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Original Article

Identification of potential biomarker genes in ulcerative colitis-associated colorectal cancer using a bioinformatics approach

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Abstract: Background: Ulcerative colitis-associated colorectal cancer (UC-CRC) is the main cause of death in UC patients. It lacks specific non-invasive molecular markers for early diagnosis and treatment, so we explored these in the present study using bioinformatics analysis of genomic data. Methods: Gene expression datasets (GSE3629 and GSE37283) were obtained from the GEO database. Differentially expressed genes (DEGs) between UC-CRC and UC samples were identified and analyzed using the GEO2R online analysis tool. GO and KEGG enrichment analysis were performed. The STRING database and Cytoscape software were used for protein-protein interactions visualization. The CytoHubba plugin was employed to calculate the degree of each protein node and identify 'hub' genes. Finally, we selected colon and rectum adenocarcinoma of the TCGA dataset to perform expression and survival analysis for each 'hub' gene using UALCAN. Results: We identified a total of 163 up-regulated DEGs and 266 down-regulated DEGs in UC-CRC, among which 20 'hub' genes with a higher degree of connectivity were selected. The low expression of *CDH1* and *B3GNT7* hub genes was associated with a poor prognosis of UC-CRC. Conclusion: Our findings indicate that CDH1 and B3GNT7 may be novel biomarkers for the early diagnosis and prognosis of UC-CRC.

Keywords: Colitis-associated cancer, hub genes, ulcerative colitis, expression profiling data, bioinformatics

Introduction

Ulcerative colitis-associated colorectal cancer (UC-CRC) is one of the major complications in UC patients, accounting for 1-2% of cases of colorectal cancer, and is an important cause of death in these patients [1]. Studies have shown that patients with UC at 10, 20, and 30 years are 1.15%, 3.56%, and 14.36% at risk of developing UC-CRC [2]. Compared with sporadic colorectal cancer, UC-CRC has an earlier age of onset and a worse prognosis [3, 4].

The mechanism of UC-CRC has not been fully defined and specific and effective treatment methods are currently lacking. Current monitoring and treatment of UC-CRC and its precancerous lesions rely largely on endoscopic endoscopy and pathological biopsy [3, 5]. Unfortunately, endoscopic screening is associated with misdiagnosis, the risks of an invasive procedure, poor patient compliance, and a financial burden, which affects the early diagnosis and treatment of UC-CRC [3]. However, there is currently no specific, sensitive, non-invasive biomarker for the early diagnosis and prognosis of UC-CRC [6].

In this study, we tested new UC-CRC prognostic indicators and UC-CRC susceptibility genes to identify UC-CRC treatment targets and predict molecular marker genes involved in the progression of UC to CRC. To detect genes that were differentially expressed (DEGs) between UC-CRC and UC tissue, a bioinformatics approach was adopted to evaluate gene expression profiling data retrieved from the Gene Expression Omnibus (GEO). Obtained DEGs then underwent functional clustering, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and Gene Ontology (GO) functional annotation analysis. A protein-protein interaction (PPI) network was constructed to select 'hub' genes associ-

Table 1. Two groups of the two microarray databases derived from the GEO database

Dataset ID	Number of UC-CRC cases	Number of UC-nonCa cases	Total number of cases
GSE3629	6	43	49
GSE37283	11	4	15

UC-CRC: ulcerative colitis-associated colorectal cancer. UC-nonCa: ulcerative colitis-non cancer.

ated with UC-CRC, and their expression and survival analyses were carried out using the online survival analysis software UALCAN.

Materials and methods

High throughput data

Gene expression datasets were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). A total of 2679 series on UC were extracted from the database. Search term: ulcerative colitis. After careful scrutiny, two gene expression profiles (GSE3629 and GSE37283) were selected [7, 8]. GSE3629 was based on the GPL570 platform (HG-U133_Plus_2, Affymetrix Human Genome U133 Plus 2.0 Array), and GSE37283 was based on platform GPL13158 (HT_HG-U133_Plus_PM, Affymetrix HT HG-U133 + PM Array Plate). All data are freely available online. This study did not involve human or animal experiments and therefore had no ethical issues.

