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High expression of CDH3 predicts a good prognosis for colon adenocarcinoma patients

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Abstract. Colon adenocarcinoma (COAD) is one of the most common types of malignancy of the digestive system, and a better understanding of the molecular mechanisms will contribute to an improvement in the quality of life for COAD patients. Cadherin 3 (CDH3), a gene encoding P-cadherin, is a major component of adherens junctions and is closely associated with the occurrence and development of a variety of tumor types. However, the current knowledge regarding the role of CDH3 in COAD is limited. The present study aimed to identify the relative mRNA and protein expression levels of CDH3 in COAD tissues, and whether CDH3 had any influence on the survival rate of patients with COAD. Analysis of differentially expressed genes using the UALCAN database revealed that CDH3 was significantly upregulated in COAD tissues, and reverse transcription-quantitative PCR analysis further confirmed that CDH3 was upregulated in 48 COAD tissues compared with that in their paired normal tissues (n=48). Consistent with this, analysis of the Human Protein Atlas database indicated that the expression levels of the CDH3 protein were upregulated in COAD tissues (n=11) compared with those in normal tissues (n=3; P=0.0245). Next, the association between the mRNA levels of CDH3 and the survival rate of the COAD patients was analyzed using the UALCAN database, and the Kaplan-Meier curves revealed that the CDH3 high expression group (n=69) had a better overall survival compared with that of the CDH3 medium/low expression group (n=210; P=0.037). Furthermore, analysis of clinical data of a cohort from our hospital indicated that the median survival time for COAD patients with high (n=20) and low (n=20) CDH3 levels was 55.5 and 43.5 months, respectively, and there was a significant difference in the survival time between the two groups (P=0.0078). The above results verified that CDH3 was significantly upregulated in the COAD tissues and that high expression of CDH3 predicts a good prognosis for COAD patients.

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Introduction

Colorectal cancer (CRC) is one of the most common human cancer types worldwide. According to the 2017 cancer statistics, the incidence rate of CRC ranks third in the US (1). Non-specific clinical symptoms, rapid advancement and metachronous metastasis have become the most common causes of colon adenocarcinoma (COAD)-associated death. A previous report indicated that >1 million novel cases of colon cancer are diagnosed and >0.5 million cancer-associated deaths occur annually (2). Although the definition of CRC patient subsets, drug development, combined application of targeted therapy and immunotherapy, and other newer technologies have allowed for great optimism for the future for patients with CRC (3), patients still have a poor outcome and there is a lack of effective prognostic markers (4). Effective diagnostic and prognostic markers may not only provide useful prognostic information but also help to guide treatment.

COAD is the most common type of CRC. Thus far this year, a large amount of research has been performed on diagnostic and prognostic biomarkers for COAD. For instance, the adenomatous polyposis coli (APC) gene encodes a multifunctional protein which negatively regulates the Wnt signaling pathway and that have various important roles, including intercellular adhesion, cell cycle regulation and apoptosis (5). Various reports have verified that mutations in the APC gene are responsible for COAD (6-8). Furthermore, a high expression of cellular communication network factor 1 was identified in CRC, and its expression was associated with a poor prognosis for CRC patients (9). Recently, an increasing number of microRNAs (miRs) have been reported to hold significant promise for the diagnosis and prognosis of CRC, including miR-25 (10), miR-21 (11), let-7 (12) and miR-126 (13). However, most of the research on diagnostic and prognostic markers for CRC is at the primary experimental exploration stage and has limited value for clinical applications. Thus, there is an enormous demand for additional valuable markers that may help predict the prognosis and guide treatments for CRC.

In the present study, an analysis of data from the UALCAN database was performed, which indicated that cadherin 3 (CDH3) was significantly upregulated in COAD tissues, and COAD patients with a high CDH3 level generally had a good prognosis. The clinical data of the present study also support the above conclusions. Furthermore, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses determined found that CDH3 is mainly involved in regulating cell-cell adhesion, which may affect the metastasis and progression of COAD. To the best of our knowledge, the present study was the first to report on the value of CDH3 in predicting the survival of COAD patients and its potential mechanistic involvement in regulating the malignant phenotype of tumors.

