Identification of four novel prognosis biomarkers and potential therapeutic drugs for human colorectal cancer by bioinformatics analysis

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Abstract

Colorectal cancer (CRC) is one of the most deadly cancers in the world with few reliable biomarkers that have been selected into clinical guidelines for prognosis of CRC patients. In this study, mRNA microarray datasets GSE113513, GSE21510, GSE44076, and GSE32323 were obtained from the Gene Expression Omnibus (GEO) and analyzed with bioinformatics to identify hub genes in CRC development. Differentially expressed genes (DEGs) were analyzed using the GEO2R tool. Gene ontology (GO) and KEGG analyses were performed through the DAVID database. STRING database and Cytoscape software were used to construct a protein-protein interaction (PPI) network and identify key modules and hub genes. Survival analyses of the DEGs were performed on GEPIA database. The Connectivity Map database was used to screen potential drugs. A total of 865 DEGs were identified, including 374 upregulated and 491 downregulated genes. These DEGs were mainly associated with metabolic pathways, pathways in cancer, cell cycle and so on. The PPI network was identified with 863 nodes and 5817 edges. Survival analysis revealed that HMMR, PAICS, ETFDH, and SCG2 were significantly associated with overall survival of CRC patients. And blebbistatin and sulconazole were identified as candidate drugs. In conclusion, our study found four hub genes involved in CRC, which may provide novel potential biomarkers for CRC prognosis, and two potential candidate drugs for CRC.

Keywords: colorectal cancer, Gene Expression Omnibus, biomarkers, bioinformatics analysis

Introduction

Nowadays, colorectal cancer (CRC) is one of the most deadly cancers and almost 900 000 CRC-related deaths were reported each year in the world¹. With the understanding of pathophysiology of the disease, different treatment options to improve the survival rates of CRC patients have been developed in the
The 5-year survival rate of CRC patients was >90% when the patients were diagnosed at early stages\cite{2}. However, due to lacking early detection methods, many CRC patients were diagnosed at an advanced stage or in the metastasis status. And the 5-year survival rate for those diagnosed with metastasis was at approximately 12\%\cite{3}. Recently, a new kind of analysis method has been used to identify the differential expression genes between CRC and normal tissues based on the high-throughput sequencing platforms, such as microarrays. This is a promising tool with extensive clinical applications, including molecular diagnosis, prognosis prediction, new drug targets discovery, etc\cite{4-6}. Furthermore, microarray assay combining bioinformatics analysis made it possible to analyze the gene expression on mRNA level in CRC progression. For example, several studies have used this method to identify key genes in CRC development through comparing with normal samples, and showed that the key genes were involved in different signal pathways, biological processes, and molecular functions\cite{7-12}.

However, with a relatively limited degree of overlap, we still can not find reliable biomarkers or drug targets. Therefore, the discovery of novel biomarkers for early detection and prognosis prediction of CRC is urgently required.

In the present study, we targeted to find key genes to develop novel biomarkers or drug targets for CRC. Therefore, we chose four Gene Expression Omnibus (GEO) datasets, GSE113513, GSE21510, GSE44076, and GSE32323, and used bioinformatics methods to screen the significant differentially expressed genes (DEGs) between CRC tissues and normal tissues. Gene ontology (GO) and KEGG pathway analyses were performed with \textit{P}-value <0.05. PPI network construction and module analysis

The DEGs profiles were submitted to STRING database for exploring their potential interactions. The interactions with a combined score >0.4 were considered significant. Subsequently, the interaction files were downloaded and imported into Cytoscape software to construct the PPI network. The MCODE plugin was used to find key modules of the whole PPI network with a degree cutoff=2, node score cutoff=0.2, K-core=2, and max depth=100. The hub genes were then selected with connectivity degree >10. Furthermore, KEGG pathway analyses of the significant modules were performed with \textit{P}-value <0.05.

Analysis and validation of hub genes

To verify the hub genes we found, we used GEPIA database to analyze their expression and clinical
prognostic information in 270 CRC patients. And the survival curve, stage analysis and box plot were performed to show the clinical implications of hub genes.

