Screening for implicated genes in colorectal cancer using whole-genome gene expression profiling

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Abstract. To identify biologically relevant genes associated with the pathogenesis of colorectal cancer (CRC), genome wide expression profiles of 17 pairs of CRC tumor and adjacent tissues, previously published in a DNA microarray study, were analyzed. Cytoscape, String tools and DAVID tools were used to investigate the biological pathways encoded by the genes identified as being either upregulated or downregulated in CRC, to determine protein-protein interactions and to identify potential hub genes associated with CRC. As a result, a total of 3,264 genes were identified as being differentially expressed in CRC and adjacent tissues, including 1,594 downregulated and 1,670 upregulated genes. Furthermore, 306 genes were revealed to be clustered in a complex interaction network, and the top 20 hub genes in this network were determined by application of the Matthews Correlation Coefficient algorithm. In addition, the patterns of the expression levels of the 20 hub genes were investigated using reverse transcription‑quantitative polymerase chain reaction. Gene Ontology analysis revealed that four of the 20 hub genes encoded small subunit processome components (UTP3 small subunit processome component; UTP14 small subunit processome component; UTP 18 small subunit processome component; and UTP20 small subunit processome component) and a further four encoded WD repeat domains (WD repeat -containing protein 3, WD repeat domain 12, WD repeat -containing protein 43 and WD repeat‑containing protein 75). In conclusion, the present DNA microarray study identified genes involved in the pathogenesis of CRC. Furthermore, it was revealed that hub genes identified from among the total identified upregulated and downregulated genes in CRC encoding subunit processome components and WD repeat domains may represent novel target molecules for future treatments of CRC.

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide (1). It is the second most prevalent cancer type among women, following breast cancer, and the third most prevalent cancer type among men (2,3). In recent decades, diagnoses and treatment of CRC have significantly improved. However, the disease remains a major public health problem worldwide. In 2016, the American Cancer Society estimated 70,820 new male cases and 63,670 new female cases of CRC would be diagnosed, and that there would be 49,190 CRC associated deaths in the USA (4). The high rate of mortality of the disease is in part due to limitations in the currently available therapies for CRC treatment, which is the result of an incomplete understanding of the biological mechanisms underlying the disease.

It has been well established that genes have an important role in tumor pathogenesis, and that the transcriptional activation or inhibition of certain genes serves an important role in the development and progression of the majority of human tumors (5). The analysis of genome-wide gene expression levels via DNA microarray experiments is a recently developed systematic approach that is used to gain comprehensive information regarding tumor associated gene transcription profiles (6). This information can lead to the identification of prognostic biomarkers (7,8), enable the discrimination between various histological subtypes of tumors (9,10).

Interactions between genes can affect the pathogenesis of CRC and tumor progression. The aim of the present study was to identify biologically relevant genes that may be associated with the pathogenesis of CRC, via analysis of genome-wide expression profiles in CRC. Following the identification of differentially expressed genes (DEGs), the present study further aimed to determine the hub genes involved in the regulatory pathways of CRC progression, by Gene Ontology (GO) analysis, and to subsequently derive a gene interaction network. Identification of hub genes in CRC and the development of a gene interaction network may reveal the biological characteristics of CRC tumor development and enable the development of novel molecular-targeted therapies for the treatment of CRC.

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Materials and methods

Data collection. The gene expression profiling dataset from previously published relevant study was retrieved from the NCBI GEO database (accession number GSE32323; www.ncbi.nlm.nih.gov/geo) (11). Data regarding a total of 17 paired samples of CRC tumors and adjacent tissues were available from the GSE32323 dataset.

Data processing. Raw RNA expression profile datasets were preprocessed using R 3.4.1 statistical software (https://www.r-project.org/) together with Affy package (12). In accordance with the R-package, the gcma algorithm (13) was used to adjust for background intensities in the Affymetrix array data by including optical noise and non-specific binding. The background adjusted probe intensities were then converted into expression measures using the normalization and summarization methods included in the multiarray average algorithm (12). The k-Nearest Neighbor algorithm (14) was subsequently used to generate the missing values.