Materials and methods

Sample collection and patient follow-up. A total of 48 paired COAD resection tissues and adjacent tissues were obtained from patients undergoing CRC surgery at HwaMei Hospital, University of the Chinese Academy of Sciences (Ningbo, China) between January 2010 and April 2011, and stored in liquid nitrogen. The cohort of 48 COAD patients comprised 27 males and 21 females with an average age of 38.53±4.84 years (age range, 33-45), and all of them were diagnosed with primary COAD by histopathology, while cases of recurrent and metastatic COAD were excluded. These tissues were used to perform reverse transcription-quantitative (RT-q)PCR analysis. The survival information of 40 of the COAD patients was obtained by telephone follow-up.

UALCAN database analysis. The differentially expressed genes from the COAD and normal tissues were analyzed by using the UALCAN online database (http://ualcan.path.uab.edu/index.html), according to the website's instructions (14). In the UALCAN database, COAD was selected, and the heatmap and differentially expressed genes were downloaded. Next, according to the average expression level of CDH3, the COAD patients from The Cancer Genome Atlas (TCGA; TCGA and UALCAN data were for the same cohort) (14) database were divided into a CDH3 high expression group and a CDH3 medium/low expression group, and the Kaplan-Meier method was used to analyze the overall survival (OS) of the COAD patients.

RT-qPCR analysis. The sequences of 20 genes (CST1, MMP7, KRT23, KRT80, CA9, FOXQ1, CDH3, KIAA1199, CLDN2, LY6G6D, ETV4, KLK10, CLDN1, GRIN2D, NKD1, C6orf223, MMP3, MMP11, TRIM29 and TESC) were acquired from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/). Primers were designed using premier 5.0 software (http://www.premierbiosoft.com/primerdesign/; Table I) and the synthesis was performed by Invitrogen (Thermo Fisher Scientific, Inc.).

Total RNA was isolated from the COAD and normal tissues with TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The total RNA was used for complementary (c)DNA synthesis with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). Next, Power SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to perform qPCR analysis. The thermocycling conditions for PCR were as follows: 95˚C for 10 min; followed by 40 cycles of 95˚C for 10 sec, 60˚C for 20 sec and 72˚C for 10 sec. All experiments were performed according to the manufacturer's protocols and GAPDH was used as a control. The fold change was determined relative to the control after normalizing to a housekeeping gene using the $2^{-\Delta\Delta Cq}$ method (15). All experiments were independently replicated three times.

Analysis of other databases. The expression of CDH3 in colon cancer tissues was assessed using the Human Protein Atlas (HPA) online database (www.proteinatlas.org). In this database, COAD data were selected, 'CDH3' was entered as a search term, the IHC images were viewed and downloaded, and the representative diagram of CDH3 expression levels in COAD and normal tissues was displayed. Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.) was used to calculate the mean integrated optical density (IOD) of these IHC images, and the sum IOD represented the relative expression level of CDH3 in COAD and normal tissues.

GO and KEGG analyses were performed by using the DAVID (https://david.ncifcrf.gov/; version 6.8) and STRING (https://string-db.org/cgi/input.pl; version 11.0) database, respectively.

Statistical methods. SPSS 21.0 software (IBM Corp.) was used for statistical analysis. RT-qPCR data were analyzed by unpaired Student's t-tests, and the IHC data were analyzed using independent-samples t-tests. The Kaplan-Meier method and log-rank test were employed to estimate OS. $P<0.05$ was considered to indicate statistical significance.

Results

CDH3 mRNA is significantly upregulated in COAD tissues. The top 20 significantly upregulated genes according to the results of the UALCAN database analysis are presented in Fig. 1; they were CST1, MMP7, KRT23, KRT80, CA9, FOXQ1, CDH3, KIAA1199, CLDN2, LY6G6D, ETV4, KLK10, CLDN1, GRIN2D, NKD1, C6orf223, MMP3, MMP11, TRIM29 and TESC. To further confirm these differentially expressed mRNAs, the relative levels of these 20 genes were detected by RT-qPCR analysis of tissues from 48 paired COAD tissues. In the present study, MMP7, KRT23, FOXQ1, CDH3, KIAA1199, CLDN2, GRIN2D, NKD1, C6orf223, MMP3, MMP11 and TRIM29 were confirmed to be continuously upregulated in the 48 COAD tissues compared with those in the normal colon tissues (Fig. 2A and B). Among these dysregulated genes, CDH3 was noted to have been examined by only few studies with regard to its expression levels and prognostic value in colon cancer. Hence, CDH3 was further investigated.

