Identification of Genes Related to Clinicopathological Characteristics and Prognosis of Patients with Colorectal Cancer

Xueren Gao¹ and Jiaojiao Yang²

The aim of this study was to identify genes with clinical significance in colorectal cancer (CRC). Gene expression profiles of 585 CRC tissues and 61 normal colorectal tissues from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases were used to identify differentially expressed genes (DEGs) between CRC and normal colorectal tissues. DAVID and KOBAS tools were used to explore Gene Ontology (GO) and KEGG pathways enriched by DEGs, respectively. In addition, TCGA data sets were also used to identify prognostic factors and develop a prognostic prediction model for CRC. A total of 353 DEGs including 117 upregulated and 236 downregulated genes in CRC were identified based on GSE32323 data set. These DEGs were significantly enriched in the biological process related to the regulation of cell proliferation and 50 signaling pathways, such as “TGF-beta signaling pathway,” “Wnt signaling pathway,” and “Jak-STAT signaling pathway.” *GCG*, *ADH1B*, *SLC4A4*, *ZG16*, and *CLCA4* were the top five downregulated in CRC. *FOXQ1*, *LGR5*, *CLDN1*, *KRT23*, and *DPEP1* were the top five upregulated in CRC. *KRT23* expression could affect tumor stage and regional lymph node metastasis in CRC patients. *FOXQ1* expression could affect tumor distant metastasis in CRC patients. Survival analysis indicated that *SLC4A4* expression was associated with the prognosis of CRC patients. Prognostic prediction model developed based on age, tumor stage, and *SLC4A4* expression exhibited an efficient performance in predicting 1-, 3-, and 5-year overall survival of CRC patients. In conclusion, the current study identified several genes and pathways related to CRC, which provided new insight in understanding molecular mechanism of tumorigenesis and development of CRC.

Keywords: colorectal cancer, gene, pathway, prognosis

Introduction

COLORRECTAL CANCER (CRC) is a major public health issue worldwide. There are over 1.8 million new CRC cases and 881,000 deaths in 2018 (Bray *et al.*, 2018). Although researchers have made great efforts to reveal molecular mechanism of tumorigenesis and development of CRC, it has not been completely clarified. Therefore, it is necessary to further explore CRC-related genes and pathways, which helps not only to understand molecular mechanism of the occurrence and development of CRC, but also to guide the development of promising diagnostic and prognostic biomarkers and optimal therapeutic strategies.

Over the past decade, microarray and high throughput sequencing technologies have been widely used in the biomedical field and have greatly promoted the development of diagnostic and prognostic biomarkers and therapeutic targets for cancer. Meanwhile, a large number of cancer-

related data are stored in publicly available databases, such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO). A reanalysis of these data could facilitate the discovery of a vast range of valuable biological information (Gao *et al.*, 2018, 2019; Liu *et al.*, 2019; Zhou *et al.*, 2019). For instance, several novel genes associated with poor prognosis of patients with pancreatic ductal adenocarcinoma (PDAC) were identified by analyzing the array data from GEO. These genes might serve as potential diagnostic and prognostic biomarkers for PDAC (Zhou *et al.*, 2019). Liu *et al.* (2019) revealed a novel robust four-gene metabolic signature predicting overall survival for hepatocellular carcinoma (HCC) by mining mRNA expression profiles and clinicopathological data from TCGA and GSE14520 data sets. The signature would be very useful for reflecting the dysregulated metabolic microenvironment and providing potential biomarkers for metabolic therapy and treatment response prediction in HCC (Liu *et al.*, 2019). In 2012,

¹School of Pharmacy, Yancheng Teachers' University, Yancheng, China.

²Department of Microbiology and Immunology, Shanxi Medical University, Tai yuan, China.

FIG. 1. Volcano plot and heatmap showing differentially expressed genes in CRC. **(a)** Volcano plot and **(b)** heatmap showing the expression levels of top five upregulated and downregulated genes. CRC, colorectal cancer. Color images are available online.

researchers comprehensively analyzed molecular characterization of human colon and rectal cancer based on TCGA data, and found that colon and rectum cancers had considerably similar patterns of genomic alteration (Cancer Genome Atlas Network, 2012). Twenty-four genes, such as ARID1A, SOX9, and FAM123B, were significantly mutated (Cancer Genome Atlas Network, 2012). In 2015, Guinney *et al.* (2015) developed a novel classification framework for predicting consensus molecular subtypes of CRC using aggregated gene expression data.

