Identification of Key Genes Involved in Colorectal Cancer: A Bioinformatics Analysis

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Abstract

Background

Colorectal cancer is the life-threatening tumor with both high prevalence and mortality worldwide. However, the molecular mechanism behind it remains unknown. Methods: Herein, the potential prognostic candidate biomarkers for colorectal cancer were tested by Bioinformatical analysis combined with the CRC clinical samples. Three data sets (GSE32323, GSE44076 and GSE43078) were collected from the gene expression omnibus (GEO). The limma and clusterProfiler packages were used to identify differentially expressed genes (DEGs) and conduct functional enrichment analysis, respectively. To retrieve Interacting Genes (STRING) database, protein–protein interaction (PPI) network was built up using Search Tool, and Cytoscape was applied to carry out the module analysis. Subsequently, the online tool GEPIA was employed to conduct overall survival analysis (<http://gepia.cancer-pku.cn/index.html>), and the Oncomine database was used to analyze prognostic candidate biomarkers. Finally, 4 key hub genes were selected for validation of their expression levels in 9 patients newly diagnosed with CRC via reverse transcription-quantitative PCR (RT-qPCR). To evaluate the accuracy of prediction, time-dependent receiver operating characteristic (ROC) was applied.

Results

In total, 547 DEGs got identified, inclusive of 475 downregulated and 72 upregulated genes with a significant enrichment in the cellular response to hypoxia, the positive control of ERK1 and ERK2 cascade and the positive control of apoptotic process. The enhanced pathways were Pathways in cancer, PI3K-Akt signaling pathway, cGMP-PKG signaling pathway. Through the extraction of critical modules from the PPI network, 10 hub genes got removed. These 10 hub genes are all up-regulated genes and are highly expressed in colorectal cancer. Survival analysis shows that only CCNB1 and CCNA2 are associated with the survival prediction of colorectal cancer. Moreover, consistence is shown between the TCGA data sets and the expression levels of the 4 hub genes. Receiver Operating Characteristic (ROC) curves showed that all CCNB1, CCNA2, AURKA and BUB1B have potential predictive value. Briefly, new hub genes identified can shed light on the underlying mechanism behind CRC carcinogenesis and development, which is conducive to detecting and treating CRC timely.

Background

Colorectal cancer remains the one of leading healthy issues worldwide, with as estimated 1.8 million new cases in 2018[1]. By 2030, the global CRC burden is expected to increase by 60%, more than 2.2 million new cases and 1.1 million deaths. It has been ranked the third and second cancer morbidity of male and female in China, respectively, and the mortality rate ranked the fifth[2]. The K-ras gene is present in approximately 45% of colorectal cancers[3] and is responsive to EGFR-targeted inhibitors. Related studies abroad have shown that for patients with early diagnosis of colorectal cancer, comprehensive treatment can make their 5-year survival rate reach to 82.9% [4], and once the tumor has metastasized, the 5-year

survival rate of the patient is <20%[5].Lacking sensitive and specific early biomarkers, a high possibility of drug resistance and metastasis is considered to contribute the high mortality of this disease. Therefore, there has a pressing need for identifying the more sensitive and specific biomarkers or drug targets of CRC for developing effective diagnosis and treatment strategies.

Microarray technology provides an all-in-one system biology solution from hardware to software systems. It can simultaneously scan the hybridization signals of tens of thousands of gene probes in the chip and carry out quantitative analysis on the transcriptome profile of samples. Recent advances especially in the algorithms of probe signal detection and analysis, such as the introduction of artificial intelligence technologies, will make the results of microarray more accurate and reliable[6-8].The microarray technique also provides a powerful tool for exploring the gene regulation pattern and molecular mechanisms involved in oncogenesis and progression of CRC.Recently, different types of biomarkers including coding genes, miRNAs, long non-coding RNAs and circRNAs have been identified in colorectal cancer. Dysregulation of these molecules is involved in the tumor progression or is associated with the prognosis of patients[9-12].In view of the complexity of the molecular regulatory network of CRC, current studies on tumor biomarkers are not sufficient. Therefore, it is still necessary to identify novel prognostic biomarkers, which will help us develop more sensitive and effective diagnostic and therapeutic strategies. However, limited sample size and significant variability among different projects make it hard to obtain credible results.In this study, three microarray datasets containing mRNA expression data between CRC and non-cancerous tissues were downloaded from Gene Expression Omnibus (GEO) and the differentially expressed genes (DEGs) were screened out. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and protein–protein interaction (PPI) network analyses were performed to explore the key modules and hub genes involved in CRC progression. In sum, 547 DEGs and 10 hub genes were screened out, which may be candidate biomarkers for CRC.