CDH3 protein is significantly upregulated in colon cancer tissues. In order to further evaluate the expression levels of CDH3 in colon cancer tissues, an analysis of IHC samples from the HPA web portal database was performed. In the colon cancer tissues and normal tissues, CDH3 was observed to be mainly expressed in the cytoplasm and not in the nucleus. According to the sum IOD value, the expression level of CDH3 in the colon cancer tissues (n=11) was higher than that in the

Table I. Primer sequences.

Gene symbol	Forward (5'-3')	Reverse (5'-3')
CST1	ACTTGGACACCTGTGCCTTC	TCACCAGGGACCTTCTGTTC
MMP7	AAGCCAAACTCAAGGAGATGC	ATGTCAGCAGTTCCCCATACA
KRT23	ACCACATTTGACAGCCCATG	CTTTCATCCCAGCACACCTC
KRT80	TCGGGCATCTCTATGAGGAATA	AAGGTGAACTCCATGTCTGTG
CA9	GAAAACAGTGCCTATGAGCAGTTG	TGCTTAAGCACTCAGCATCAC
FOXQ1	TGACAACTACTGGATGCTCATTCAAGAGATG	TCGAGGAAAAAAGACAACTACTGGATGCTC
	AGCATCCAGTAGTTGTCTTTTTTC	ATCTCTTGAATGAGCATCCAGTAGTTGTCA
CDH3	AAACTTGGGGACAGCAACATCAG	TCTTTTGGTTTGCAGAGACAGGG
KIAA1199	GGCTGTGGCCTATGCAGTCA	TGTGACAAGGTTCCCACTGCTTAC
CLDN2	CTGCCAACACAGTCTCCTCA	GGATTTGTTGCCTAGGGTGA
LY6G6D	ATGAAACCCCAGTTTGTTGGG	CTATCCGCTCCACAGTCCTGG
ETV4	CACCCGTCCTGCCCCTTCACCTT	GGCTTCCCACGTGCGCAGCAGGA
KLK10	CTCTGGCGAAGCTGCTG	ATAGGCTTCGGGGTCCAA
CLDN1	GGCAGATCCAGTGCAAAGTC	TCTTCTGCACCTCATCGTCTT
GRIN2D	CCTTCTTTGCCGTCATCTTTCTTGC	AAACTTCAGGGGTGGGTATTGCTCC
NKD1	TCGCCGGGATAGAAAACTACA	CAGTTCTGACTTCTGGGCCAC
C6orf223	ATCGCTTCGCCTCCTACAACA	GACAGCGGCGGTGGGTAAT
MMP3	CGGTGGCTTCAGTACCTTTC	ACCTCCTCCCAGACCTTCA
MMP11	TGGCTGTACGACGGTGAAAA	CATGGGTCTCTAGCCCTGATA
TRIM29	GACATCATACCAGCCCTCGT	ATCCCGTTGCCTTTGTTGAC
TESC	AATTGTTCGTGCCTTCTTCG	TCCGAGTCGTACATGTGGAA
GAPDH	CTTCACCACCATGGAGAAGGC	GGCATGGACTGTGGTCATGAG

