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CDH1 genetic variants and its aberrant expression are the risk factors for colorectal cancer metastasis

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Abstract

E-cadherin, encoded by the *CDH1* gene, plays an essential role in epithelial cellular adhesion, and the loss of it has been reported to be associated with tumor progression and metastasis, potentially offer a glimpse in to the development of colorectal cancer. The present study aimed to explore effect of *CDH1*-160 polymorphism, *CDH1* transcription and its protein E-cadherin expression on colorectal cancer, meanwhile uncovering the underlying mechanism. Specimens from cancer loci, adjacent cancer tissue, and distal normal tissue from colorectal cancer patients were collected for Hematoxylin-eosin staining to detect the histopathological change of colorectal mucosa. Direct sequencing and Quantitative Real-Time PCR were used to detect the *CDH1* genotype and its mRNA expression, respectively. E-cadherin expression was detected using the ElivisionTM plus method. As a result, we found that the A allele of the *CDH1*-160 may be a protective gene against colorectal cancer, and the C > A polymorphism may regulate its transcription activity and expression of E-cadherin. The decrease of the *CDH1* mRNA transcription level and the absence of E-cadherin on the cytomembrane may promote intestinal mucosal carcinogenesis and accelerate cancer cell metastasis. Deficiency of cytomembrane expression of E-cadherin protein may have some early warning signs for malignant lesions of the gut mucosa.

Keywords Colorectal cancer, *CDH1*, E-cadherin, Single nucleotide polymorphism, Metastasis

Introduction

Colorectal cancer (CRC) ranks as the third most prevalent cancer and the second leading cause of cancer-related mortality globally, with an incidence rate of approximately 10.0% and a mortality rate of 9.4% [1]. Lack of early diagnosis and tumor metastasis are the two leading causes of CRC-related fatalities [2]. The etiology of CRC is multifactorial, encompassing gender, environment, and genetic factors [3]. Notably, a low penetrance genetic polymorphism has been identified as a factor in CRC susceptibility [4], with single nucleotide polymorphisms (SNPs) being a focal point of current research.

E-cadherin, encoded by the *CDH1* gene, is a transmembrane glycoprotein that plays a crucial role in cell-cell adhesion of epithelial cells and the maintenance of normal tissue morphology [5]. Numerous studies have reported that E-cadherin deficiency is linked to tumor progression and metastasis. Specifically, the loss of E-cadherin disrupts intercellular adhesion, facilitates the dissociation and metastasis of tumor cells, mediates

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epithelial-mesenchymal transition, and thereby accelerates cancer progression [6, 7]. Additionally, E-cadherin deficiency results in the loss of contact inhibition, promoting the cancer cell proliferation [8]. Abnormal expression of E-cadherin has been reported in several cancers such as gastric cancer, bladder cancer, and breast cancer [9–11].

E-cadherin downregulation is usually a result of transcriptional repression and is associated with its coding gene, *CDH1*. Several SNPs within the *CDH1* promoter region have been reported to influence its transcriptional regulation and contribute to cancer susceptibility. Among these, two extensively studied loci, *CDH1*-160 C > A (rs16260) and *CDH1*-347 G > GA (rs5030625), are implicated in the development and prognosis of several cancers [12, 13]. However, research on the association between the *CDH1*-160 C > A polymorphism and CRC susceptibility is limited and yields inconclusive results.

In this study, we investigate the association of the *CDH1*-160 SNP with CRC by examining its impact on mRNA transcript levels and the qualitative localized expression of its protein, E-cadherin. Our aim is to elucidate the molecular mechanisms underlying the carcinogenesis and metastasis of intestinal mucosal tissue, which could potentially serve as an early biomarker for the diagnosis of CRC.

Materials and methods

Study population and specimen collection

A total of 99 CRC patients were recruited from gastrointestinal surgery of the first affiliated hospital of Guangzhou University of Chinese Medicine from August 1st, 2016 to May 1st, 2020. Specimens were collected from cancerous loci, adjacent tissue, and distal normal tissue (four specimens per site) for subsequent analysis. The study protocol received approval from the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (Ethics Approval No. [2015] 009). Informed consent was obtained from all participants.