Methods

In line with the process shown in Fig. 1, bioinformatics analysis was carried out. Through getGEO function in package GEOquery, three data sets (GSE32323, GSE44076 and GSE23878) containing the gene expression data on CRC and normal tissues were obtained from GEO (<https://www.ncbi.nlm.nih.gov/geo/>) [13]. Table 1 shows the specifics of GEO data sets. With the aforementioned method applied, the raw expression files of three microarray datasets were processed preliminarily [14]. The selected chips were downloaded by the online tool GEO2R ([https:// www.ncbi.nlm.nih.gov/geo/geo2r/](https://www.ncbi.nlm.nih.gov/geo/geo2r/)) and the differentially expressed genes were screened. The screening criterion is $\text{adj. P. Val} < 0.01$, $|\log_2 \text{Fold Change}| \geq 1$, and the probe name was transformed into the standard gene name.Then, based on annotation information, the array probes were transformed into matching genetic symbols. When there are a number of probes matching the same gene, the value of gene expression was treated as the mean of the probes. When the same probe matches a number of genes, the probe gets removed. The possible regulatory mechanisms behind the occurrence and development of the disease can be revealed by the identification of DEGs at various states and investigation into their correlations. A web tool called ClustVis was applied to build up heatmaps. Differentially up-regulated and differentially

down-regulated genes were subject to screening for the three datasets. Finally, in the online Veeny tool (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>), as were the differential intersection genes of the three microarrays.

GO and KEGG enrichment analysis

GO functional analysis is a useful method for annotating genes and identifying characteristic biological attributes for high-throughput genome or transcriptome data [15]. KEGG incorporates a wide range of databases, including those on genomes, biological pathways, diseases, drugs and chemical substances [16]. Gene Ontology and KEGG analyses were conducted using enrich GO and enrich KEGG functions of Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.ncifcrf.gov/>), an online bioinformatics database that aims to provide tools for the functional interpretation of genes or proteins [17], respectively. $P_{\text{adjust}} (\text{FDR}) < 0.05$ was considered to be statistically significant.

Construction of PPI network and module analysis

The PPI network was constructed by Search Tool for the Retrieval of Interacting Genes (STRING) (<https://string-db.org/>) with interaction score of 0.9 as the threshold [18]. Subsequently, Cytoscape software [19] was used to construct a PPI network and analyze the interactions of the DEGs. The cytoHubba plug-in was used to identify hub genes. The Molecular Complex Detection (MCODE) plug-in was used to screen modules of the PPI network in Cytoscape with a degree cut-off = 2, node score cut-off = 0.2, k-core = 2 and max depth = 100. The KEGG pathway enrichment analysis of genes in the top module was performed using DAVID.

Hub gene analysis

The Gene Expression Profiling Interactive Analysis (GEPIA; <http://gepia.cancer-pku.cn/index.html>) is an online tool, which delivers fast and customizable analysis for RNA sequencing expression data based on The Cancer Genome Atlas and GTEx databases, which include data on 9,736 tumors and 8,587 normal samples [20]. GEPIA provides key interactive and customizable functions, including differential expression analysis between cancer and normal tissues. The seed genes in modules referred to hub genes. The overall survival analyses were performed using online tool GEPIA (<http://gepia.cancer-pku.cn/>). The logrank $P < 0.05$ was considered statistically significant. The association of expression level of hub genes with clinical traits were analysis using the Oncomine Database [21].

Tissue specimens, RNA extraction and qRT-PCR analysis.

9 tumor tissues and tumor-adjacent tissues from CRC donors were enrolled in our study to validate the expression levels of co-expressed DEGs. Prior patient consent and ethical approval from the ethics committee of the Jiangsu Provincial Hospital of Traditional Chinese Medicine. All methods were performed in accordance with the ethics guidelines and regulations. We selected 4 co-expressed DEGs, including CCNB1, CCNA2, AURKA and BUB1B, for validation. All tissues were histologically diagnosed. Total RNA from the tissue specimens was isolated using TRIzol reagent (Invitrogen, Carlsbad, California,

USA), and qRT-PCR was performed with SYBR® Green dye (TaKaRa, Shiga, Japan), following the manufacturer's instructions. The primer sequences are provided in Supplementary Table S1. β -actin was used as a reference gene.