Screening for DEGs

DEGs between UC-CRC and UC samples in each chip were identified and analyzed using the GEO2R online analysis tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/), and the adjusted *P*-value (Benjamini-Hochberg method) and |logFC|, as well as the absolute value of the logarithm of fold change, were calculated. The cutoff criterion of DEG was adjusted to $P<0.05$ and $|logFC| \geq 1.0$. Volcano plots of DEGs were drawn using SangerBox software (http://sangerbox.com/). Datasets were statistically processed and intersecting DEGs were identified using an online Venn diagram tool (http://bioinformatics.psb.ugent.be/webtools/Venn/).

Functional clustering

GO enrichment analysis, mainly including the three functional annotations of biological process (BP), molecular function (MF), and cellular component (CC), is one of the most important methods to quickly understand the functional tendency of target genes in biological information analysis. The KEGG database integrates genomic, chemical, and system function information. This study used the Database for Annotation, Visualization and Integrated Discovery tools (v.6.8) for GO and KEGG analysis (https://david.ncifcrf.gov/) [9, 10], with $P<0.01$ and gene counts ≥5 regarded as statistically significant.

Interacting network

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING v.11.0, https://string-db.org/) was used to identify protein-protein interactions (PPI). Previously obtained DEGs were imported into the STRING database, selecting those with a combined score > 0.4. Next, Cytoscape software (v.3.6.0) was used for PPI visualization [11]. Finally, the CytoHubba plugin in Cytoscape was employed to calculate the degree of each protein node and identify 'hub' genes [12]. The top 20 genes were defined as 'hub' genes.

Clinical significance

UALCAN is a comprehensive and interactive web resource for analyzing cancer OMICS data (http://ualcan.path.uab.edu/index.html) [13]. This online tool provides graphs depicting gene expression or patient survival information. We selected UC-CRC subtypes, colon adenocarcinoma and rectum adenocarcinoma of the TCGA dataset to perform expression and survival analysis for each 'hub' gene using UALCAN. $P<0.05$ represented a significant difference.

Results

DEGs

We identified two gene expression profiles (GSE3629 and GSE37283). GSE3629 contained six UC-CRC and 43 UC-nonCa samples, while GSE37283 included 11 UC-CRC and four UC-nonCa specimens (**Table 1**). According to $P<0.05$ and $|logFC| \geq 1$, 18,870 DEGs, including 10,202 up-regulated genes and 8,668 down-regulated genes, were found in