Microarray and statistical analysis. The R-package and limma (www.bioconductor.org.uk) (15) were used to investigate gene expression microarray data. Limma uses linear models, as well as empirical Bayesian methods, to investigate the differential expression levels in the GSE32323 dataset. Fold-change (FC) values were calculated using the ratio of the geometric means of the gene expression levels between the two treatment groups (CRC tissue vs. adjacent tissue). A Wilcoxon Rank-Sum Test was performed to determine the statistical significance of differences between the groups (P<0.05 was considered statistically significant). Genes were selected for if the log2-FC values were >1 (equivalent to a FC value of >2.0 or <0.5), and P-values determined by the Wilcoxon Rank-Sum Test were P<0.0002. Hierarchical clustering of the selected genes was performed with R software using the Euclidean distance and complete linkage method. Regarding clustering, the expression data were standardized via conversion to z scores (mean=0, variance=1) for each probe. Following this, protein-protein interactions among the selected genes were analyzed using the reference data contained in the String database of known protein-protein interactions, the Cytoscape (www.cytoscape.org/) plug-in CytoHubba, version 3.5.1 (apps.cytoscape.org/apps/mcode) (16). In addition, edge of the gene clusters was calculated using molecular complex detection (MCODE) software (version 1.4.2) from Cytoscape, version 3.5.1 (apps.cytoscape.org/apps/mcode) (16). In addition, the Cytoscape (www.cytoscape.org/) plug-in CytoHubba, version 0.1 (http://apps.cytoscape.org/apps/cytohubba) was used to identify hub genes among the selected genes. Finally, BiNGO software version 3.0.3 (http://apps.cytoscape.org/apps/bingo) from Cytoscape was used to determine which GO categories represented identified genes, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (http://www.genome.jp/kegg/) were analyzed using DAVID functional annotation tools (version 6.8) (17,18).

Collection of CRC samples. The present study also investigated 3 patients (2 males and 1 female; aged 48-56 years old) with CRC who had previously undergone radical resection of CRC in the Gastroenterology Department of Renji Hospital Affiliated to Shanghai Jiaotong University School of Medicine (Shanghai, China) between November 2017 and December 2017. The inclusion criteria for patients were as follows: i) colorectal adenocarcinoma confirmed by pathological examination; ii) the patients must have not received a radical resection prior and must not have been treated with preoperative chemoradiotherapy. Characteristics of patients included in the present study are presented in Table I. Written informed consent was obtained from all patients included in the present study. The Ethics Committee of Renji Hospital Affiliated to Shanghai Jiaotong University School of Medicine approved the present study and protocols. CRC tissue samples (diameter of 0.5 cm) were obtained via tumor dissection. Paracancerous tissue (distal tissue 3 cm away from the tumor) was also obtained from patients via dissection. Paracancerous tissues and cancerous tissues were collected as normal tissues and tumor tissues, respectively. RNA was extracted from these tissues by TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) for future experiments.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). In order to further investigate the results of the microarray analysis, the 20 hub genes identified to be associated with CRC were subjected to RT-qPCR analysis. A total of 1 µg of RNA from tumor or normal tissue samples was reverse transcribed to cDNA using SuperScript™ II reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) and primers (2.5 µM). The reaction was performed using the Bio-Rad RT-qPCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) under the following PCR conditions: Initial denaturation at 95°C for 5 min; 35 cycles at 95°C for 30 sec; 60°C for 30 sec; 72°C for 30 sec; and final extension at 72°C for 5 min. Expression levels of these genes were normalized to the house-keeping gene, β-actin via the 2-ΔΔCq method (19). Primer sequences used were as follows: WD repeat-containing protein 3 (WDR3) forward, 5'-AATCTCAGGGGTGAC TAA-3' and reverse, 5'-ACAGGCCTAGAGTTAGGC-3'; WD repeat-containing protein 43 (WDR43) forward, 5'-TGACCT ATGGCTCTTG-3' and reverse, 5'-GGCTGATACATA GGGAAC-3'; UTP14 small subunit processome component (UTP14A) forward, 5'-GGTGATGGCGATGTAAG-3', and reverse, 5'-GCCAATGTTGGTAAATG-3'; damage specific DNA binding protein 1 and cullin4 associated factor 13 (DCAF13) forward, 5'-GGGATGACAAAGAACTA-3', and reverse, 5'-AGATTTATCGAATACTCG-3'; KR1 small subunit processome component (KR1) forward, 5'-GGAGACGTACGTTGGTG-3' and reverse, 5'-TAAGCCCTTGGTTCCT GT-3'; digestion organ expression factor (DIEXF) forward, 5'-GAGAGCATCCGCATCTCA-3', and reverse, 5'-TAA AAC CTCGGCATCAATC-3'; HEAT repeat containing 1 (HEATR1) forward, 5'-ATTACATTGTGGCCTTAC-3', and reverse, 5'-TCTTGTGTCCTGTTAGTG-3'; WD repeat-containing protein 75 (WDR75) forward, 5'-ATGCATTGCAGAATTCCA AAAGAC-3', and reverse, 5'-GCTTTTTGATATCCG AATTGAT-3'; UTP 18 small subunit processome component (UTP18) forward, 5'-GTTTATGTGGGAGATGTGA-3', and...
Table I. Characteristics of patients with colorectal cancer in the present study.