Ethics statement. The research protocol was subject to review and approval from the Research Ethics Committee of the First Affiliated Hospital. All of the experiments were carried out on the basis of the instructions from the First Affiliated Hospital, JiangSu Provincial Hospital of Traditional Chinese Medicine. Basic information of clinical patients were shown in supplement Table S2.

Ethical approval. All processes conducted in the research with human participants involved conformed to the ethical standards set out by the ethical committee of the First Affiliated Hospital, Nanjing University of Chinese Medicine, the 2019NL-KS1129 Declaration and the subsequent amendments to it. NO tissues were derived from prisoners.

Ethical standards. Expressed consent was received from all of those participating in this research.

With Graphpad Prime 8.3 used, the ROC curves were visualized to evaluate how accurate the prediction would be of independent prognostic hub genes such as CCNB1, CCNA2, AURKA and BUB1B. For comparing the discriminatory ability of above-mentioned prognostic genes, the area under the ROC curve (AUC) was calculated.

Statistical analysis. Data are indicated by the mean $\pm$ SD for continuous variables. Both t-test and analysis of variance were conducted to assess the disparities of demographic data. All P values were two-sided, and $P < 0.05$.

Results

Identification of DEGs between CRC and tumor-adjacent tissues

The three data sets were subject to standardization, with the results presented in Figure 2. Subsequently, the duplicate genes and values with no specific genetic symbols were removed. Totally, 3387 DEGs were obtained in GSE23878. Among them, 784 genes experienced up-regulation and 2603 genes underwent down-regulation. Besides, 4883 DEGs were obtained from GSE32323. There were 919 genes experiencing up-regulation and 3964 genes undergoing down-regulation. In GSE44076, 2485 DEGs were collected. Of them, 1254 were subject to up-regulation and 1231 were subject to down-regulation. Figure 3 presents the DEGs obtained from each data set. Clustering was performed using Euclidean distance. Figure 4 shows the top 100 DEGs conducted by heatmap.

Data preprocessing and DEG screening

Three GEO datasets were downloaded, pre-processed and merged into a global dataset which contained 160 CRC and 189 normal samples. Totally, 547 DEGs were identified by online tool GEO2R, including 72

up-regulated genes and 475 down-regulated genes(Figure.5). The most statistically significant up-regulated and down-regulated genes are listed in Table 2.

GO and KEGG analysis

The biological functions and pathways analyses were conducted using DAVID 3.8.0 database. The GO categories of biological process (BP), cellular component (CC) and molecular function (MF) were enriched respectively (Figs. 6A–6C) . The up-regulated DEGs were mainly associated with cellular response to hypoxia (BP, GO:0071456), extracellular exosome (CC, GO:0070062) and heparin binding (MF, GO:0008201). The down-regulated DEGs were most significantly related to positive regulation of protein phosphorylation (BP, GO:0001934), Z disc (CC, GO:0030018) and magnesium ion binding(MF, GO:0000287).The DEGs were mainly enriched in pathways of Mineral absorption(hsa04978),cGMP-PKG signaling pathway (hsa04022), Proximal tubule bicarbonate reclamation(hsa04964), Aldosterone-regulated sodium reabsorption(hsa04960), Insulin secretion(hsa04911),Bile secretion (hsa04976), Pathways in cancer (hsa05200),Thyroid hormone signaling pathway (hsa04919) and Cell cycle(hsa04110)(Table 3; Fig. 6D).

Construction of PPI network and module analysis

Protein–protein interaction network reflect the spatiotemporal relationship of macromolecules within the cell which will provide valuable information about molecular mechanisms in physiological and pathological process. To explore the molecular mechanisms underlying CRC progression, the online STRING database was applied to construct the PPI network. The interaction score of 0.4 (highest confidence) was set as threshold, and nodes without connections were removed from network. Finally,the PPI network consisted of 542 nodes with 1338 edges, and average local clustering coefficient was 0.367 (PPI enrichment P-value < 1.0E-16) (Fig. 7A). Then,the hub genes were identified via CytoHubba plugin.The top 10 hub genes, including minichromosome maintenance3(MCM3), minichromosome maintenance7(MCM7), budding uninhibited by benzimidazoles 1 homolog beta(BUB1B), Cyclin A2(CCNA2),Cyclin B1(CCNB1), aurora kinase A(AURKA), targeting protein for Xklp2(TPX2), threonine and tyrosine kinase(TTK), Flap endonuclease I(FEN1), kinesin family member 4A(KIF4A), were identified using the cytoHubba plug-in, with a higher degree of connectivity (Fig.7B) .Finally,the key modules were identified via MCODE plugin. A total of 22 functional clusters of modules and related hub genes were detected. The top three significant modules were presented in Figs. 7C–7E. The KEGG analysis of module genes revealed that the top three modules were mainly associated with the cell cycle(hsa04110), Neuroactive ligand-receptor interaction(hsa04080), DNA replication(hsa03030),chemokine signaling pathway (hsa04062) and Progesterone-mediated oocyte maturation(hsa04914) (Fig. 7F)