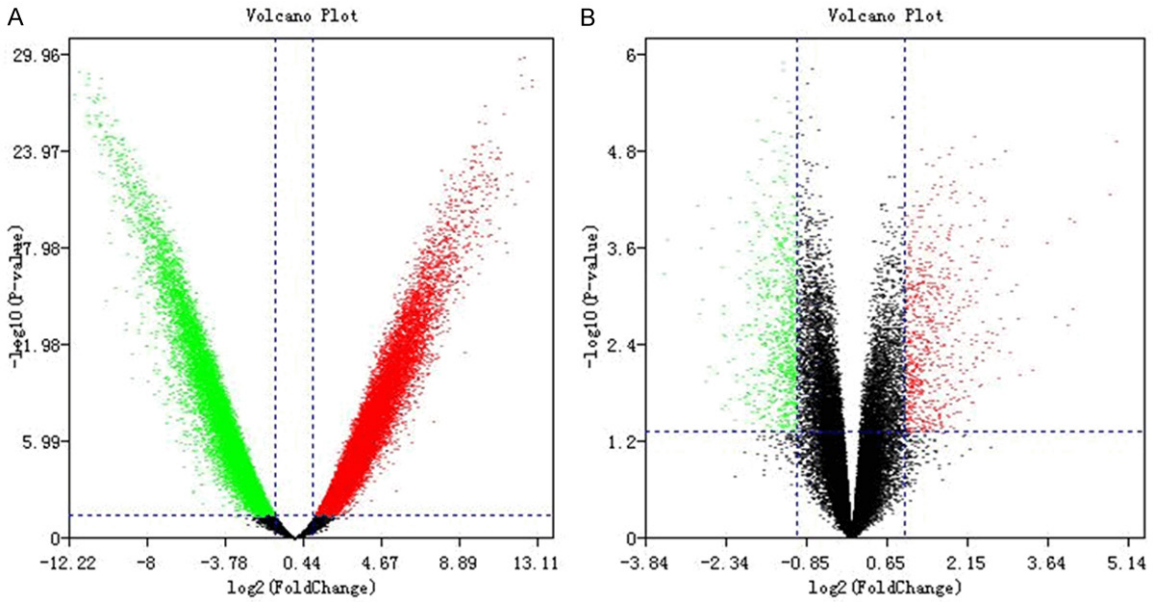

Figure 1. A. Volcano plot of GSE3629. B. Volcano plot of GSE37283. The cutoff *P* value was 0.05, and logFC was -1/1. Red: up-regulated genes, green: down-regulated genes, black: non-statistically significant genes.

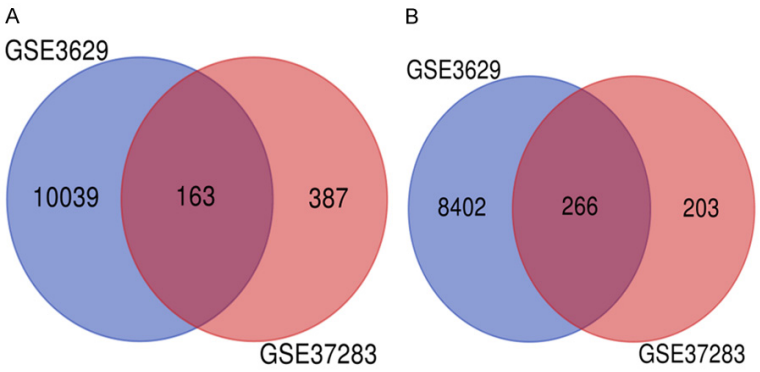

Figure 2. Venn diagrams of differentially expressed genes. A. Up-regulated genes. B. Down-regulated genes.

GSE3629. A total of 1,019 DEGs were identified in GSE37283, of which 550 were up-regulated and 469 were down-regulated. **Figure 1** shows volcano plots of the data. The intersection of the DEG profiles is shown in **Figure 2**; 429 DEGs were obtained, of which 163 were up-regulated and 266 were down-regulated.

GO and KEGG enrichment analysis

Table 2 shows the findings of GO and KEGG enrichment analyses. BP and MF enrichment analysis showed no statistical significance, and DEGs were only found to be enriched in CC, including extracellular space, lateral plasma membrane, and extracellular exosome. Subsequent KEGG pathway enrichment analysis indicated that DEGs were enriched in pathways associated with proximal tubule bicarbonate reclamation and choline metabolism in cancer and melanoma.

PPI network and 'hub' genes

Using the STRING tool, we found 56 nodes and 174 edges in the PPI network, as shown in **Figure 3A**. The top 20 'hub' genes for the connectivity degree are shown in **Table 3**. Eight of these were up-regulated and 12 were down-regulated in UC-CRC. The 'hub' gene interaction network is shown in **Figure 3B**.

Hub genes expression and survival analysis

To explore the prognostic values of the 20 potential 'hub' genes, UALCAN analysis was performed on TCGA samples. We found that low expression of the cadherin 1 'hub' gene (*CDH1*) was associated with the unfavorable overall survival of rectum adenocarcinoma (*READ*) patients (*P*=0.031, **Figure 4A**). How-

Table 2. Significantly enriched GO terms and KEGG pathways of differentially expressed genes