| Patient | Age | Sex  | Stage | Preoperative chemoradiotherapy | Surgical resection |
|---------|-----|------|-------|--------------------------------|-------------------|
| 1       | 48  | Male | 2a    | No                             | Radical correction |
| 2       | 53  | Male | 3c    | No                             | Radical correction |
| 3       | 56  | Female | 2b   | No                             | Radical correction |

Characterization of differentially expressed genes. A total of 3,264 genes were identified by the Affymetrix Human Genome Array analysis as being differentially expressed in CRC tissues compared with adjacent tissue (P<0.05; data not shown). This included 1,594 genes that were downregulated (FC<0.5 in expression in CRC vs. adjacent tissue) and 1,670 genes that were upregulated (FC>2.0 in expression in CRC vs. adjacent tissue) compared with the adjacent tissue. The former group was defined as the ‘lower group’ and the latter group as the ‘higher group’. GO and KEGG enrichment analyses were then performed to identify the biological characteristics of each of these gene subgroups.

In the lower group, a total of 1,594 downregulated genes were revealed to be significantly enriched in 75 KEGG pathways, the top 20 of which are presented in Fig. 1A (P<0.05). The protein kinase, guanosine monophosphate-protein kinase G signaling pathway (hsa04022) was the most notably enriched pathway in the lower group (Fig. 1A). In addition, numerous well-established signaling pathways were also included, including the mitogen-activated kinase-like protein signaling pathway (hsa04101), cyclic adenosine monophosphate signaling pathway (hsa04024) and the phosphoinositide 3 kinase-protein kinase B signaling pathway (hsa04151). Furthermore, pathways associated with melanoma (hsa05218) and bladder cancer (hsa05219) were also enriched in CRC, with 10 and 7 genes identified as being associated with these diseases, respectively. In addition, the results of GO classification of the identified genes into the categories ‘biological process’, ‘molecular function’ and ‘cellular component’, as performed by BiNGO from Cytoscape, revealed that in the ‘biological process’ category, the genes were notably enriched in the following GO terms: ‘Positive regulation of cell growth’ (GO: 0030308) and ‘negative regulation of cell growth’ (GO: 0045926; Fig. 1B). GO terms associated with the identified enriched genes belonging to the ‘cellular component’ and ‘molecular function’ categories are presented in Fig. 1C and D, respectively.

In the higher group, a total of 1,670 upregulated genes were revealed to be significantly enriched in 28 KEGG signaling pathways, the top 20 of which are presented in Fig. 2A. The ‘cell cycle’ (hsa04110) pathway represents the most enriched pathway within this group, followed by the ‘cellular tumor antigen p53 signaling pathway’ (hsa04115) and the ‘Wnt protein signaling pathway’ (hsa04310). In the ‘biological process’ GO category, genes were notably enriched in the following GO terms: ‘Mitotic nuclear division’ (GO: 0007067), ‘DNA replication’ (GO: 0006260), ‘sister chromatid cohesion’ (GO: 0007062) and ‘G1/S transition of mitotic cell cycle’ (GO: 0000082; Fig. 2B). In the ‘cellular component’ category, genes were significantly enriched in the GO term associated with the ‘nucleolus’ (GO: 000654; Fig. 2C), and in the ‘molecular function’ category, the genes were most significantly enriched in GO terms associated with ‘protein binding’ (GO: 0005515) and ‘pol (A) RNA binding’ (GO: 0044822; Fig. 2D).

