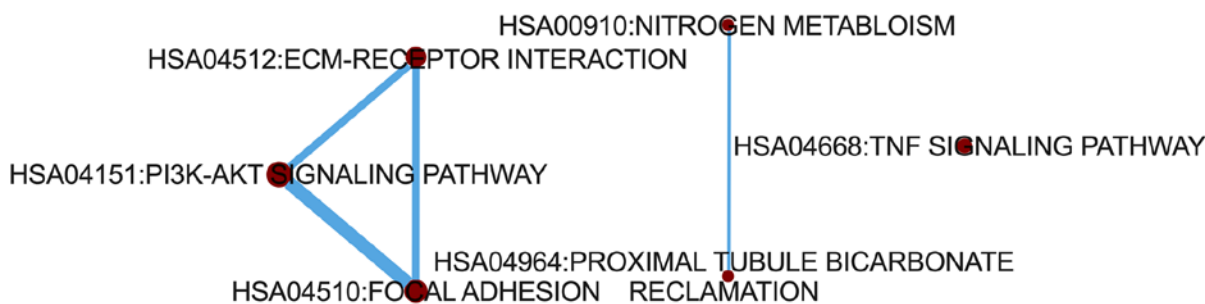


Figure 3. Crosstalk analysis for significant pathways. ECM, extracellular matrix; TNF, tumor necrosis factor.

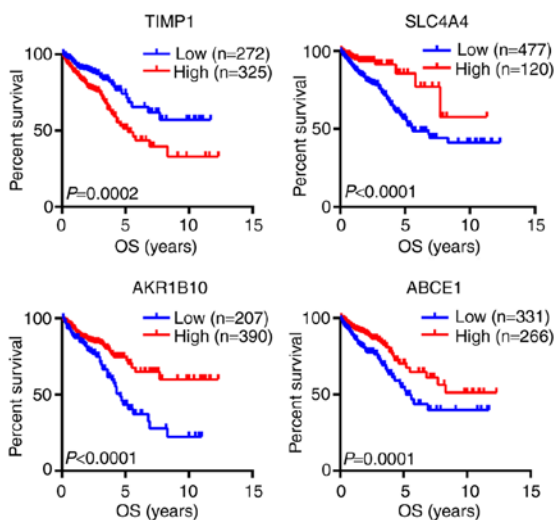


Figure 4. Kaplan-Meier OS curves according to the expression levels of TIMP1, SLC4A4, AKR1B10 and ABCE1. OS curves demonstrated that high TIMP1 expression and low SLC4A4/AKR1B10/ABCE1 expression were significantly associated with low OS in patients with colorectal cancer. ABCE1, ATP binding cassette subfamily E member 1; AKR1B10, aldo-keto reductase family 1 member B10; OS, overall survival; SLC4A4, solute carrier family 4 member 4; TIMP1, TIMP metalloproteinase inhibitor 1.

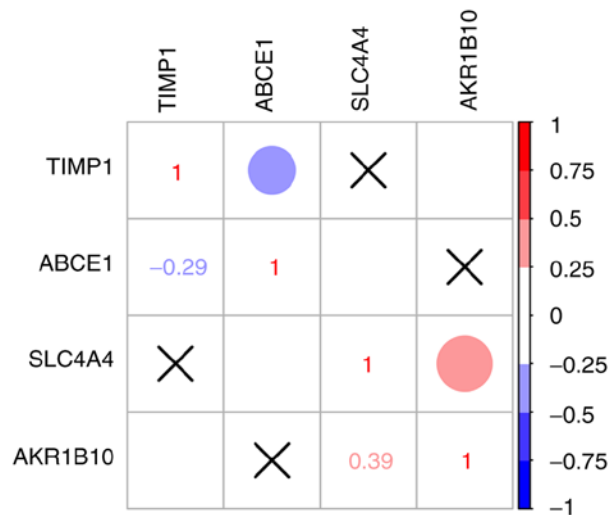


Figure 5. Correlations among the four hub genes (TIMP1, SLC4A4, AKR1B10 and ABCE1) evaluated using Spearman's correlation analysis based on Spearman's correlation coefficient (|r|<0.3) and P<0.05. ABCE1, ATP binding cassette subfamily E member 1; AKR1B10, aldo-keto reductase family 1 member B10; SLC4A4, solute carrier family 4 member 4; TIMP1, TIMP metalloproteinase inhibitor 1.

the importance of AKR1B10 in evaluating the prognosis of patients with CRC.

The results of the present study may have clinical significance for CRC; however, the current study presented certain

limitations. Firstly, since the candidate prognosis-associated hub DEGs were detected using data from the GEO and TCGA databases, there was no validation for these genes based on the data generated in the present study. Secondly, the expression levels of the prognosis-associated hub DEGs were not validated using PCR, western blotting or other experimental methods. Thirdly, since the potential roles of AKR1B10, SLC4A4, ABCE1 and TIMP1 in CRC remain unknown, future investigations will determine the effects of these hub genes in CRC by using *in vivo* and *in vitro* experiments.

In conclusion, through GEO and TCGA data analyses, four hub DEGs (SLC4A4, ABCE1, AKR1B10 and TIMP1) were identified as being significantly associated with the OS of patients with CRC. These four genes may serve as novel independent prognostic biomarkers that could be used to predict the clinical outcomes of patients with CRC. However, further investigations using cancer cell lines and xenograft models are required in order to determine the underlying mechanisms of these four hub genes and their roles in the prognosis of CRC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

GPD and LPW collected the data, analyzed the data and drafted the manuscript. YQW, XQR and SGZ designed the study and critically revised the manuscript. All authors read and approved the final version of the 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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