Category	Term	Count	Percentage	*P*-Value
GOTERM_CC_DIRECT	GO:0005615: extracellular space	58	13.679	7.74E-07
GOTERM_CC_DIRECT	GO:0016328: lateral plasma membrane	10	2.358	1.80E-06
GOTERM_CC_DIRECT	GO:0070062: extracellular exosome	96	22.642	3.63E-06
KEGG_PATHWAY	hsa04964: Proximal tubule bicarbonate reclamation	5	1.179	0.002
KEGG_PATHWAY	hsa05231: Choline metabolism in cancer	9	2.123	0.003
KEGG_PATHWAY	hsa05218: Melanoma	7	1.651	0.007

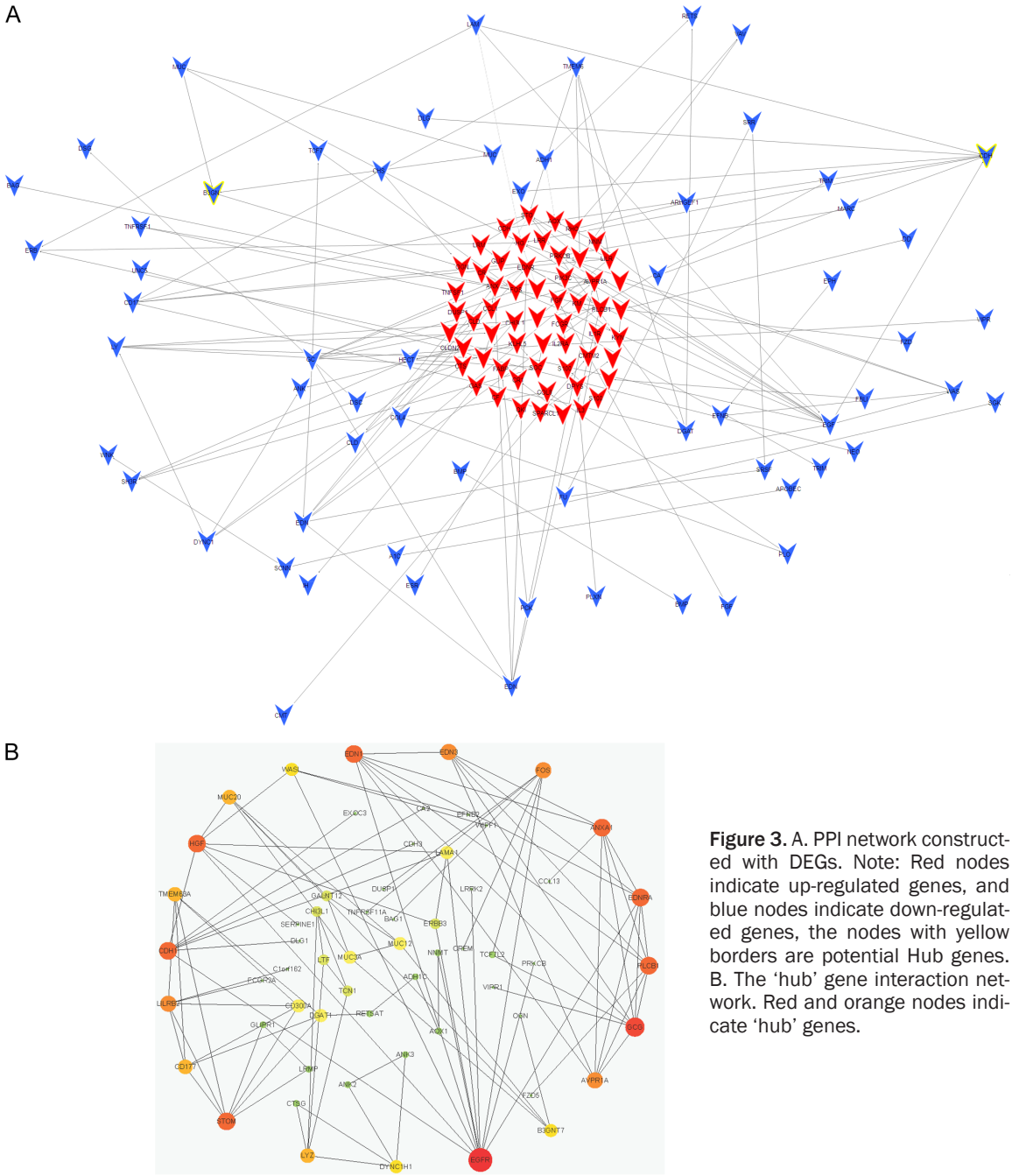

Figure 3. A. PPI network constructed with DEGs. Note: Red nodes indicate up-regulated genes, and blue nodes indicate down-regulated genes, the nodes with yellow borders are potential Hub genes. B. The 'hub' gene interaction network. Red and orange nodes indicate 'hub' genes.