Analysis of protein-protein interactions of the differentially expressed genes. To determine the protein-protein interactions...
of the selected genes, the String tool was used. The results revealed that 306 of the genes were clustered in a complex interaction network (Fig. 3A), thus suggesting close interactions between the proteins encoded by such genes. Furthermore, calculation of the weighted index between each encoded protein revealed the existence of three subgroups within the larger network, which were closely associated with each other (subgroups are represented in blue, green and light blue shading in Fig. 3A). Each subgroup was extracted and their interactions are separately presented in Fig. 3B.

Identification of hub genes for CRC. To identify potential hub genes among the 306 genes previously identified, Matthew's correlation coefficient algorithms were performed using the CytoHubba software plug-in. As presented in Fig. 4, 20 potential hub genes for CRC were identified, all of which were closely associated with each other. The characteristics of the 20 hub genes are presented in Table II. Four of the 20 hub genes are genes that encode for small subunit proces- some components (UTP3, UTP14A, UTP18 and UTP20) and four are genes that encode for WD-repeat domains (WDR3, WDR12, WDR43, WDR75). The remaining 12 genes encode binding domains or proteins.

Verification of Affymetrix gene expression data. To further investigate the gene expression profiles, the expression levels of the 20 hub genes were determined via RT-qPCR analysis. The results demonstrated that the majority of the expression levels of the 20 hub genes were significantly upregulated in CRC tissues compared with control tissues (Fig. 5), which is consistent with the results of the microarray data analyses.

Discussion

CRC progression is characterized by the transformation of the normal mucosa of the bowel into an adenoma and then into a malignant tumor. This progression involves the acquisition of gene mutations that enable the tumor cells to proliferate and migrate within the tissues (20).

In the present study, a previously published microarray dataset (11) was used to identify significant genes in CRC tumor tissues via comparison of gene expression profiles in CRC tissues with those in adjacent paired tissue samples. A total of 1,594 genes were revealed to be downregulated, and 1,670 genes were revealed to be upregulated, in CRC tumor tissues. KEGG enrichment analysis of each of these subgroups provided information regarding the function and other biological characteristics of associated genes. The upregulated genes were predominantly enriched in ‘cell cycle’ (hsa04110), ‘RNA transport’ (hsa03013) and ‘DNA replication’ (hsa03030) pathways, which is consistent with the findings of a previous study investigating gene involvement in
The mammalian cell cycle is highly organized and regulated to ensure the correct functioning of cell division and other biological activities (22). The four phases of the cell cycle (G0/G1, S, G2, and M) are regulated by numerous cyclin-dependent kinases (CDKs) (23). Aberrant cell cycle activity, which may occur as a result of genetic lesion within genes encoding cell cycle proteins, represents one of the typical characteristics of cancer (24). Therefore, synthetic inhibitors of CDKs are widely used as anticancer drugs in current cancer treatment therapies (25, 26).

Notably, in the present study, the upregulated genes identified in CRC were also revealed to be enriched in ‘small cell lung cancer’ (hsa05222), ‘bladder cancer’ (hsa05219) and ‘basal cell carcinoma’ (hsa05217) KEGG pathways, suggesting that CRC may exhibit similar gene regulation and molecular mechanisms with small cell lung cancer, bladder cancer and basal cell carcinoma. Similarly, the results from the enrichment analysis of the downregulated genes revealed that there were 10 and 7 downregulated DEGs enriched in the ‘melanoma’ (hsa05218) term and ‘bladder cancer’ (hsa05219) term, respectively. Merlo et al (27) revealed that one of the microsatellite alterations exhibited in small cell lung cancer is also exhibited in CRC. Furthermore, a clinical study in Japan has suggested that patients with bladder cancer have the potential to develop colon cancer (1.44%) during anticancer therapy (28), and an immunotherapy study have demonstrated that type I and II interferons can be used to treat both CRC and melanoma due to their dual role in promoting proliferation and inhibiting growth (29). However, the extent to which genes that have been previously revealed to be implicated in these other cancers are expressed in CRC remains largely unknown, and warrants further research.