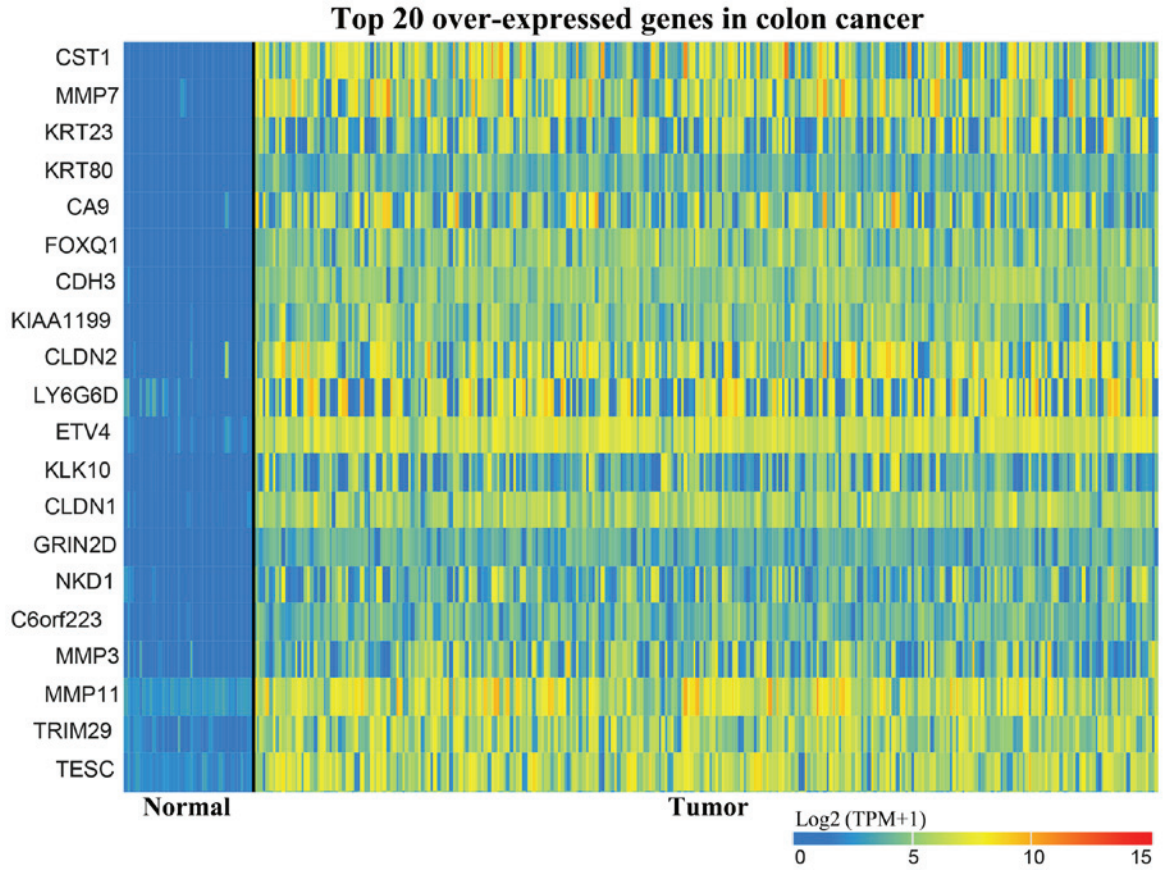

Figure 1. Heatmap of upregulated genes in colon adenocarcinoma and normal tissues from UALCAN database. TPM, transcripts per kilobase of exon model per million mapped reads.

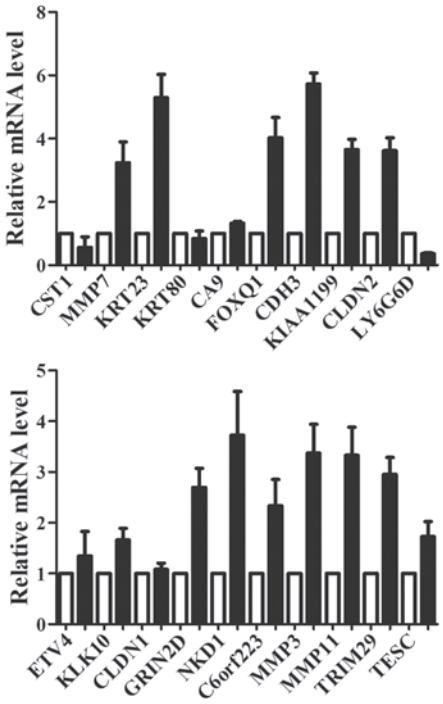

Figure 2. Expression levels of the top 20 significantly differentially expressed genes in 48 paired COAD and normal tissues. The mRNA levels were determined using reverse transcription-quantitative PCR data. The white columns indicate normal tissues, black columns indicate COAD tissues and error bars indicate the standard deviation. All mRNA expression levels of normal tissues were set to 1 and the data reflect the relative mRNA levels (COAD vs. normal tissues). COAD, colon adenocarcinoma.