Hub genes analysis

A total of 10 genes were identified as hub genes. We selected 4 of the hub genes that are most closely related to the occurrence and development, which were CRC, CCNB1, CCNA2, AURKA and BUB1B, and analyzed their expression in colorectal cancer (Fig.8A-D).Sometimes,the overall survival analysis of the

hub genes was performed using the online tool GEPIA(<http://gepia.cancer-pku.cn/>). Except CCNB1 and CCNA2, CRC patients with upregulated MM3, MCM7, BUB1B, AURKA, TPX2, TTK, FEN1, KIF4A showed no statistically significant on overall survival (Figs. 9A–H). It is worth noting that CCNB1 and CCNA2 are upregulated in CRC patients, but the high expression level is associated with better overall survival (Fig. 9I–J). Subsequently, the expression status of hub genes with Logrank $P \leq 0.05$ were further validated using the Oncomine database. The result showed that CCNB1 and CCNA2 were markedly overexpressed in CRC in the different datasets (Fig. 10A–B). In the Oncomine dataset, the alternation of CCNB1 and CCNA2 were associated with tumor grade (Fig. 11A–B), implicating vital roles of these genes in the carcinogenesis or progression of CRC.

The validation of core genes expression in clinical samples.

For ascertaining which gene is crucial to the development of CRC, real-time qPCR was applied to examine the expression of 4 DEGs through clinical sampling, inclusive of CCNB1, CCNA2, AURKA and BUB1B. Notably, relative to tumor-adjacent tissues, CCNB1, CCNA2, AURKA and BUB1B were normally subject to upregulation in colorectal cancer tissues, which conforms to the aforementioned results of bioinformatics analysis (Fig. 12A–D). As revealed by the ROC curve, all CCNB1 (AUC = 0.790, 95% CI: 0.570 to 1.01, $P = 0.038$) CCNA2 (AUC = 0.864, 95% CI: 0.697 to 1.03, $P = 0.009$) AURKA (AUC = 0.840, 95% CI: 0.653 to 1.03, $P = 0.0152$) and BUB1B (AUC = 0.901, 95% CI: 0.754 to 1.05, $P = 0.0041$) are potentially suitable for prediction (Fig. 12A–D).

Discussion

A number of pre-clinical and clinical studies have been conducted to reveal the underlying mechanisms of CRC in the past decades; however, the incidence and mortality of CRC remain high. This is primarily due to the majority of the studies focusing on a single genetic event, or the results were generated from a single cohort study [1]. In this study, three GEO datasets were analyzed and 547 DEGs were identified, including 72 up-regulated and 475 down-regulated genes. The 547 DEGs were classified into three groups (BP, CC and MF groups) by GO terms, and the KEGG pathway enrichment analysis of the DEGs was conducted using the DAVID database. Finally, the DEGs PPI network was constructed, and the top 10 hub genes and the most significant module was filtered from the PPI network.

The KEGG analysis revealed some DEGs were significantly enriched in Pathways in cancer, PI3K-Akt signaling pathway, cGMP-PKG signaling pathway. These annotation results provided valuable clues to reveal molecular interactions in the development of CRC. Additionally, the most significant module was filtered from the PPI network, among which the majority of the corresponding genes were mostly associated with cell cycle, Neuroactive ligand-receptor interaction and DNA replication. Studies have shown [1–4] that there are countless signaling pathways that play an important role in the cell cycle of cancer and the process of cell proliferation. The signaling pathway of the protein kinase B (PI3K)/ protein kinase B (PKB/ Akt) mammalian target of rapamycin (mTOR) is abnormally induced in a variety of human tumors, including the breast gland cancer, the pancreatic gland cancer, the non-small cell lung