Table 3. Top twenty hub genes with higher degrees

Gene symbol	Degree	Up- or down-regulation	Description of gene symbol
EGFR	11	down	epidermal growth factor receptor
GCG	8	down	glucagon
ANXA1	7	up	annexin A1
STOM	7	up	stomatin
EDN1	7	down	endothelin 1
EDNRA	7	up	endothelin receptor type A
CDH1	7	down	cadherin 1
HGF	7	up	hepatocyte growth factor
PLCB1	7	up	phospholipase C beta 1
EDN3	6	down	endothelin 3
FOS	6	up	Fos proto-oncogene
AVPR1A	6	up	arginine vasopressin receptor 1A
LILRB2	6	up	leukocyte immunoglobulin like receptor B2
TMEM63A	5	down	transmembrane protein 63A
CD177	5	down	CD177 molecule
MUC20	5	down	mucin 20, cell surface associated
LYZ	5	down	lysozyme
DYNC1H1	4	down	dynein cytoplasmic 1 heavy chain 1
B3GNT7	4	down	UDP-GlcNAc: betaGal beta-1, 3-N-acetylglucosaminyltransferase 7
WASL	4	down	WASP like actin nucleation promoting factor

ever, the expression of *CDH1* had no statistical significance in READ (P=8.031E-01). Additionally, low expression of B3GNT7 was an unfavorable prognostic factor of survival in colon adenocarcinoma (COAD) patients (P= 0.013, **Figure 4B**), and the expression of *B3GNT7* had statistical significance in COAD and READ patients (P<0.05, **Figure 4C** and **4D**). Moreover, promoter methylation levels of *B3GNT7* were significantly increased in COAD and READ patients (P<0.05, **Figure 4E** and **4F**).

Discussion

We found that 163 up-regulated and 266 down-regulated DEGs were associated with GO CC terms such as extracellular space, lateral plasma membrane, and extracellular exosome, and were significantly enriched in the KEGG terms proximal tubule bicarbonate reclamation and choline metabolism in cancer and melanoma. Our interaction network and clinical significance analyses found that low expression of *CDH1* and *B3GNT7* 'hub' genes was an unfavorable prognostic factor in UC-CRC patients.

Different subtypes of CRC have diverse markers that correlate with patient survival time [14, 15]. The pathogenesis of UC-CRC differs from that of sporadic CRC [6, 16]. Long-term chronic inflammatory stimulation appears to be the main factor of UC oncogenesis, and has a clear genetic component [17]. However, because of the time and spatial heterogeneity of tumors, it remains unclear which UC-CRC genetic mutations and epigenetic factors are associated with chronic inflammation.

CDH1 is located on human chromosome 16q22.1 and encodes E-cadherin, which is involved in regulating cell adhesion, migration, and epithelial cell proliferation. *CDH1* mutations are associated with gastric, breast, colorectal, thyroid, and ovarian cancers. Moreover, *CDH1* deficiency is closely related to poor prognosis, metastasis, and tumor progression in a variety of human tumors [18-23]. A meta-analysis suggested that *CDH1* promoter methylation plays an important role in colorectal carcinogenesis [24]. However, one study found that the *CDH1*-160C > A polymorphism does not contribute to the genetic susceptibility of CRC and may not directly affect progression of the disease in Turkish patients [25]. Another study documented hypermethylation of the *CDH1* promoter region in 46% of colorectal cancers, but found no difference in