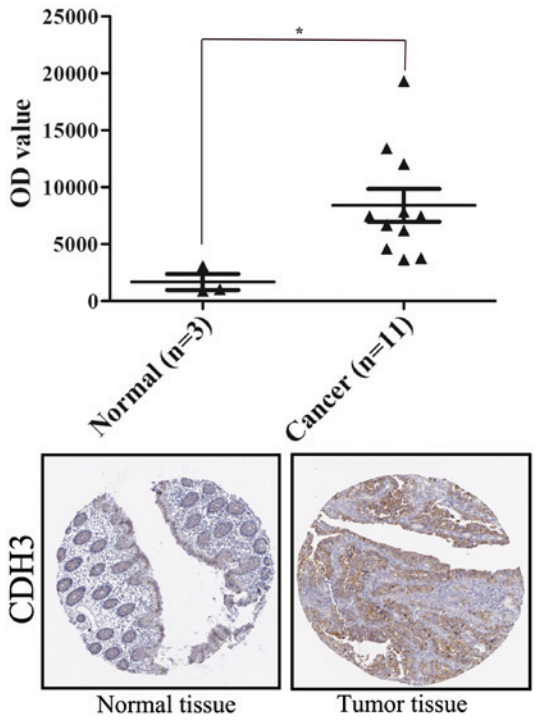

Figure 3. Protein expression levels of CDH3 in COAD and normal tissues from the HPA database (magnification unavailable). Black lines indicate mean expression levels and the triangles represent the IOD value of each sample. Representative IHC images were obtained from the HPA database and the mean IOD value of these IHC images was determined, and there was a statistically significant difference between COAD and normal tissues. *P<0.05. IHC, immunohistochemistry; IOD, integrated optical density; CDH3, cadherin 3; HPA, Human Protein Atlas.

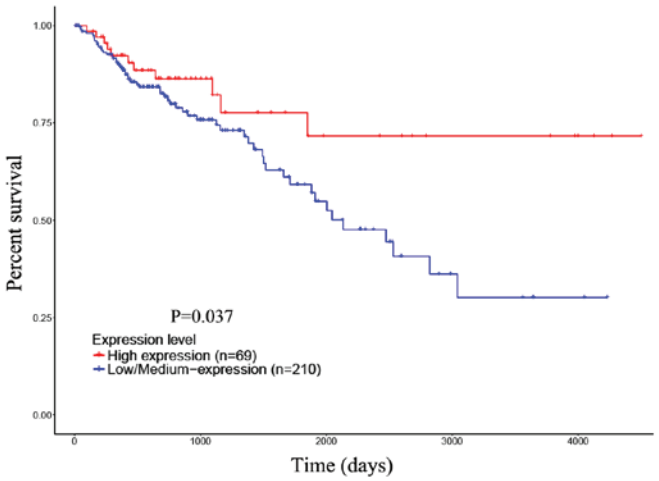

Figure 4. Association between cadherin 3 mRNA levels and survival time of COAD patients from TCGA database. Patients were divided into a CDH3 high expression group and a CDH3 medium/low expression group. TCGA, The Cancer Genome Atlas; COAD, colon adenocarcinoma.

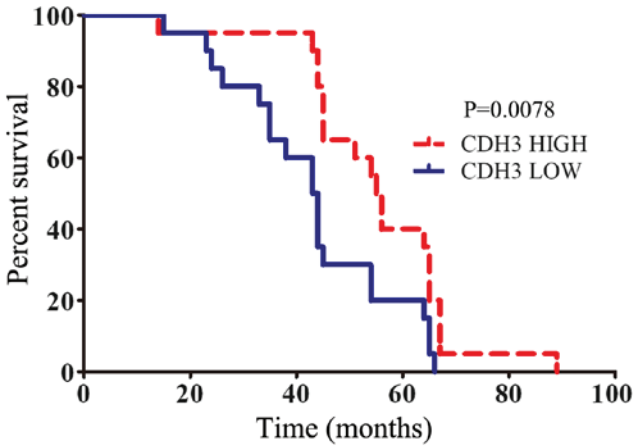

Figure 5. Association between CDH3 mRNA levels and survival of 40 COAD patients. Red lines indicate high expression and blue lines indicate low expression of CDH3 according to median CDH3 expression level. COAD, colon adenocarcinoma; CDH3, cadherin 3.

normal tissues (n=3; P=0.0245). The above results suggested that the protein levels of CDH3 were significantly upregulated in colon cancer tissues (Fig. 3).

High CDH3 expression is associated with a high survival rate for patients with COAD. The association between the expression of CDH3 and other clinical characteristics was analyzed in TCGA database. The results suggested that there was no significant association between the expression level of CDH3 and age, gender, ethnicity, tumor stage and tumor type (adenocarcinoma vs. mucinous-adenocarcinoma; data not shown; P<0.05).