In this study, we reanalyzed gene expression profiles from GEO and TCGA databases and identified several genes and

pathways related to CRC based on bioinformatics methods. In addition, we also developed a clinical model for predicting 1-, 3-, and 5-year overall survival of CRC patients.

Materials and Methods

Gene expression profiles

The RMA normalized gene expression profiles of cancerous and non-cancerous tissues from 17 CRC patients were acquired from the GEO. These gene expression profiles were contributed by Khamas *et al.* (2012) and generated by Affymetrix HG-U133 Plus 2.0 arrays. In addition, the FPKM

TABLE 1. GENE ONTOLOGY ENRICHMENT ANALYSIS OF THE DIFFERENTIALLY EXPRESSED GENES

Categories	Terms	FDR
GOTERM_BP_DIRECT	GO:0008285 ~ negative regulation of cell proliferation	0.004
GOTERM_CC_DIRECT	GO:0005615 ~ extracellular space	<0.001
GOTERM_CC_DIRECT	GO:0005578 ~ proteinaceous extracellular matrix	<0.001
GOTERM_CC_DIRECT	GO:0070062 ~ extracellular exosome	0.001
GOTERM_CC_DIRECT	GO:0005576 ~ extracellular region	0.001
GOTERM_CC_DIRECT	GO:0005887 ~ integral component of plasma membrane	0.009
GOTERM_CC_DIRECT	GO:0016324 ~ apical plasma membrane	0.011
GOTERM_MF_DIRECT	GO:0045236 ~ CXCR chemokine receptor binding	0.019
GOTERM_MF_DIRECT	GO:0008009 ~ chemokine activity	0.044

BP, biological process; CC, cellular component; FDR, false discovery rate; GO, Gene Ontology; MF, molecular function.

normalized gene expression profiles of 568 CRC tissues and 44 normal tissues and clinicopathological data from CRC patients were downloaded from TCGA database and processed by the Perl language.

Identification of differentially expressed genes

GEO2R tool was used to identify differentially expressed genes (DEGs) between cancerous and non-cancerous tissues from 17 CRC patients in GSE32323 data set. Genes with $|\log_2FC| > 2$ and adjusted p -value < 0.05 were considered as DEGs. According to $\log_2FC$ value, top five upregulated and downregulated genes were selected for further analysis. A volcano plot of DEGs was constructed by using ggplot2 and ggrepel packages. Furthermore, a heatmap of genes expression was also constructed by using HemI toolkit (Deng *et al.*, 2014).

Enrichment analysis of DEGs

To understand the biological significance of DEGs, the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 (Huang *et al.*, 2009, 2019) and KOBAS 3.0 (Wu *et al.*, 2006; Xie *et al.*, 2011; Ai and Kong, 2018) online tools were used for enrichment analysis. There into DAVID was used to reveal Gene Ontology (GO) enriched by DEGs. KOBAS 3.0 was used to reveal KEGG pathways enriched by DEGs. GO terms with the false discovery rate < 0.05 and KEGG pathways with the corrected p -value < 0.05 were considered to be significantly enriched by DEGs.

Validation and analysis of gene expression levels in CRC

Wilcoxon test was used to validate the expression levels of top five upregulated and downregulated genes based on TCGA data sets. In addition, the association between expression levels of top five upregulated and downregulated genes and microsatellite instability (MSI) status of CRC patients was assessed by Wilcoxon test. p -value < 0.05 was considered significant.

The relationship between gene expression and clinicopathological characteristics

Logistic regression analysis was used to explore the relationship between the expression levels of top five upregulated and downregulated genes and clinicopathological characteristics of CRC patients. The univariate cox regression analysis was used to identify factors associated with the overall survival of CRC patients. p -value < 0.05 was considered significant.

Development and evaluation of a prognostic prediction model for CRC patients

A prognostic prediction model for CRC patients was developed based on potential prognostic factors and displayed in a nomogram. The concordance index (C-index) and receiver operating characteristic (ROC) curve were used to evaluate the performance of the prognostic prediction model. The risk score of each CRC patient was calculated based on the model. All CRC patients were divided into high- and