cancer, CRC, etc.[5]. The activation of PI3K-Akt signaling pathway is closely related to colorectal cancer metastasis and poor prognosis [6-7]. Research shows [8] that a deregulated PI3K-AKT signaling pathway in CRC patients, which might serve as therapeutic target(s). The cell cycle is controlled by various mechanisms, which ensure correct cell division; loss of normal cell cycle control is a hallmark of cancer[9]. An increasing number of studies has revealed that targeting the deregulation of the cell cycle in cancer is a potential therapeutic strategy [10]. Therefore, investigating the cell cycle pathway may promote the understanding of carcinogenic mechanisms and insights into CRC treatment options..These data may provide new ideas and directions for the mechanism research and therapeutic strategy of CRC.GO term enrichment analysis showed that the up-regulated DGEs were significantly enriched in cellular response to hypoxia, extracellular exosome, heparin binding , suggesting that some of these DEGs could locate in the nucleus and be involved in cell cycle processes to promote cell proliferation by enhancing DNA helicase activity in colorectal cancer. Increasing experimental evidence indicates that hypoxia is prevalent in the development of solid tumors.In order to ensure their survival, tumor cells under hypoxia can undergo a series of changes in biological behavior to adapt to hypoxia, specifically: (choose genotypes (such as TP53 mutation) that are conducive to survival of hypoxia repair [11] ,changes in expression of pro-survival genes [12] and inhibition of apoptosis [13], cell support for enhanced autophagy [14] and anabolic conversion [15], etc. Hypoxia can also enhance tumor angiogenesis [16], epithelial cells Interstitialization [17], immunosuppression [18], invasion and metastasis [19-20], etc. Through these changes, tumor cells can fight the body's autoimmunity and existing medical treatments, affecting their prognosis.It can be seen that the up-regulated DGEs play an important role in the occurrence and development of colorectal cancer.

Through integrated bioinformatics analysis, 10 hub genes were identified, including MCM3,MCM7, BUB1B,AURKA,TPX2, TTK,FEN1, cyclin B1(CCNB1),cyclin A2(CCNA2) and KIF4A with a high degree of connectivity.Among them, MCM3 and MCM7 belong to the MCMs family, and their biological functions are primarily involved in the DNA replication[2].Multiple previous studies have demonstrated that minichromosome maintenance(MCM) protein additionally serve a role in the occurrence and development of varies malignancies[3-5].The MCM participates in DNA synthesis [6] and can be used as a biomarker of oral squamous cell carcinoma [7], melanoma [8], glioma [9] and colon cancer [10]. In this study, the KEGG pathway showed that the DEGs were mainly involved in the cell cycle and DNA replication. CCNB1, one of the highly conserved cyclin family members, is involved in regulating cell cycle at the G2/M transition by forming maturation-promoting factor (MPF) with p34, suggesting that its over-expression can promote the progression of cancers [11-12]. AURKA gene mainly exists on chromosome 1 and encodes cell cycle regulated protein kinase, which is involved in chromosome mitosis and plays a role in tumorigenesis and development.BUB1B is an important constituent protein of the mitotic checkpoint, and is a multidomain protein kinase that responds to centromere tension [13-14]. Studies have demonstrated that BUB1B is overexpressed in various different types of tumor, such as colorectal cancer, renal and breast carcinoma, and its [15-16]. Therefore, further investigation on BUB1B may lead to a greater understanding of its importance in the CRC process, and novel ideas for investigating its molecular mechanisms and establishing more effective treatments[17].The TPX2 gene is located at

20q11.2 and encodes a microtubule-associated protein involved in spindle assembly during cell mitosis. TPX2 overexpression is common to many tumor types. In hepatocellular carcinoma, it was correlated with increased proliferation, apoptosis inhibition, and induction of EMT [18]. In breast cancer, TPX2 silencing repressed PI3K/AKT and activated p53 signaling, which inhibited proliferation and promoted apoptosis [19]. As a DNA repair protein, Flap endonuclease 1 (FEN1) plays crucial parts in preventing carcinogenesis. Two functional germ line variants (-69G > A and 4150G > T) in the FEN1 gene have been associated with DNA damage levels in coke oven workers and lung cancer risk in general populations. Studies showed lower FEN1 expression may lead to higher risk for malignant transformation of gastrointestinal cells [20-21]. TTK is a class of bispecific kinases first discovered in *Saccharomyces cerevisiae*, which can phosphorylate serine, threonine, and tyrosine residues. It is the basic component of the spindle assembly checkpoint (SAC), and plays an important role in the replication of mitotic centrosomes and the correct separation of chromosomes [22]. TTK is overexpressed and proposed as a therapeutic target for CRC and other cancers, and closely related to poor clinical prognosis [23-26].