Next, to evaluate the association between upregulated CDH3 and prognosis, a survival analysis of COAD patients with different CDH3 mRNA levels was performed. First, according to the CDH3 mRNA levels, the COAD patients from the TCGA database were divided into a high expression group (n=69) and a medium/low expression group (n=210).

Table II. Gene Ontology analysis of genes interacting with cadherin 3.

A, Category cellular component

Rank	Pathway description	FDR
1	Cell-cell adherens junction	$5.29x10^{-6}$
2	Catenin complex	$5.29x10^{-6}$
3	Plasma membrane	$1.30x10^{-5}$
4	Cell periphery	$1.30x10^{-5}$
5	Apical junction complex	0.000118
6	Adherens junction	0.000364
7	Zonula adherens	0.0015
8	Cell-cell junction	0.00353
9	Focal adhesion	0.00353
10	Membrane part	0.00353
11	Extrinsic component of plasma membrane	0.00405
12	Cell junction	0.0118
13	Intercalated disc	0.0277

B, Category molecular function

Rank	Pathway description	FDR
1	Calcium ion binding	4.49E-07
2	Cadherin binding	4.49E-07
3	Cell adhesion molecule binding	5.45E-06
4	Beta-catenin binding	0.00275
5	Gamma-catenin binding	0.0119

C, Category biological process

Rank	Pathway description	FDR
1	Adherens junction organization	1.70E-20
2	Cell junction assembly	1.22E-14
3	Cell-cell junction organization	1.22E-14
4	Cell-cell adhesion	1.22E-14
5	Homophilic cell adhesion via plasma membrane adhesion molecules	1.47E-12
6	Cellular response to indole-3-methanol	1.77E-06
7	Cellular component assembly	0.000299
8	Vascular endothelial growth factor receptor signaling pathway	0.000307
9	Cellular component biogenesis	0.000496
10	Single organismal cell-cell adhesion	0.00137
11	Cell adhesion	0.00227
12	Epithelial cell-cell adhesion	0.0157
13	Cellular component organization	0.0181
14	Regulation of cell proliferation	0.019

FDR, false discovery rate.

Table III. Kyoto Encyclopedia of Genes and Genomes analysis of genes interacting with cadherin 3.

Rank	Pathway description	FDR
1	Adherens junction	0.00178
2	Leukocyte transendothelial migration	0.00376
3	Cell adhesion molecules	0.00436
4	Endometrial cancer	0.0228
5	Bacterial invasion of epithelial cells	0.0277
6	Pathways in cancer	0.0277
7	Arrhythmogenic right ventricular cardiomyopathy	0.0277

FDR, false discovery rate.

The prognostic implication of CDH3 was investigated with the UALCAN online database. The Kaplan-Meier curves indicated that the COAD patients with a high CDH3 level had a better OS compared with that of the CDH3 medium/low expression group (P=0.037; Fig. 4). Next, follow-up was performed for 48 COAD patients who received surgery at our hospital and the survival information of 40 patients was obtained. According to the median CDH3 expression level, the 40 colon cancer patients were divided into a high expression group (n=20) and a low expression group (n=20), and Kaplan-Meier analysis was again performed. The results indicated that the median survival time for the COAD patients with a high or low CDH3 level was 55.50 or 43.50 months, respectively. There was an obvious difference in the survival rate between the two groups (P=0.0078; Fig. 5). Based on the above results, it was confirmed that a high CDH3 level indicates a better prognosis for COAD patients.

CDH3 is associated with cell-cell adherens junction. To explore the potential function of CDH3 in the occurrence and development of tumors, GO and KEGG analyses were performed. As presented in Table II, the GO terms in the biological process category for genes interacting with CDH3 mainly included adherens junction organization and cell-cell adhesion. The GO terms in the category molecular function mainly included calcium ion binding, cadherin binding and cell adhesion molecular binding, while the terms in the category cellular component mainly included cell-cell adherens junction and catenin complexing. As presented in Table III, the KEGG analysis results confirmed that CDH3 is mainly involved in adherens junctions, leukocyte transendothelial migration, cell adhesion molecules and pathways involved in cancer.

The above comprehensive results confirmed that CDH3 is closely associated with cell-cell adherens junctions. Hence, CDH3 may be involved in the progression and metastasis of colon cancer by regulating cell adhesion.