TABLE 2. KEGG PATHWAY ENRICHMENT ANALYSIS OF THE DIFFERENTIALLY EXPRESSED GENES

<i>IDs</i>	<i>Terms</i>	<i>Corrected p-value</i>
hsa04060	Cytokine-cytokine receptor interaction	5.98E-07
hsa04978	Mineral absorption	1.11E-05
hsa00910	Nitrogen metabolism	1.46E-05
hsa04972	Pancreatic secretion	4.30E-05
hsa04610	Complement and coagulation cascades	1.12E-04
hsa04960	Aldosterone-regulated sodium reabsorption	3.64E-04
hsa05146	Amoebiasis	3.94E-04
hsa05202	Transcriptional misregulation in cancer	4.06E-04
hsa04976	Bile secretion	4.99E-04
hsa04062	Chemokine signaling pathway	5.13E-04
hsa04151	PI3K-Akt signaling pathway	6.07E-04
hsa04964	Proximal tubule bicarbonate reclamation	6.81E-04
hsa04350	TGF-beta signaling pathway	1.04E-03
hsa01100	Metabolic pathways	1.07E-03
hsa05323	Rheumatoid arthritis	1.45E-03
hsa05200	Pathways in cancer	1.87E-03
hsa04924	Renin secretion	2.24E-03
hsa04550	Signaling pathways regulating pluripotency of stem cells	2.25E-03
hsa04510	Focal adhesion	3.33E-03
hsa03320	PPAR signaling pathway	3.38E-03
hsa05219	Bladder cancer	3.68E-03
hsa04919	Thyroid hormone signaling pathway	4.35E-03
hsa04270	Vascular smooth muscle contraction	4.68E-03
hsa04512	ECM-receptor interaction	5.33E-03
hsa05144	Malaria	6.10E-03
hsa04068	FoxO signaling pathway	7.27E-03
hsa04530	Tight junction	8.28E-03
hsa04310	Wnt signaling pathway	9.20E-03
hsa00561	Glycerolipid metabolism	9.84E-03
hsa05205	Proteoglycans in cancer	1.14E-02
hsa00830	Retinol metabolism	1.29E-02
hsa04022	cGMP-PKG signaling pathway	1.63E-02
hsa04670	Leukocyte transendothelial migration	1.74E-02
hsa04971	Gastric acid secretion	1.82E-02
hsa05160	Hepatitis C	2.54E-02
hsa00071	Fatty acid degradation	2.70E-02
hsa04640	Hematopoietic cell lineage	2.85E-02
hsa04540	Gap junction	2.85E-02
hsa04974	Protein digestion and absorption	3.03E-02
hsa01212	Fatty acid metabolism	3.18E-02
hsa04514	Cell adhesion molecules (CAMs)	3.32E-02
hsa04015	Rap1 signaling pathway	3.75E-02
hsa04390	Hippo signaling pathway	3.91E-02
hsa04933	AGE-RAGE signaling pathway in diabetic complications	4.05E-02
hsa05217	Basal cell carcinoma	4.20E-02
hsa05134	Legionellosis	4.20E-02
hsa04630	Jak-STAT signaling pathway	4.23E-02
hsa05150	Staphylococcus aureus infection	4.50E-02
hsa00500	Starch and sucrose metabolism	4.50E-02
hsa00140	Steroid hormone biosynthesis	4.66E-02

FIG. 2. The expression levels of top five upregulated and downregulated genes in CRC identified based on TCGA data sets. TCGA, The Cancer Genome Atlas. Color images are available online.

TABLE 3. THE EFFECT OF THE EXPRESSION LEVELS OF GENES ON CLINICOPATHOLOGICAL CHARACTERISTICS OF COLORECTAL CANCER

Gene	Stage (I+II, III+IV)			T (T1+T2, T3+T4)			N (N0,N1)			M (M0,M1)		
	n = 526			n = 540			n = 540			n = 478		
	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI
<i>ADH1B</i>	0.067	1.073	0.995–1.156	0.079	1.185	0.980–1.432	0.069	1.064	0.995–1.138	0.283	1.044	0.965–1.129
<i>ZG16</i>	0.660	0.999	0.996–1.003	0.647	1.001	0.997–1.006	0.610	0.999	0.996–1.003	0.051	0.990	0.980–1.000
<i>CLCA4</i>	0.381	1.003	0.996–1.009	0.530	0.998	0.992–1.004	0.937	1.000	0.995–1.006	0.756	0.998	0.986–1.011
<i>SLC4A4</i>	0.130	0.943	0.874–1.017	0.898	0.995	0.919–1.077	0.070	0.932	0.863–1.006	0.061	0.856	0.728–1.007
<i>CLDN1</i>	0.584	1.002	0.994–1.010	0.404	0.996	0.987–1.005	0.765	1.001	0.993–1.009	0.419	1.004	0.994–1.015
<i>LGR5</i>	0.604	1.002	0.993–1.011	0.584	1.003	0.992–1.015	0.884	1.001	0.992–1.010	0.260	1.007	0.995–1.018
<i>KRT23</i>	0.030	1.005	1.000–1.009	0.547	1.001	0.997–1.006	0.036	1.004	1.000–1.008	0.275	1.002	0.999–1.005
<i>FOXQ1</i>	0.063	1.007	1.000–1.015	0.963	1.000	0.991–1.009	0.248	1.004	0.997–1.012	<0.001	1.022	1.013–1.032
<i>DPEP1</i>	0.263	1.001	0.999–1.002	0.471	0.999	0.998–1.001	0.376	1.001	0.999–1.002	0.172	1.001	1.000–1.003
<i>GCG</i>	0.507	1.036	0.933–1.152	0.506	1.090	0.846–1.404	0.507	1.038	0.929–1.160	0.249	1.034	0.977–1.094