Inclusion and exclusion criteria

The inclusion and exclusion criteria for patient selection were as follows: Inclusion criteria: (1) Patients meeting the diagnostic criteria for CRC; (2) Age between 18 and 80 years; (3) Voluntary participation with signed informed consent. Exclusion criteria: (1) Presence of other serious systemic diseases; (2) Pregnant or breastfeeding women; (3) History of severe mental illness or intellectual disability, rendering them unable to cooperate with data collection and informed consent procedures.

Histopathological evaluation

Histopathological changes in colorectal mucosa were examined using hematoxylin-eosin staining. All slides were reviewed independently by two trained pathologists in a blinded manner. Based on histopathologic changes, including the severity of inflammation, inflammatory activity, and dysplasia, specimens were categorized into four pathological groups: (1) Normal control group (NOR): Normal colorectal mucosa or mild inflammation; (2) Chronic inflammation group (INF): Moderate to severe inflammation without dysplasia, characterized by mucosal edema and disorderly glands infiltrated with inflammatory cells; (3) Dysplasia group (DYS): Mild, moderate, severe dysplasia, and in situ carcinoma; (4) Colorectal cancer group (CRC): Mucosa exhibiting carcinomatous changes.

Genotyping

Genomic DNA was extracted from cancerous tissue using the Magen Hipure Tissue DNA Kits (D3121-02, Magen). Nested PCR was employed to amplify the extracted DNA fragments. Primer sequences for *CDH1* were as follows: External amplification: 5'-CTGTACTCCCAGCTACTAGAG-3' (forward) and 5'-CGTACCGCTGATTGGCTGAG-3' (reverse); Internal amplification: 5'-CTTGAGCCCAGGAGTTCGAG-3' (forward) and 5'-GCCACAGCCAATCAGCAG-3' (reverse). The PCR reaction conditions included an initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The reaction mixture (25 μL) comprised Taq polymerase (0.25 μL), 2x GC Buffer (12.5 μL, TaKaRa LA Taq® with GC Buffer, TakaRa, RR02AG), dNTPs (4 μL), 15 pmol/μL primers (1 μL each), ddH2O (4.5 μL), and DNA template (2 μL). Agarose gel electrophoresis was used to verify the DNA fragments. Direct sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (ABI, 4311320) according to standard ABI procedures, and sequences were analyzed using BioEdit Sequence Alignment Editor.

Quantitative real-time polymerase chain reaction (Q-PCR)

Total RNA was extracted from biopsy specimens using the RNAiso Plus Kits (AKA1202, TaKaRa) and reverse-transcribed into cDNA using the ReverTra Ace qPCR RT Kit (FSQ-101, TOYOBO). Q-PCR amplification was conducted with SYBR Premix Ex TaqTM (AK6006, TaKaRa) and ROX Reference Dye (50x) under the following conditions: Initial denaturation at 95°C, followed by 40 cycles at 95°C for 5 seconds, 60°C for 30 seconds, and 72°C for 1 minute. Melting curve analysis was performed at 95°C for 15 seconds, 55°C for 1 minute, and 95°C for 15 seconds. Primer sequences were 5'-CCTTAGAGGTGGGTGACTAC-3' (forward) and 5'-CAAGAATCCCCAGAATGGC

AG-3' (reverse). The 2-ΔΔCq method was used to quantify miRNA expression levels, with β-actin as the internal control.