Song et al (30) downloaded CRC microarray data from the GEO database (GSE17538) and screened for genes associated with enhancer of zeste homolog 2 (EZH2). Song et al (30) revealed that EZH2 may represent a potential prognostic marker of patients with CRC. By contrast, the present study was performed using all DEGs in CRC, and aimed to uncover the molecular mechanisms underlying the progression of CRC. Liang et al (31) analyzed 141 samples (132 CRC and 9 normal colon epitheliums) and 3,500 DEGs were identified. In addition, Liang et al (31) performed GO and KEGG pathway enrichment analyses, and the top 10 hub genes from PPI network were identified, including G protein subunit γ2, angiotensin precursor, serum amyloid A1, adenylate cyclase 5, lysosphosphatidic acid receptor 1, neuromedin-U, interleukin-8, C-X-C motif chemokine ligand 12, G protein subunit α1, and C-C motif chemokine receptor 2. There was no intersection between the 10 hub genes and 20 hub genes detected in the present study, which suggested that the novel CRC-associated genes were identified. Furthermore, Guo et al (32) acquired overlapping DEGs in CRC from four GEO datasets.
GSE28000, GSE21815, GSE44076 and GSE75970) (33-36) and performed GO enrichment analysis, KEGG pathway analysis and PPI network analysis. Guo et al (32) found also that cell cycle term may serve an important role in CRC; 31 hub genes were acquired in a PPI network. However, there was no intersection between these 31 hub genes and the results of the present study. A total of 17 pairs of cancer tissues and adjacent tissues were analyzed in the present study, which made obtained DEGs more reliable and accurate relative to unpaired samples. The follow-up analyses, including GO enrichment analysis, KEGG pathway enrichment analysis, PPI network analysis and MCODE software analysis, could be more reliable based on these DEGs. In addition, CRC tissues and adjacent tissues from patients with CRC were collected, and RT-qPCR was performed to verify the expression levels of the identified 20 hub genes in CRC tissue compared with normal tissue.

In the present study, DEGs in CRC and adjacent tissues were identified and were separated into two subgroups (upregulated and downregulated groups), but the accounts of DEGs are still enormous. Furthermore, the present study aimed to construct a protein interaction network based on the selected genes, and following this, a total of 306 genes were revealed to be involved in this network. From these 306 genes, 20 hub genes, which represent genes exhibiting close interactions with each other, were identified using the CytoHubba plug-in analysis. These hub genes may serve important molecular roles in the pathogenesis of CRC.
GO analysis of the 20 hub genes revealed that there are four subunit processome component encoded genes: UTP3, UTP14A, UTP18 and UTP20 differentially expressed in CRC. The subunit processome component is an essential part of the ribonucleoprotein complex that once bound to the U3 small nuclear RNA, participates in ribosome biogenesis and 18S ribosomal RNA synthesis (37). It has been demonstrated that alternative splicing of said genes may result in multiple transcript variants; which can promote cancer development (38). For example, UTP18 has been revealed to be localized in the cytoplasm of cells, and serum withdrawal has been revealed to increase cytoplasmic UTP18, which can associate with the translation complex and Hsp90 to upregulate the translation of HIF1α, Myc and VEGF, thus inducing a cellular stress response (39). UTP18 overexpression promotes transformation and tumorigenesis; however, UTP18 knockdown can inhibit these processes (23). In addition, the subunit processome component is represented by a large gene family (uridine triphosphate, UTP), which exhibits different mechanisms in cell proliferation and cancer pathogenesis (40). Furthermore, the associations between the four subunit processome component encoded genes and CRC, to the best of our knowledge, have not previously been investigated. The present study demonstrated that the aforementioned four genes may have important roles in the progression of CRC and represent potential biomarkers for CRC.

Table II. List of 20 hub genes associated with colorectal cancer.

| Rank | Gene       | Location      |
|------|------------|---------------|
| 1    | WDR3       | 1p12          |
| 2    | WDR43      | 2p23.2        |
| 3    | UTP14A     | Xq26.1        |
| 4    | DCAF13     | 8q22.3        |
| 5    | KRR1       | 12q21.2       |
| 6    | DIEXF      | 1q32.2        |
| 7    | HEATR1     | 1q43          |
| 8    | WDR75      | 2q32.2        |
| 9    | UTP18      | 17q21.33      |
| 10   | UTP3       | 4q13.3        |
| 11   | RCL1       | 9p24.1        |
| 12   | UTP20      | 12q23.2       |
| 13   | TEX10      | 9q31.1        |
| 14   | WDR12      | 2q33.2        |
| 15   | EXOSC5     | 19q13.2       |
| 16   | NOB1       | 16q22.1       |
| 17   | SDAD1      | 4q21.1        |
| 18   | DDX27      | 20q13.13      |
| 19   | RPF2       | 6q21          |
| 20   | GTPBP4     | 10p15.3       |