Bold values indicate significant effects.

CI, confidence interval; OR, odds ratio.

low-risk group based on median risk score. Kaplan–Meier survival curves and a scatter diagram were used to show prognostic difference between two groups. These analyses were performed by survival, rms, survcomp, and timeROC packages in R software.

Results

Identification and analysis of DEGs

A total of 117 upregulated and 236 downregulated genes in CRC were identified based on GSE32323 data set. *FOXQ1*, *LGR5*, *CLDN1*, *KRT23*, and *DPEP1* were top five up-regulated genes and *GCG*, *ADH1B*, *SLC4A4*, *ZG16*, and *CLCA4* were top five down-regulated genes (Fig. 1). The functional enrichment analysis showed that DEGs were significantly enriched in the following nine GO terms: GO:0008285 ~ negative regulation of cell proliferation, GO:0005615 ~ extracellular space, GO:0005578 ~ proteinaceous extracellular matrix, GO:0070062 ~ extracellular exosome, GO:0005576 ~ extracellular region, GO:0005887 ~ integral component of plasma membrane, GO:0016324 ~ apical plasma membrane, GO:0045236 ~ CXCR chemokine receptor binding, and GO:0008009 ~ chemokine activity (Table 1). The enrichment analysis based on KEGG pathway showed that DEGs were significantly enriched in 50 pathways (Table 2). There into hsa04060 ~ cytokine-cytokine receptor interaction, hsa04978 ~ mineral absorption, hsa00910 ~ nitrogen metabolism, hsa04972 ~ pancreatic secretion, and hsa04610 ~ complement and coagulation cascades were top five pathways significantly enriched by DEGs (Table 2).

Validation and analysis of gene expression levels in CRC

TCGA data sets showed that the expression levels of *FOXQ1*, *LGR5*, *CLDN1*, *KRT23*, and *DPEP1* were up-regulated in CRC tissues, and the expression levels of *GCG*, *ADH1B*, *SLC4A4*, *ZG16*, and *CLCA4* were downregulated in CRC tissues, which were consistent with the results of GSE32323 data set (Fig. 2). In addition, logistic regression analysis indicated that *KRT23* expression in CRC tissues

could affect tumor stage (OR=1.005, 95% CI=1.000–1.009, $p=0.03$) and regional lymph node metastasis (OR=1.004, 95% CI=1.000–1.008, $p=0.036$) (Table 3). *FOXQ1* expression in CRC tissues could affect tumor distant metastasis (OR=1.022, 95% CI=1.013–1.032, $p<0.001$) (Table 3). The univariate cox regression analysis showed that *SLC4A4* expression was correlated with the overall survival of CRC patients (HR=0.872, 95% CI=0.762–0.999, $p=0.048$) (Table 4). After adjusting for *BRAF* mutation status, *SLC4A4* expression still could affect the overall survival of CRC patients (HR=0.87, 95% CI=0.75–0.99, $p=0.041$).

Development and evaluation of a prognostic prediction model for CRC patients

Age, tumor stage, and *SLC4A4* expression were used to develop a prognostic prediction model (Fig. 3a). The C-index was 0.737 for the prognostic prediction model, suggesting that the model had moderate discrimination ability. The area under the ROC curve for 1-, 3-, and 5-year