Identification of small molecules

To find potential small active molecules to develop new drugs for treating CRC, we uploaded DEGs probe profiles into the CMap database. This database can help to predict small molecules that induce or reverse gene expression signature with a score from −1 to 1. And the molecules which value from 0 closer to −1 were functioned as reversing the cancer cell status.

Results

Identification of DEGs in CRC

Analyzed with the GEO2R online tool, a total of 1763, 4411, 2428, and 2276 DEGs were extracted from GSE113513, GSE21510, GSE44076, and GSE32323, respectively, using adjusted P-value <0.05 and |log2(fold change)| >1 as cutoff criteria. The volcano plots of DEGs in each dataset were shown in Fig. 1A. And the Venn diagrams showed that 865 overlap DEGs were identified from these four datasets, including 374 significantly upregulated genes and 491 downregulated genes (Fig. 1B and Supplementary Table 1, available online).

Enrichment analysis of DEGs

To explore the biological functional roles of the overlap DEGs, GO and KEGG analyses were performed on DAVID database. And the top 20 terms were listed in the charts (Fig. 2A–D and Supplementary Table 2, available online). The GO analysis results consist of three functional categories, including BP, CC, and MF. In the BP group, DEGs were mainly enriched in cell proliferation (Fig. 2A). In the CC group, DEGs were enriched in cytoplasm (Fig. 2B). And in the MF group, DEGs were enriched in protein binding (Fig. 2C). KEGG pathway analysis showed that DEGs were enriched in metabolic pathways, pathways in cancer and cell cycle (Fig. 2D). The details of the top 20 terms were listed in Supplementary Table 2.

PPI network construction and module analysis

Using the STRING online database and Cytoscape software, a total of 865 DEGs were filtered into the PPI network complex, containing 863 nodes and 5817 edges (Fig. 3). Based on degree scores using the MCODE plugin, two key modules were detected from the whole PPI network complex. Module 1 contained 61 nodes and 1648 edges, and DEGs were enriched in cell cycle, oocyte meiosis, progesterone-mediated oocyte maturation, DNA replication and p53 signaling pathway (Fig. 4A and B). Module 2 had 55 nodes and 625 edges, and these DEGs were enriched in chemokine signaling pathway, ribosome biogenesis in eukaryotes, cytokine-cytokine receptor interaction, pathways in cancer, purine metabolism, RNA polymerase, retrograde endocannabinoid signaling, TNF signaling pathway, legionellosis, regulation of lipolysis in adipocytes, NOD-like receptor signaling pathway, cytosolic DNA-sensing pathway and gastric acid secretion (Fig. 4C and D). Additionally, the top 20 hub genes, CDK1, CCNB1, MYC, CCNA2, MAD2L1, AURKA, TOP2A, CDC6, UBE2C, CHEK1, RRM2, BUB1B, TTK, TRIP13, TPX2, BUB1, NCAPG, KIF2C, KIF23, and MCM4 were identified with higher degrees of connectivity. These hub genes were enriched in cell cycle, progesterone-mediated oocyte maturation, oocyte meiosis, p53 signaling pathway, and HTLV-I infection (Fig. 4E and F).

Analysis and validation of hub genes

To validate the hub genes we got from this study, we uploaded the hub genes list into GEPIA database and explored the correlation between hub genes expression and the clinical characteristics of CRC. It was found that HMMR, PAICS, ETFDH, and SCG2 were significant DEGs in 270 CRC samples from GEPIA (Fig. 5A). And these four genes could represent the important prognostic biomarkers for predicting the survival of CRC patients (Fig. 5B). Meanwhile, PAICS and SCG2 were related to the stages of CRC progression (Fig. 5C). The summaries of four hub genes were shown in Table 1.

Identification of related active small molecules

To search candidate small molecules for developing potential drugs to treat CRC, we uploaded DEGs probe profiles into the CMap database. And the predicted results were download and filtered with enrichment score <0 and P-value <0.05. The results were shown in Table 2. And Fig. 5D listed the top 20 small molecules with their enrichment scores and P-values. Therefore, these small molecules may be the targets to develop new drugs or therapies of CRC. Among these molecules, Blebbistatin and Sulconazole may be selected for new clinical trials.
Fig. 1  Identification of the DEGs in CRC. A: Volcano plots of gene expression profiles between CRC and normal samples from GSE113513, GSE21510, GSE44076 and GSE32323. Red dots: significantly upregulated genes in CRC; Green dots: remarkably downregulated genes in CRC. Adjusted \( P \) value <0.05 and \(|\log_2(\text{fold change})| >1\) were considered as significant criteria. B: Venn diagrams show that 865 overlap DEGs were found through GEO2R in the four datasets, including 374 upregulated DEGs and 491 downregulated DEGs. DEGs: differentially expressed genes; CRC: colorectal cancer.