Discussion

The present study first examined the expression level and prognostic value of CDH3 in COAD with the TCGA database. It was revealed that CDH3 is a significantly upregulated gene

in COAD tissues compared with normal tissues. Furthermore, COAD patients with a high CDH3 level in their tumor tissues usually had a longer survival time compared with that of patients with medium/low levels. Based on the results from the database analysis, the relative expression level of CDH3 was further verified in 48 COAD tissues and the association between CDH3 and survival time was assessed 4 in 0 COAD patients. Of note, the results were consistent with those of the database analysis. The GO and KEGG analyses further indicated that CDH3 is mainly involved in regulating cell-cell adhesion, which may be one of the reasons for affecting the prognosis of COAD patients.

With the development of proteomics and genomics, high-throughput technology has been applied to explore biomarkers associated with diagnosis and prognosis. In colon cancer, an increasing number of prognostic markers have been identified, and these biomarkers have contributed to the determination of the prognosis and provide a theoretical basis for individualized treatment (8). These biomarkers are contained in various body fluids and include multiple proteins, mRNA, non-coding RNA and exosomes. For instance, a high level of SOX9 has recently been reported to be associated with a good prognosis for stage II CRC (16). Furthermore, in colon cancer tissues, various lncRNAs were downregulated in COAD tissues and contributed to a poor prognosis for colon cancer; they were CASC2, CTD903, GASS, MEG3, RPI-13P20.6 and TUSC7 (4). Exosomal CD151 expression has been investigated in CRC tissues and elevated expression was observed in patients with advanced disease; higher levels of expression were retrospectively identified to be associated with a poorer prognosis (17). However, only few studies have reported on the expression of CDH3 in colon cancer and its association with prognosis. A previous study confirmed that CDH3 expression was elevated in COAD tissues compared with that in normal tissues (18). However, the association between the expression levels of CDH3 and patient prognosis was not previously described. To the best of our knowledge, the present study revealed that CDH3 was significantly upregulated in COAD tissues and predicted a good prognosis for COAD patients.

An increasing number of studies have confirmed that CDH3 was more frequently demethylated in advanced colorectal carcinomas (19), and this phenomenon was also observed in advanced gastric carcinomas (20). Hence, CDH3 is a potential diagnostic and prognostic marker for various tumor types. The mRNA expression levels of CDH3 were reported to be able to distinguish between malignant and benign biliary strictures in brush cytology specimens (21). However, CDH3 expression was closely associated with a clinicopathological features and poor prognosis in gallbladder cancer (22). However, the potential mechanism by which CDH3 affects the prognosis of COAD patients remains elusive.

In the present study, it was revealed that CDH3 is highly expressed in colon cancers, but patients with a higher CDH3 expression usually had a better prognosis. As an explanation for this contradiction, it is possible that high CDH3 expression may have a protective function in colon cancer. A previous, similar study also reported that high expression of Beclin-1 was associated with a better OS and disease-free survival in CRC, which is possible due to the promotion of the formation of the autophagic vesicle (23). A previous study confirmed that the mRNA levels of several genes encoding adherens junction proteins were dysregulated in 26 CRC, 42 adenoma and 24 normal mucosa samples. Among these genes, CDH3 was significantly upregulated in CRC tissues (24). In the present study, the GO and KEGG analyses indicated that CDH3 was closely associated with a cell adhesion function, which may be the major reason for its influence on the prognosis of COAD patients. However, the direct empirical evidence provided by the present study is sparse. Furthermore, owing to the lack of cohort clinical characteristic determination and an analysis assessing its association with CDH3 levels, the present study is limited.

In conclusion, the present study indicated that CDH3 was significantly upregulated in COAD tissues, and a high CDH3 level was predictive of a good prognosis. In addition, Bioinformatics revealed that CDH3 is mainly involved in regulating cell-cell adhesion. To the best of our knowledge, the present study was the first to report on the role of CDH3 in the prognosis of COAD and provided novel insight into the mechanism by which CDH3 affects the prognosis of COAD patients. However, whether CDH3 is a possible candidate marker for guiding targeted or individualized treatment still requires to be verified with a larger number of samples.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YX performed the experiments and collected and analyzed the data. JZ designed the study, coordinated the experiments and acquired the data. XD, MD and YX designed the present study, collected and analyzed the, data, and wrote the manuscript. All authors read and approved the final manuscript.

Ethical approval and consent to participate

The present study was approved by the Ethics Committee of the HwaMei Hospital, University of the Chinese Academy of Sciences (Ningbo, China), and all of the patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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