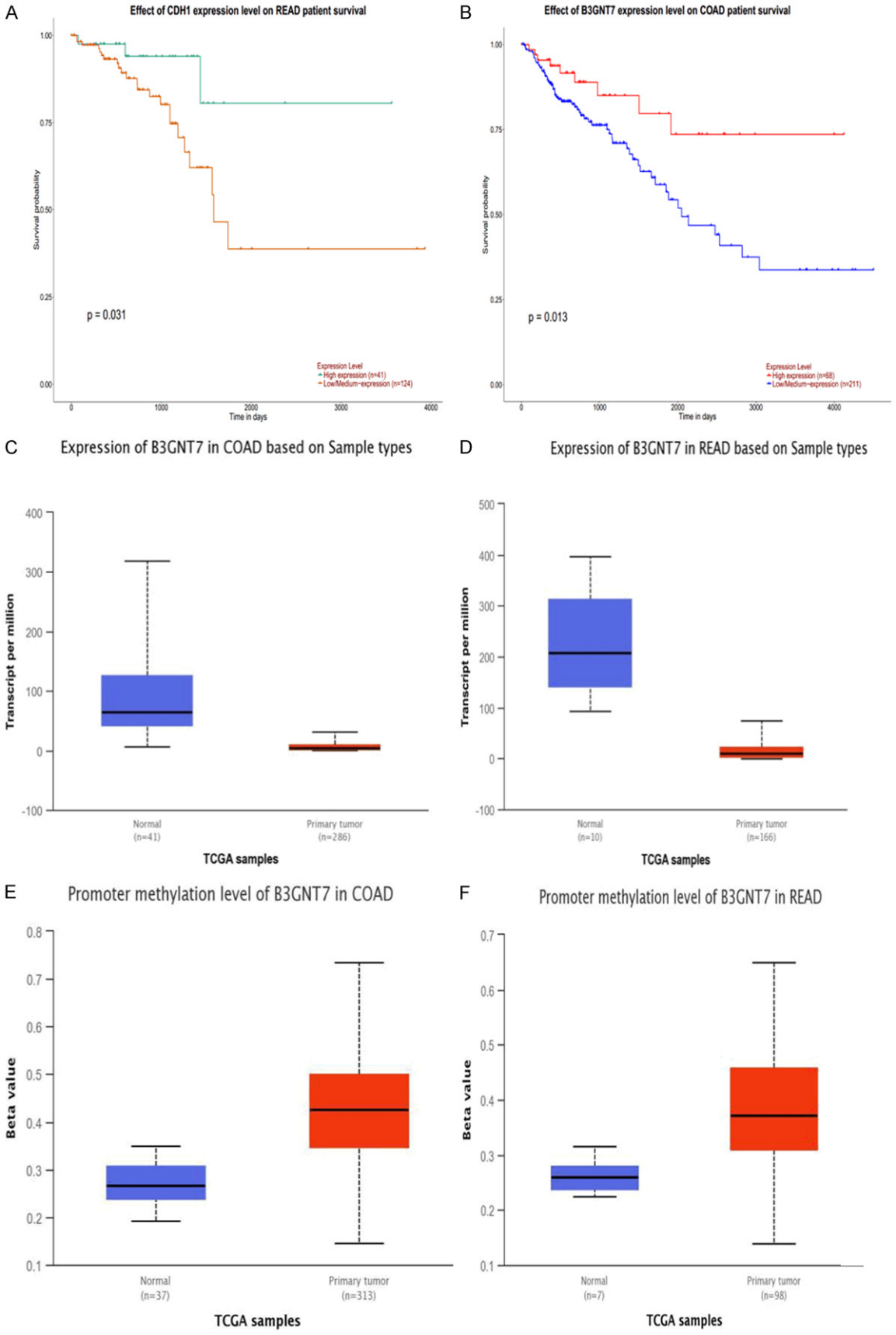
A
Effect of CDH1 expression level on READ patient survival
Survival probability
p = 0.031
Expression Level
High expression (n=41)
Low/Medium-expression (n=124)
Time in days
B
Effect of B3GNT7 expression level on COAD patient survival
Survival probability
p = 0.013
Expression Level
High expression (n=68)
Low/Medium-expression (n=211)
Time in days
C
Expression of B3GNT7 in COAD based on Sample types
Transcript per million
Normal (n=41)
Primary tumor (n=286)
TCGA samples
D
Expression of B3GNT7 in READ based on Sample types
Transcript per million
Normal (n=10)
Primary tumor (n=166)
TCGA samples
E
Promoter methylation level of B3GNT7 in COAD
Beta value
Normal (n=37)
Primary tumor (n=313)
TCGA samples
F
Promoter methylation level of B3GNT7 in READ
Beta value
Normal (n=7)
Primary tumor (n=98)
TCGA samples