Immunochemistry

Immunohistochemical staining for E-cadherin was performed using the ElivisionMT plus method. Paraffin-embedded sections were deparaffinized, rehydrated, and subjected to antigen retrieval by heating at 100 °C for 20 min in citrate buffer. Sections were then incubated in 3% hydrogen peroxide for 10 min, followed by five washes with 0.01 M PBS *(P*H 7.4) for 2 min each. Subsequently, sections were incubated with the Anti-E-cadherin antibody (EP700Y) (dilution 1:1500, ab40772, Abcam) at 4 °C overnight. After washing with PBS, sections were incubated with Goat Anti-Rabbit IgG H&L (HRP) (ab6721, Abcam) as the secondary antibody for 30 min at 37 °C. The DAB reaction was used for visualization, and sections were counterstained with hematoxylin and mounted with neutral resin. Immunostaining intensity and the percentage of positive cells were assessed semi-quantitatively and classified as negative (-), mild positive (+), moderate positive (++) or strong positive (+++). The Immunostaining evaluation will be scored by two independent observer and the concordance testing will be conducted, with the discrepancies will be resolved by consensus.

Statistical analysis

Statistical analyses were performed using SPSS software (version 25.0). Genotypic distribution and E-cadherin expression were assessed using the Chi-Square test or Fisher's exact test. Binary logistic regression was employed to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between *CDH1* SNP and CRC. *CDH1* mRNA levels were analyzed using one-way analysis of variance or the Kruskal-Wallis test. Spearman's rank correlation analysis was conducted to examine the relationship between *CDH1* mRNA expression and pathological changes in colorectal mucosa. A p-value of <0.05 was considered statistically significant. Additionally, post-hoc power analysis was conducted to test the power of statistical results.

Table 1 Basic characteristic of study subjects

Parameters	*N* (%)
Gender	
Male	57(57.6%)
Female	42(42.4%)
Age, years (mean ± SD)	62.14 ± 12.92
Tumor location	
Right-sided colon	22(22.2%)
Left-sided colon	68(68.7%)
Rectum	9(9.1%)
Histopathological classification	
Adenocarcinoma	84(84.8%)
Others	15(15.2%)
Tumor differentiation	
Well differentiated	9(9.1%)
Moderately differentiated	80(80.8%)
Poor differentiated	10(10.1%)
TNM stage	
I	13(13.2%)
II	44(44.4%)
III	38(38.4%)
IV	4(4.0%)

Results

Study population

The baseline characteristics of subjects are presented in Table 1. A total of 99 CRC patients were included in the study, with a gender distribution of 57 male (57.6%) and 42 female (42.4%), and an average age of 62.14 ± 12.92 years. A total of 288 specimens were collected from cancer loci, cancer adjacent tissue, and distal normal tissue for further analyzed (9 specimens were missing or damaged). The location was mainly in the left-sided colon (68.7%), while the adenocarcinoma was the predominant type (84.8%), and most cases were moderately differentiated (80.8%). The majority of patients were in stage II (44.4%) and stage III (38.4%) with respect to TNM stage.

CDH1 genetic polymorphism and CRC

The results of gene polymorphism are illustrated in Fig. 1. The association between *CDH1*-160 gene polymorphism and the clinicopathologic features of CRC is detailed in Table 2. The genotype distribution was in Hardy-Weinberg equilibrium *(*$P>0.05$). Gene polymorphism at this locus was found to be associated with tumor location *(*$P=0.034$) and clinical TNM stage *(*$P<0.001$). Specifically, patients with the A/A genotype had a higher likelihood of developing right-sided CRC compared to those with the C/C genotype (55.6% vs. 13.6%, $P=0.014$). Additionally, patients with the C/C genotype were more likely to present with an advanced TNM stage than those with the C/A genotype *(*$P<0.001$) and the A/A genotype *(*$P=0.044$).

The results of the regression analysis are summarized in Table 3. After adjusting for age and gender, patients with the C/A or A/A genotypes exhibited a reduced risk of progressing to stage III and IV (adjusted OR = 0.237, 95% CI = 0.101–0.555) and developing lymph node metastasis (adjusted OR = 0.260, 95% CI 0.111–0.606) compared with those with the C/C genotype. Regarding alleles, the presence of the A allele, compared to the C allele, was associated with a lower likelihood of progression to stages III and IV CRC (adjusted OR = 0.364, 95% CI = 0.190–0.700) and lymph node metastasis (adjusted