TABLE 4. IDENTIFICATION OF FACTORS ASSOCIATED WITH THE OVERALL SURVIVAL OF COLORECTAL CANCER PATIENTS

Characteristics	HR	95% CI	p
Age	1.036	1.016–1.056	<0.001
Gender	1.133	0.751–1.709	0.552
Stage	2.103	1.668–2.653	<0.001
<i>ADH1B</i>	1.029	0.967–1.096	0.360
<i>ZG16</i>	0.994	0.986–1.001	0.111
<i>CLCA4</i>	1.000	0.992–1.008	0.994
<i>SLC4A4</i>	0.872	0.762–0.999	0.048
<i>CLDN1</i>	1.002	0.993–1.012	0.611
<i>LGR5</i>	1.002	0.992–1.012	0.715
<i>KRT23</i>	0.997	0.991–1.002	0.253
<i>FOXQ1</i>	1.005	0.997–1.013	0.252
<i>DPEP1</i>	1.000	0.998–1.002	0.867
<i>GCG</i>	1.001	0.992–1.011	0.800

Bold values indicate significant associations.

CI, confidence interval; HR, hazard ratio.

FIG. 3. The nomogram and ROC curve analysis of prognostic prediction model for CRC patients. (a) Nomogram and (b) ROC curve. ROC, receiver operating characteristic. Color images are available online.

overall survival were 0.741, 0.773, and 0.790, respectively, suggesting that the prognostic prediction model had a moderate sensitivity and specificity (Fig. 3b). In addition, Kaplan–Meier plot showed that the overall survival of the high-risk group was significantly poorer than that of the low-risk group ($p = 2.121 \times 10^{-12}$, Fig. 4a). The scatter diagram showed that mortality of the high-risk group was significantly higher than that of the low-risk group (29% vs. 6.5%, $p < 0.001$, Fig. 4b).

Discussion

The incidence and mortality of CRC were still on the rise in many countries. Identification of genes and pathways

related to CRC would be helpful for the diagnosis and treatment of the disease. In the current study, we identified 353 CRC-related genes by mining gene expression profiles of GSE32323 data set. These genes were significantly enriched in cancer-related GO terms and signaling pathways. For instance, PI3K-Akt pathway was one of the most frequently activated signal transduction pathways in human cancer, and strongly implicated in cancer pathogenesis (Guo *et al.*, 2015; Chen *et al.*, 2016a; Wang *et al.*, 2019). TGF- β signaling pathway could promote tumor growth, invasion, and metastasis through inducing angiogenesis, epithelial-mesenchymal transition, and immune escape (Roberts and Wakefield, 2003). Targeting the effectors of these pathways might be a promising therapeutic approach.

FIG. 4. Survival analysis of CRC patients in the high- and low-risk group. **(a)** Survival curve of CRC patients in the high- and low-risk group and **(b)** scatter diagram for risk score, survival time, and status of CRC patients. Color images are available online.

Top five upregulated (*FOXQ1*, *LGR5*, *CLDN1*, *KRT23*, and *DPEP1*) and downregulated (*GCG*, *ADH1B*, *SLC4A4*, *ZG16*, and *CLCA4*) genes in CRC tissues were further analyzed based on TCGA data sets. The expression levels of *FOXQ1*, *LGR5*, *CLDN1*, *KRT23*, *DPEP1*, *GCG*, *SLC4A4*, and *CLCA4* were associated with MSI status of CRC patients (Fig. 5). The expression levels of *FOXQ1*, *LGR5*, *CLDN1*, *KRT23*, and *DPEP1* in CRC patients with MSI were significantly lower than those in CRC patients without MSI. The expression levels of *GCG*, *SLC4A4*, and *CLCA4* in CRC patients with MSI were significantly higher than those in CRC patients without MSI. *FOXQ1* was a member of the forkhead box protein family, which was characterized by a conserved 110-amino acid DNA-binding motif called the forkhead or winged helix domain. It was involved in many important biological processes, such as embryonic development, metabolism, and cell cycle regulation (Zhu, 2016). In addition, *FOXQ1* had been recognized as an oncogene that was overexpressed in a variety of cancers, such as CRC, liver cancer, and esophageal cancer (Kaneda *et al.*, 2010; Wang *et al.*, 2013; Pei *et al.*, 2015). The current research found that high expression of *FOXQ1* in CRC tissues could increase the risk of tumor distant metastasis. *LGR5* was an established cancer stem cell marker and could promote the proliferation of CRC cells via the Wnt/ β -catenin signaling pathway, which was a critical pathway in the process of tumor initiation and growth (Lin *et al.*, 2015). *CLDN1*, a member of the claudin family, was an integral membrane protein and a component of tight junction strands. It had been found abnormally expressed in a variety of tumors, such as cervical cancer, oral squamous cell carcinoma, and CRC (Tang *et al.*, 2011; Sappayatosok and Phattar-