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A

- GO: 0006508–proteolysis
- GO: 0043066–negative regulation of apoptotic process
- GO: 0045893–positive regulation of transcription: DNA-templated
- GO: 0001666–response to hypoxia
- GO: 0006629–lipid metabolic process
- GO: 0030198–extracellular matrix organization
- GO: 0008284–positive regulation of cell proliferation
- GO: 0010629–negative regulation of gene expression
- GO: 0042493–response to drug
- GO: 0006260–DNA replication
- GO: 0000086–G2/M transition of mitotic cell cycle
- GO: 0042127–regulation of cell proliferation
- GO: 0006364–rRNA processing
- GO: 0000082–G1/S transition of mitotic cell cycle
- GO: 0030855–epithelial cell differentiation
- GO: 0008283–cell proliferation
- GO: 0007067–mitotic nuclear division

B

- GO: 0045121–membrane raft
- GO: 005886–plasma membrane
- GO: 0031965–nuclear membrane
- GO: 0048471–perinuclear region of cytoplasm
- GO: 0009986–cell surface
- GO: 0005737–cytoplasm
- GO: 0005730–nucleolus
- GO: 0016020–membrane
- GO: 0005887–integral component of plasma membrane
- GO: 0005788–proteinaceous extracellular matrix
- GO: 0031012–extracellular matrix
- GO: 0016324–apical plasma membrane
- GO: 0005615–extracellular space
- GO: 0070062–extracellular exosome

(Continued)
Fig. 2 GO and KEGG analysis of the overlap differentially expressed genes in colorectal cancer through DAVID online-tools. Top 20 terms of biological processes (A), cellular components (B), molecular functions (C), and KEGG signaling pathways (D) were shown in the charts, and \( P \)-value <0.05 was considered as selection criteria.
In summary, we chose GSE113513, GSE21510, GSE44076, and GSE32323 GEO datasets and found 865 significant DEGs between CRC tissues and normal tissues. Subsequently, the biological roles of these DEGs were confirmed with enrichment pathway analysis. Furthermore, the four hub genes, HMMR, PAICS, ETFDH, and SCG2 were identified as important prognostic biomarkers for predicting the survival of CRC patients based on the GEPIA database. Finally, blebbistatin and sulconazole were picked out to develop new drugs through CMap database (Fig. 6).

**Discussion**

In our study, we chose four GEO datasets and used bioinformatics methods to get 865 DEGs (374 upregulated and 491 downregulated). KEGG pathway analysis showed that the key modules were mainly metabolic pathways, pathways in cancer, cell cycle, purine metabolism, pancreatic secretion, thyroid hormone signaling pathway and Wnt signaling pathway. The PPI network was constructed including 863 nodes and 5817 edges. The four hub genes, HMMR, PAICS, ETFDH, and SCG2 were remarkably
A B

C

hsa04115: p35 signaling pathway

hsa03030: DNA replication

hsa04914: Progesterone-mediated oocyte maturation

hsa04114: Oocyte meiosis

hsa04110: Cell cycle

hsa04971: Gastric acid secretion

hsa04623: Cytosolic DNA-sensing pathway

hsa04923: Regulation of lipolysis in adipocytes

hsa04621: NOD-like receptor signaling pathway

hsa05134: Legionellosis

hsa04668: TNF signaling pathway

hsa04723: Retrograde endocannabinoid signaling

hsa03020: RNA polymerase

hsa00230: Purine metabolism

hsa05200: Pathways in cancer

hsa04060: Cytokine-cytokine receptor interaction

hsa03008: Ribosome biogenesis in eukaryotes

hsa04062: Chemokine signaling pathway

D

Count

–log10(P-value)

0 0.0005 0.0010 0.0015

0 0.01 0.02 0.03 0.04

Rich factor

(Continued)

28

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related to the prognosis of patients. Furthermore, two small molecules, blebbistatin and sulconazole, also have been identified as potential candidates to develop new drugs.