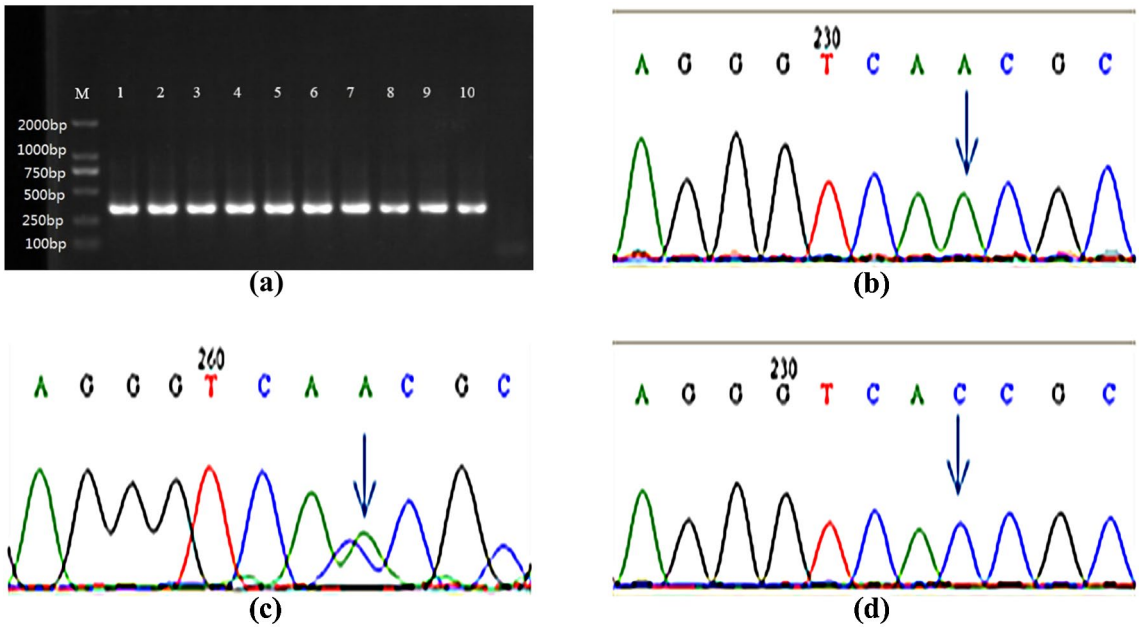

Fig. 1 *CDH1*-160 genotypes by the PCR-direct sequencing method. (**a**) Agarose gel (1%) electrophoresis of PCR product, M: Marker; 1 and 10 are positive and negative control, respectively; Lane 2 to 9 represents 8 different individuals; (**b**) CC homozygous wild genotype; (**c**) CA heterozygous mutant genotype; and (**d**) AA homozygous mutant genotype

Table 2 *CDH1*-160 genotype and basic character of CRC patients

Parameters	Genotype, *N* (%)			χ^2	*P*	Allele, *N* (%)		χ2	*P*
	C/C	C/A	A/A			C	A		
Gender									
Male	25(56.8)	24(52.2)	8(88.9)	4.173	0.118	79(59.0)	35(54.7)	0.323	0.570
Female	19(43.2)	22(47.8)	1(11.1)			55(41.0)	29(45.3)		
Tumor location									
Left-sided colon	32(72.7) *	33(71.7)	3(33.3)	-	0.034	92(68.7)	44(68.8)	0.233	0.890
Right-sided colon	6(13.6)	11(23.9)	5(55.6)			29(21.6)	15(23.4)		
Rectum	6(13.6)	2(4.3)	1(11.1)			13(9.7)	5(7.8)		
Histopathological classification									
Adenocarcinoma	41(93.2)	35(76.1)	8(88.9)	5.238	0.073	115(85.8)	64(82.8)	0.305	0.581
Others	3(6.8)	11(23.9)	1(11.1)			19(14.2)	11(17.2)		
Tumor differentiation									
Well-differentiated	7(15.9)	2(4.3)	1(11.0)	-	0.124	10(7.5)	8(12.5)	1.485	0.476
Moderately differentiated	35(79.5)	39(84.8)	6(66.7)			111(82.8)	49(76.6)		
Poorly differentiated	2(4.5)	5(10.9)	2(22.2)			13(9.7)	7(10.9)		
TNM stage									
T1	5(11.4)	8(17.4)	0	-	< 0.001	15(11.2)	11(17.2)	1.539	0.673
T2	12(27.3)	25(54.3)	7(77.8)			61(45.5)	27(42.2)		
T3	25(56.8)	11(23.9)	2(22.2)			54(39.6)	23(35.9)		
T4	2(4.5)	2(4.3)	0			5(3.7)	3(4.7)		