Figure 4. A. Survival analysis for the *CDH1* 'hub' gene expressed in rectum adenocarcinoma patients (high expression vs low/medium expression, $P<0.05$). B. Survival analysis for the *B3GNT7* 'hub' gene expressed in colon adenocarcinoma patients (high expression vs low/medium expression, $P<0.05$). C. Expression of B3GNT7 in colon adenocarcinoma patients (colon adenocarcinoma group vs normal group, $P<0.05$). D. Expression of *B3GNT7* in rectum adenocarcinoma patients (rectum adenocarcinoma group vs normal group, $P<0.05$). E. Promoter methylation level of *B3GNT7* in colon adenocarcinoma patients (colon adenocarcinoma group vs normal group, $P<0.05$). F. Promoter methylation level of *B3GNT7* in rectum adenocarcinoma patients (rectum adenocarcinoma group vs normal group, $P<0.05$).

expression between UC-CRC and sporadic CRC groups [26]. In our study, *CDH1* was expressed at lower levels in UC-CRC compared with UC tissue, and correlated with the unfavorable survival of rectal cancer patients. However, we found no significant difference in colon cancer survival analysis.

As a down-regulated 'hub' gene in GEO, *CDH1* was verified in the TCGA database. Its expression was shown to be reduced in CRC, but the difference was not significant. This may be associated with the different disease subtypes included in different databases. Although Saito et al reported that active inflammation was an independent factor of methylation for *CDH1* and *GDNF* in UC, further studies are needed to expand the role of *CDH1* in UC-CRC and UC-related dysplasia, and the effects of non-coding RNA and exosomes on *CDH1* expression [27].

B3GNT7 was first reported by Kataoka and Huh in 2002 and shown to be involved in tissue invasion [28]. In humans, *B3GNT7* is expressed in the brain, thymus, esophagus, pancreas, intestine, connective tissue, lung, muscle, ovary, spleen, testis, trachea, and vascular system. It appears to affect the adhesiveness and motility of certain cancer cells [28-31], while the expression of *B3GNT7* was markedly down-regulated during colon cancer tumorigenesis [32]. We found that *B3GNT7* was down-regulated in UC-CRC, which is meaningful for colon cancer survival analysis, but not in rectal cancer. Additionally, we showed that *B3GNT7* expression was decreased in both colon and rectal cancer, and that this difference was statistically significant. *B3GNT7* was also significantly methylated in colorectal adenocarcinoma.

The present study has some limitations, including the failure to analyze selective bias factors caused by UC medication, lesion sites, course, race, and dysplasia. Nevertheless, based on our combined GO and KEGG enrichment analyses, *B3GNT7* may be a prognostic factor and potential therapeutic target for UC-CRC. Insights from our gene function enrichment analysis suggest that it will be necessary to further study the role of *B3GNT7* in the choline metabolism pathway of the intestinal nervous system and exosomes in UC-CRC.

In conclusion, the present study was designed to identify aberrantly DEGs that may be involved in the carcinogenesis of UC. A total of 20 'hub' genes were selected, of which *CDH1* and *B3GNT7* were identified as potential novel biomarkers for the early and accurate diagnosis and prognosis of UC-CRC. Further research should pay close attention to the epigenetic mutations involved in driving tumorigenesis in UC patients.

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Disclosure of conflict of interest

None.

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