Recently, findings about DEGs or molecular biomarkers of CRC have been increasingly reported. Based on integrated analysis of GSE32323, GSE74602, and GSE113513 datasets, and TCGA databases, CCL19, CXCL1, CXCL5, CXCL11, CXCL12, GNG4, INSL5, NMU, PYY, and SST were identified as hub genes. And 9 genes including SLC4A4, NFE2L3, GLDN, PCOLCE2, TIMP1, CCL28, SCGB2A1, AXIN2, and MMP1 was related to predicting overall survivals of CRC patients[8]. Moreover, TOP2A, MAD2L1, CCNB1, CHEK1, CDC6, and UBE2C were indicated as hub genes, and TOP2A, MAD2L1, CDC6, and CHEK1 may serve as prognostic biomarkers in CRC[10]. In addition, CEACAM7, SLC4A4, GCG, and CLCA1 genes were associated with unfavorable prognosis in CRC[11].

According to analysis of GEO datasets and survival analysis by GEPIA database, AURKA, CCNB1, CCNF, and EXO1 were significantly associated with longer overall survival. Moreover, CMap predicted that DL-thiorphan, repaglinide, MS-275, and quinostatin have the potential to treat CRC[9]. In this study, we have identified four hub genes as new potential biomarkers to predict the prognosis of CRC patients and two new small molecules. Further studies are needed to develop new drugs to treat CRC.

Several studies have reported that these hub genes play important roles in cancer development. For instance, HMMR expression level was remarkably correlated with the progression and prognosis of breast cancer[13], bladder cancer[14], prostate cancer[15–16], lung cancer[17–19], hepatocellular carcinoma (HCC)[20–22], and gastric cancer[23]. Furthermore, HMMR was confirmed to maintain its oncogenic properties and resistance to chemotherapy through activating TGF-β/Smad-2 signaling pathway[24]. And HMMR was highly expressed in glioblastoma and related to support the self-renewal and tumorigenic potential of glioblastoma stem cells[25]. PAICS was also upregulated in several kinds of cancer tissues and it promotes cancer cells proliferation, migration, and invasion[26–31]. The expression level of ETFDH was found significantly decreased in HCC tissues, and this low expression was related to poor overall survival in patients[32]. However, the role of SCG2 in cancer remains unclear.

In the present study, HMMR, PAICS, ETFDH, and SCG2 were significantly up or down regulated in CRC.

Fig. 4  Module and KEGG analyses of the hub genes. A and C: Two top modules screened from the whole PPI network of DEGs analyzed by MCODE plugin in Cytoscape. E: Top 20 hub genes with a higher degree of connectivity of DEGs were selected with MCODE plugin. B, D, and F: KEGG pathway analysis of the two top modules and top 20 hub genes. DEGs: differentially expressed genes.
Overall survival

Low HMMR TPM
Hign HMMR TPM
Log-rank P=0.019

HR(high)=0.56
Pr(HR)=0.0324

n(high)=135
n(low)=134

Overall survival

Low PAICS TPM
Hign PAICS TPM
Log-rank P=0.0097

HR(high)=0.52
Pr(HR)=0.03

n(high)=135
n(low)=135

Overall survival

Low ETFDH TPM
Hign ETFDH TPM
Log-rank P=0.028

HR(high)=0.638
Pr(HR)=0.006

n(high)=134
n(low)=135

Overall survival

Low SCG2 TPM
Hign SCG2 TPM
Log-rank P=0.005

HR(high)=2
Pr(HR)=0.006

n(high)=135
n(low)=135

(Continued)
Fig. 5  Analysis and validation of hub genes and identification of related active small molecules. A and B: The expression level and prognostic value of four hub genes based on the GEPIA database. C: PAICS and SCG2 were related with the stages of CRC progression through GEPIA database. D: Top 20 potential small active molecules reverse DEG of CRC predicted by CMap database. TPM: transcripts per million.