WDR3, WD repeat domain 3; WDR43, WD repeat domain 43; UTP14A, UTP14 small subunit processome component; DCAF13, damage specific DNA binding protein 1 and cullin4 associated factor 13; KRR1, KRR1 small subunit processome component; DIEXF, digestive organ expansion factor; HEATR1, HEAT repeat containing 1; WDR75, WD repeat domain 75; UTP18, UTP 18 small subunit processome component; TEX10, testis expressed 10; WDR12, WD repeat domain 12; EXOSC5, exosome component 5; NOB1, neutral invertase (regulatory particle non-ATPase 12) binding protein 1; SDAD1, SDA1 domain containing 1; DDX27, DEAD-box helicase 27; RPF2, ribosome production factor 2; GTPBP4, GTP binding protein 4.
knockdown of FBXW7 via application of small interfering RNA could enhance cell sensitivity to anticancer agents.

Akdi et al (44) revealed that mRNA and protein levels of WDR3 are dysregulated in human thyroid cancer cells. In addition, a further study demonstrated that WDR3 can regulate genome stability in patients with thyroid cancer (45). However, studies investigating the associations between WD repeats and CRC are currently very limited. In the present study, it was revealed that these WD repeats are hub genes in progression of CRC. Therefore, further in vitro and in vivo studies on this topic are required. He et al (46) demonstrated that knockdown of NOB1 can induce apoptosis of human colorectal cells. In addition, Zeng et al (47) demonstrated that knockdown of SDAD1 can suppress the proliferation, migration and invasion of colon cancer cells (47). The results of the previous two studies are consistent with the results of the present study. In addition, a previous study demonstrated that overexpression of DCAF13 in hepatocellular carcinoma is correlated with poor survival of patients (48). Dyachenko et al (49) demonstrated that KRR1 may represent a potential biomarker of particular histological types (invasive ductal) of breast tumor. Furthermore, Liu et al (50) revealed that HEATR1 can negatively regulate protein kinase B and further decrease resistance to gemcitabine and other chemotherapeutics. Tsukamoto et al (51) demonstrated that DDX27 is upregulated in gastric cancer tissues and may represent a potential therapeutic target for patients with gastric cancer. In addition, Liu et al (52) revealed that GTPBP4 serves an important role in hepatocellular carcinoma development, and that increased GTPBP4 expression is correlated with poor survival of patients with hepatocellular carcinoma. However, the functions of aforementioned five genes (DCAF13, KRR1, HEATR1, DDX27 and GTPBP4) have, to the best of our knowledge, not been investigated with regards to CRC. The results of the present study demonstrated that the aforementioned five genes and the remaining 15 genes in the top 20 hub genes (DIEXF, RCL1, TEX10, EXOSC5 and RPF2) may serve important roles in CRC development and represent potential biomarkers for CRC.

In conclusion, the present study identified significant genes associated with the pathogenesis of CRC via analysis of genome-wide expression profiles of CRC as well as comparison of expression levels of significant genes in CRC tissues compared with healthy adjacent tissue samples. Furthermore, 20 hub genes were revealed via genetic analysis as validated by RT-qPCR. GO analysis revealed that ‘small subunit processsome component’ and ‘WD repeat domains’ were two protein family subgroups encoded by the 20 hub genes, and could represent novel molecular markers associated with CRC. The patterns of the expression levels of the 20 hub genes in CRC were further verified by RT-qPCR. However, a small sample size (three paired samples) represents a limitation of the present study, and therefore this should be further investigated using a greater sample size in future studies. Therefore, additional studies are required to further investigate the associations between identified hub genes and CRC.

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Availability of data and materials

The datasets used and analyzed during the current study are available from GEO database (GSE32323, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32323).

Authors' contributions

All the research was conducted by SL. The design of the present study was conceived by QH. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients included in the present study. The Ethics Committee of Renji Hospital Affiliated to Shanghai Jiaotong University School of Medicine approved the present study and protocols.

Consent for publication

Written informed consent was obtained from all patients for publication of reverse transcription-quantitative polymerase chain reaction results in this study; personal identifying information was not included in this article.

Competing interests

The authors declare that they have no competing interests.

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