ataratip, 2015; Zhang *et al.*, 2016). In human CRC tissues, *CLDN1* expression was upregulated (Tang *et al.*, 2011). Furthermore, it could regulate cellular transformation and metastatic behavior in colon cancer (Dhawan *et al.*, 2005). *KRT23* was a member of the keratin family that was an intermediate filament protein responsible for the structural integrity of epithelial cells. *KRT23* could act as an important cellular factor that promoted CRC growth by activating the expression of human telomerase reverse transcriptase (Zhang *et al.*, 2017). The current research indicated that high expression of *KRT23* in CRC tissues could increase the risk of advanced tumor stages and regional lymph node metastasis. *DPEP1* was a zinc-dependent metalloproteinase involved in the metabolism of glutathione and other similar proteins by dipeptide hydrolysis. It was initially considered as a tumor suppressor gene in PDAC and breast cancer (Green *et al.*, 2009; Zhang *et al.*, 2012). On the contrary, several studies demonstrated that *DPEP1* was highly expressed in CRC and promoted metastasis by regulating expression level of E-cadherin in colon cancer (Toiyama *et al.*, 2011; Park *et al.*, 2016). *GCG* was a preproprotein that was cleaved into four distinct mature peptides. One of these, glucagon, was a ligand for a specific G-protein linked receptor whose signaling pathway controlled cell proliferation. The current results showed that *GCG* expression was significantly downregulated in CRC tissues. Thus, we inferred that *GCG* might play a major role in suppressing the development of CRC. *ADH1B* was a member of the alcohol dehydrogenase family that metabolized a wide variety of substrates, such as ethanol, hydroxysteroids, and lipid peroxidation products. Alcohol carcinogenesis mostly affected tissues directly exposed to alcohol ingestion, such as

FIG. 5. The association between expression levels of top five upregulated and downregulated genes and MSI status of CRC patients (15 CRC patients with MSI and 92 CRC patients without MSI). MSI, microsatellite instability. Color images are available online.

esophagus, larynx and colon. Although the molecular function of ADH1B in CRC was not directly reported, genetic polymorphism (rs1229984) in *ADH1B* gene was revealed to be associated with CRC risk (Crous-Bou *et al.*, 2013; Chen *et al.*, 2014), suggesting that *ADH1B* might be an important genetic susceptibility gene for CRC. SLC4A4 was a sodium bicarbonate cotransporter responsible for the regulation of bicarbonate secretion and absorption and intracellular pH. Xiao *et al.* (2019) found that miR-223-3p could enhance cell proliferation and metastasis by downregulating SLC4A4 in clear cell renal cell carcinoma, indicating that *SLC4A4* might serve as a tumor suppressor gene. We found that SLC4A4 expression was downregulated in CRC tissues, and high expression of SLC4A4 could reduce the risk of death in CRC patients. ZG16 was a secreted protein downregulated in liver cancer and CRC (Zhou *et al.*, 2007). Chen *et al.* (2016b) demonstrated that loss of ZG16 was helpful for stemness and progression of CRC, which might provide a promising therapeutic strategy for advanced CRC. CLCA4 belonged to the calcium sensitive chloride conductance protein family and could act as a tumor suppressor in tumorigenesis and progression of several human cancers, including HCC, CRC, and bladder cancer (Hou *et al.*, 2017; Liu *et al.*, 2018; Chen *et al.*, 2019).

Recently, the development of a prognostic prediction model has become a research hotspot. In this study, we observed that age, tumor stage, and SLC4A4 expression were associated with overall survival of CRC patients. Subsequently, a model for predicting prognosis of CRC patients was developed base on these three prognostic factors. The model had a good performance in predicting 1-, 3-, and 5-year overall survival of CRC patients, which would help clinicians make better clinical decisions and follow-up surveillance. However, due to limitations of research, systemic therapies and other known prognostic factors were not included in survival analysis. Thus the prognosis model needed to be further improved.

Conclusions

This study identified several genes and pathways related to CRC, which provided not only new diagnostic markers and therapeutic targets for CRC, but also a promising prognostic indicator for CRC patients.

Authors' Contributions

X.G., the first author and the corresponding author, put forward the ideas of this article, wrote the article, and analyzed the data. As a coauthor, J.Y. acquired the data and helped in revising the article.

Disclosure Statement

The authors declare no conflict of interest.

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Address correspondence to:

Xueren Gao, PhD

School of Pharmacy

Yancheng Teachers' University

Yancheng 224007

Jiangsu

China

E-mail: gxr871230@126.com

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