Fig. 6  Workflow model of the present study.
tissues compared with those in normal samples, and the survival rate of CRC patients was positively correlated with the expression of these genes. Besides, several small molecules with potential therapeutic efficacy were identified through bioinformatics analyses, including blebbistatin and sulconazole. Blebbistatin has been reported to inhibit cell migration and invasiveness of pancreatic adenocarcinoma[31], and decrease spreading and migration of breast cancer cells[33]. Moreover, blebbistatin has shown its antitumorigenic properties in HCC cells[34]. Another small molecule, sulconazole, also inhibited the proliferation and formation of breast cancer stem cells through blocking the NF-κB/IL-8 signaling pathway[35]. Although these two molecules have significant antitumor activity, their specific roles in CRC development need to be further clarified.

Acknowledgments
This work was supported by grants from the National Natural Science Foundation of China (No. 81672748 and No. 81871936).

| Table 1 Gene summaries of the four hub genes | Gene | Summary |
|---|---|---|
| | | Microarray datasets [P-value, log2(fold change)] |
| | | GSE113513 | GSE21510 | GSE44076 | GSE32323 |
| HMMR | The protein encoded by this gene is involved in cell motility. It is expressed in breast tissue and together with other proteins, it forms a complex with BRCA1 and BRCA2, thus is potentially associated with higher risk of breast cancer. Alternatively spliced transcript variants encoding different isoforms have been noted for this gene. | 6.82e−04, 1.11 | 3.84e−35, 3.22 | 5.32e−22, 1.20 | 2.63e−04, 1.63 |
| PAICS | This gene encodes a bifunctional enzyme containing phosphoribosylaminomimidazole carboxylase activity in its N-terminal region and phosphoribosylaminomimidazole succinocarboxamide synthetase in its C-terminal region. It catalyzes steps 6 and 7 of purine biosynthesis. The gene is closely linked and divergently transcribed with a locus that encodes an enzyme in the same pathway, and transcription of the two genes is coordinately regulated. The human genome contains several pseudogenes of this gene. Multiple transcript variants encoding different isoforms have been found for this gene. | 4.77e−08, 1.31 | 5.71e−47, 2.48 | 2.14e−55, 1.49 | 1.38e−06, 1.73 |
| ETFDH | This gene encodes a component of the electron-transfer system in mitochondria and is essential for electron transfer from a number of mitochondrial flavin-containing dehydrogenases to the main respiratory chain. Mutations in this gene are associated with glutaric acidemia. Alternatively spliced transcript variants that encode distinct isoforms have been observed. | 1.78e−08, −1.47 | 1.67e−27, −1.92 | 1.80e−71, −1.85 | 3.14e−08, −1.78 |
| SCG2 | The protein encoded by this gene is a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins. Studies in rodents suggest that the full-length protein, secretogranin II, is involved in the packaging or sorting of peptide hormones and neuropeptides into secretory vesicles. The full-length protein is cleaved to produce the active peptide secretoneurin, which exerts chemotaxic effects on specific cell types, and EM66, whose function is unknown. | 2.37e−07, −2.50 | 1.13e−17, −1.86 | 4.83e−25, −1.90 | 2.91e−07, −2.28 |
| CMap name            | Count | Enrichment | P-value | CMap name            | Count | Enrichment | P-value |
|----------------------|-------|------------|---------|----------------------|-------|------------|---------|
| DL-thiorphan         | 2     | -0.976     | 0.00127 | 8-azaguaine          | 4     | -0.644     | 0.03811 |
| Quinostatin          | 2     | -0.878     | 0.02990 | Acepromazine          | 4     | -0.640     | 0.04008 |
| 1,4-chrysenequinone  | 2     | -0.866     | 0.03575 | Doxazosin            | 4     | -0.639     | 0.04048 |