Some reports are contradictory to our findings, and this is potentially attributed to the heterogeneity of the tumor. Thus, it is necessary to further conduct sample functional verification. For the further verification of the expression of the 4 hub gene, RT-qPCR was carried out in 9 clinical samples. As illustrated in Figure 12, we noted up-regulation on the expression of the 4 hub genes in colorectal cancer tissues comparing to tumor-adjacent tissues, which were consistent with our bioinformatics analysis results above, indicating that they would be one of the effective biomarkers for cancer treatment.

Herein, a set of candidate biomarkers possibly significant to the development of CRC were identified. They can be taken as research subjects to investigate their roles in the development of diseases, which can improve our understanding as to the mechanism of CRC on a molecular level. Besides, to figure out their prognostic effects, clinical validation study can be conducted with them as possible prognostic biomarkers. Nevertheless, the study remains subject to some limitations. Firstly, it is premised on bioinformatics analysis of published data. Secondly, it is uncertain whether the correlation between the differential expression of hub genes and disease progression is a causal one. Finally, despite the combination of four GEO data sets, the sample size remains small, which can render the results unreliable. Thus, it is essential to conduct further bioinformatics analysis and experimental verification with a larger sample size.

Conclusion

In general, we have excavated biomarkers for predicting the recurrence, metastasis and prognosis of colorectal cancer through the big biological data (GEO, TCGA, Ocomine database). Among these biomarkers, CCNA2 and CCNB1 are closely related to the survival and prognosis of colorectal cancer, and can predict the patient's OS and DFS. More importantly, we selected CCNB1, CCNA2, AURKA and BUB1B for verification on clinical colorectal tumor samples, which were consistent with our bioinformatics analysis results above, indicating that they would be one of the effective biomarkers for cancer

treatment. However, further prospective and multi-center, large-sample studies should be conducted to ensure these results and provide evidence for individualized treatment.

Declarations

Ethical Approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethical committee of the First Affiliated Hospital, Nanjing University of Chinese Medicine were obtained and with the 2019NL-KS1129 Declaration and its later amendments or comparable. NO tissues were procured from prisoners. All procedures performed in the present study involving human participants were in accordance with the ethical standards of institutional. Written informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of Data and Materials

The Primer sequence and clinical sample information used to support the findings of this study are included within the supplementary information file.

Competing interests

The authors declare that they have no conflicts of interest.

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Tables

Table 1 The detail information of three GEO Datasets				
ID	Tissue	Platform	Normal	Tumor
GSE32323	CRC	GPL570	17	27
GSE44076	CRC	GPL13667	148	98
GSE23878	CRC	GPL570	24	35

GEO: gene expression omnibus;CRC:colorectal cancer

Table 2 Top ten up- and down-regulated DEGs.					
Up-regulated DEGs			Down-regulated DEGs		
Gene symbol	logFC	Adjust P	Gene symbol	logFC	Adjust P
FABP6	2.5236035	0.00000002	SST	-5.0158362	6.83E-12
SLCO1B3	3.1640579	0.000142	CHRD1	-4.7149542	9.14E-12
SLC7A5	2.4605643	2.09E-10	CA4	-4.7812383	0.000000286
CLDN1	3.8365233	0.000347	LGALS2	-4.8012694	0.000000015
ASCL2	2.7737687	0.000138	MMP28	-4.5484346	7.19E-13
CRNDE	4.2755368	1.59E-12	AKR1B10	-5.1512725	0.00000145
TRIB3	2.866854	1.32E-09	MT1F	-5.2634923	1.22E-08
ADH1B	2.4665976	0.000947	PADI2	-6.2011828	2.27E-08
ABCA8	2.722522	0.00324	LAMA1	-5.3343777	0.000000294
GCNT2	2.3043753	0.00435	SLC25A34	-4.7128069	2.61E-08

Table 3 KEGG enriched pathways of DEGs			
ID	Description	Count	p adjust (FDR)
hsa04978	Mineral absorption	9	6.96E-05
hsa04022	cGMP-PKG signaling pathway	13	0.005089719
hsa04964	Proximal tubule bicarbonate reclamation	5	0.005859887
hsa04960	Aldosterone-regulated sodium reabsorption	6	0.008022768
hsa04911	Insulin secretion	8	0.019882876
hsa04976	Bile secretion	7	0.023805229
hsa05200	Pathways in cancer	21	0.029453784
hsa04151	PI3K-Akt signaling pathway	19	0.03041787
hsa04919	Thyroid hormone signaling pathway	9	0.032753173
hsa04110	Cell cycle	9	0.047771444

Figures

Figure 1

Flow diagram of bioinformatics analysis.