| Menadione            | 2     | -0.852     | 0.04324 | Mefloquine           | 5     | -0.637     | 0.01628 |
| Blebbistatin         | 2     | -0.844     | 0.04859 | Trifluridine         | 4     | -0.637     | 0.04187 |
| trazodone            | 3     | -0.813     | 0.01320 | Clomipramine        | 4     | -0.636     | 0.04251 |
| Thioguanosine        | 4     | -0.809     | 0.00259 | Trioxysalen          | 4     | -0.633     | 0.04424 |
| Sulconazole          | 4     | -0.805     | 0.00282 | Corbdrine            | 4     | -0.629     | 0.04673 |
| 0297417-0002B        | 3     | -0.792     | 0.01827 | Etofenamate          | 4     | -0.624     | 0.04927 |
| Etacrynic acid       | 3     | -0.776     | 0.02299 | Phthalylosulfathiazole| 5     | -0.616     | 0.02271 |
| Latanoxef            | 3     | -0.759     | 0.02876 | Oxetacaine           | 5     | -0.610     | 0.02523 |
| GW-8510              | 4     | -0.757     | 0.00712 | Amiodarone           | 5     | -0.601     | 0.02900 |
| Triflusal            | 3     | -0.755     | 0.03011 | Meticranec           | 5     | -0.599     | 0.02962 |
| Piperidolate          | 3     | -0.743     | 0.03463 | Vorinostat           | 12    | -0.596     | 0.00018 |
| Daunorubicin         | 4     | -0.733     | 0.01019 | Tranleypromine       | 5     | -0.592     | 0.03264 |
| Repaglinide          | 4     | -0.727     | 0.01142 | Bromocriptine        | 5     | -0.573     | 0.04246 |
| Camptothecin         | 3     | -0.725     | 0.04285 | Zimeldine            | 5     | -0.572     | 0.04338 |
| Norcyclobenzaprine   | 4     | -0.723     | 0.01202 | Clemizole            | 5     | -0.568     | 0.04548 |
| Ronidazole           | 3     | -0.711     | 0.04919 | Astemizole           | 5     | -0.567     | 0.04614 |
| Ellipticine          | 4     | -0.708     | 0.01514 | Meclozole            | 5     | -0.565     | 0.04708 |
| Gliclazide           | 4     | -0.707     | 0.01528 | Procaine             | 5     | -0.565     | 0.04746 |
| Phenoxybenzamine     | 4     | -0.704     | 0.01576 | Clopenthixazone      | 6     | -0.551     | 0.03156 |
| 0175029-00000        | 6     | -0.701     | 0.00163 | Famprofazone         | 6     | -0.543     | 0.03621 |
| Tyloxapol            | 4     | -0.694     | 0.01852 | Dipyridamolol        | 6     | -0.537     | 0.03963 |
| Skimmianine          | 4     | -0.690     | 0.02001 | Promazine            | 6     | -0.523     | 0.04785 |
| Bisacodyl            | 4     | -0.690     | 0.02002 | Trifluoperazine      | 16    | -0.504     | 0.00020 |
| Resveratrol          | 9     | -0.684     | 0.00004 | Prochloperazine      | 16    | -0.434     | 0.00299 |
| Medrysone            | 6     | -0.680     | 0.00292 | LY-294002            | 61    | -0.428     | 0.00000 |
| Bepridil             | 4     | -0.674     | 0.02522 | Trichostatin A       | 182   | -0.425     | 0.00000 |
| Pyrvinium            | 6     | -0.672     | 0.00334 | Fluphenazine         | 18    | -0.406     | 0.00380 |
| Nortriptyline        | 4     | -0.669     | 0.02707 | Thiiodizine          | 20    | -0.388     | 0.00327 |
| Apigenin             | 4     | -0.667     | 0.02765 | Alpha-estradiol      | 16    | -0.357     | 0.02535 |
| Methylergometrine    | 4     | -0.664     | 0.02871 | Geldanamycin         | 15    | -0.349     | 0.03926 |
| Bufexamac            | 4     | -0.660     | 0.03010 | Sirolimus            | 44    | -0.335     | 0.00008 |
| Prestwick-1084       | 4     | -0.656     | 0.03241 | Chlorpromazine       | 19    | -0.329     | 0.02417 |
| Deptropine           | 4     | -0.651     | 0.03495 | Wortmannin           | 18    | -0.314     | 0.04691 |
| Dextromethorphan     | 4     | -0.647     | 0.03686 | Tanespimycin         | 62    | -0.311     | 0.00000 |
| Protriptyline        | 4     | -0.646     | 0.03736 | Fulvestrant          | 40    | -0.225     | 0.02999 |
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