Figure 2

Standardization of gene expression (A) Standardization of GSE23878, (B) standardization of GSE32323, (C) standardization of GSE44076. The blue bar represents the data before normalization, and the red bar represents the data after normalization.

Figure 3

Heatmap of the top 100DEGs Heatmap of DEGs identified in (A) GSE23878, (B) GSE32323 and (C) GSE44076. Genes up-regulated are in red. Genes down-regulated are in blue. The differences are set as $|FC| > 2$ and corrected P-value < 0.05 .

Figure 4

Volcano plots of DEGs between CRC and tumor-adjacent tissues (A) GSE23878, (B) GSE32323 and (C) GSE44076. . Data points in red represent up-regulated and green represent down-regulated genes. Genes without any significant difference are in grey The differences are set as $|FC| > 2$ and corrected P-value < 0.05 .

Figure 5

Identification of consistent DEGs between primary CRC tumor tissues and normal tissues. (A) Venn plot for consistent up-regulated DEGs. (B) Venn plot for consistent down-regulated DEGs

Figure 6

GO and KEGG analysis of DEGs. (A–C) The top 15 terms of GO categories of biological process (BP), cellular component (CC) and molecular function MF, respectively. (D) KEGG pathway analysis of DEGs, p.adjust (FDR) < 0.05 was considered significantly. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

Figure 7

Protein–protein interaction network of DEGs and modules analysis.(A) PPI network of DEGs; (B) Top 10 hub genes with a higher degree of connectivity.(C–E) the top 3 modules of PPI network.(F) The KEGG enrichment analysis of the genes in the top three modules. PPI, protein–protein interaction; DEG, differentially expressed gene; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 8

The expression level of the selected 4 hub genes in the CRC tissues and tumor-adjacent tissues from TCGA.(A)CCNB1;(B)CCNA2;(3)AURKA;(4)BUB1B.

Figure 9

The overall survival analysis of the 10 hub genes(A-J).

Figure 10

Oncomine analysis of cancer vs. normal tissue of CCNB1 and CCNA2. Heat maps of CCNB1 (A), CCNA2 (B) gene expression in colorectal cancer samples vs. normal tissues. 1. Rectal Adenocarcinoma vs. Normal Gaedcke Colorectal, Genes Chromosomes Cancer, 2010;2. Colorectal Adenoma Epithelia vs. Normal Gaspar Colon, Am J Pathol, 2008;3. Colon Adenoma vs. Normal Sabates-Bellver Colon, Mol Cancer Res, 2007;4. Rectal Adenoma vs. Normal Sabates-Bellver Colon, Mol Cancer Res, 2007;5. Cecum Adenocarcinoma vs. Normal TCGA Colorectal, No Associated Paper, 2011;6. Colon Mucinous

Adenocarcinoma vs. Normal TCGA Colorectal, No Associated Paper, 2011;7. Rectal Mucinous Adenocarcinoma vs. Normal TCGA Colorectal, No Associated Paper, 2011;8. Rectosigmoid Adenocarcinoma vs. Normal TCGA Colorectal, No Associated Paper, 2011.

Figure 11

The association between the expression of selected hub genes and tumor stage. (A-B) The expressions of CCNB1 and CCNA2 were correlated with tumor stage in the Oncomine dataset. 0: No value; 1: Stage I; 2: Stage II; 3: Stage IIA; 4: Stage IIB; 5: Stage III; 6: Stage IIIA; 7: Stage IIIB; 8: Stage IIIC; 9: Stage IV; 10: Stage IVA.

Figure 12

The expression level of the selected 4 hub genes in the CRC tissues and tumor-adjacent tissues from clinical samples and ROC curve analysis of the 4 hub genes.(A)CCNB1;(B)CCNA2;(3)AURKA;(4)BUB1B .Data are presented as mean $\pm$ S.D. $P \leq 0.05$ was considered as significant.