OR = 0.389, 95% CI = 0.203–0.747). Furthermore, patients carrying C/A or A/A genotypes had a higher probability of developing mucinous adenocarcinoma or indolent cell carcinoma compared to those with the C/C genotypes clinically (adjusted OR = 3.936, 95% CI = 1.027–15.092).

CDH1 mRNA expression and CRC

The histopathological characteristic of intestinal mucosa is shown in Fig. 2A, while the amplification and dissociation curve of *CDH1* mRNA are illustrated in Fig. 2B. The levels of *CDH1* mRNA (Fig. 2C) were significantly reduced in the cancerous tissue compared to distal normal tissue (mean:1.97 vs. 0.95, $P = 0.031$). Notably, the INF group exhibited higher *CDH1* mRNA expression

Table 3 *CDH1*-160 genotype frequencies and clinical statuses in CRC patients

Variable	C/C (*n*, %)	C/A + A/A (*n*, %)	OR (95%CI)	AOR (95%CI)	C (*n*, %)	A (*n*, %)	OR (95%CI)	AOR (95%CI)
Clinical stage								
I + II	17(38.6)	40(72.7)	1.00 (Reference)	1.00 (Reference)	67(50.0)	47(73.4)	1.00 (Reference)	1.00 (Reference)
III + IV	27(61.4)	15(27.3)	**0.236 (0.101–0.552)**	**0.237 (0.101–0.555)**	67(50.0)	17(26.6)	**0.362 (0.189–0.693)**	**0.364 (0.190–0.700)**
Tumor size								
T1 + T2	36(81.8)	46(83.6)	1.00 (Reference)	1.00 (Reference)	24(17.9)	54(84.4)	1.00 (Reference)	1.00 (Reference)
T3 + T4	8(18.2)	9(16.4)	1.136 (0.398–3.238)	1.150 (0.402–3.288)	110(82.1)	10(15.6)	1.178 (0.526–2.639)	1.190 (0.529–2.676)
Lymph node metastasis								
No	18(40.9)	40(72.7)	1.00 (Reference)	1.00 (Reference)	69(51.5)	47(73.4)	1.00 (Reference)	1.00 (Reference)
Yes	26(59.1)	15(27.3)	**0.260 (0.112–0.604)**	**0.260 (0.111–0.606)**	65(48.5)	17(26.6)	**0.384 (0.200-0.735)**	**0.389 (0.203–0.747)**
Distal metastasis								
No	2(4.5)	2(3.6)	1.00 (Reference)	1.00 (Reference)	128(95.5)	62(96.9)	1.00 (Reference)	1.00 (Reference)
Yes	42(95.5)	53(96.4)	0.792 (0.107–5.863)	0.845 (0.111–6.428)	6(4.5)	2(3.1)	0.688 (0.135–3.508)	0.653 (0.133–3.550)
Histopathological character								
Adenocarcinoma	41(93.2)	43(78.2)	1.00 (Reference)	1.00 (Reference)	117(87.3)	51(79.7)	1.00 (Reference)	1.00 (Reference)
Others	3(6.8)	12(21.8)	**3.814 (1.003–14.501)**	**3.936 (1.027–15.092)**	17(12.7)	13(20.3)	1.754 (0.793–3.879)	1.843 (0.825–4.115)

Annotation: OR: odd ratios; AOR: adjusted odd ratios, adjusted for age and gender; CI: confidence interval

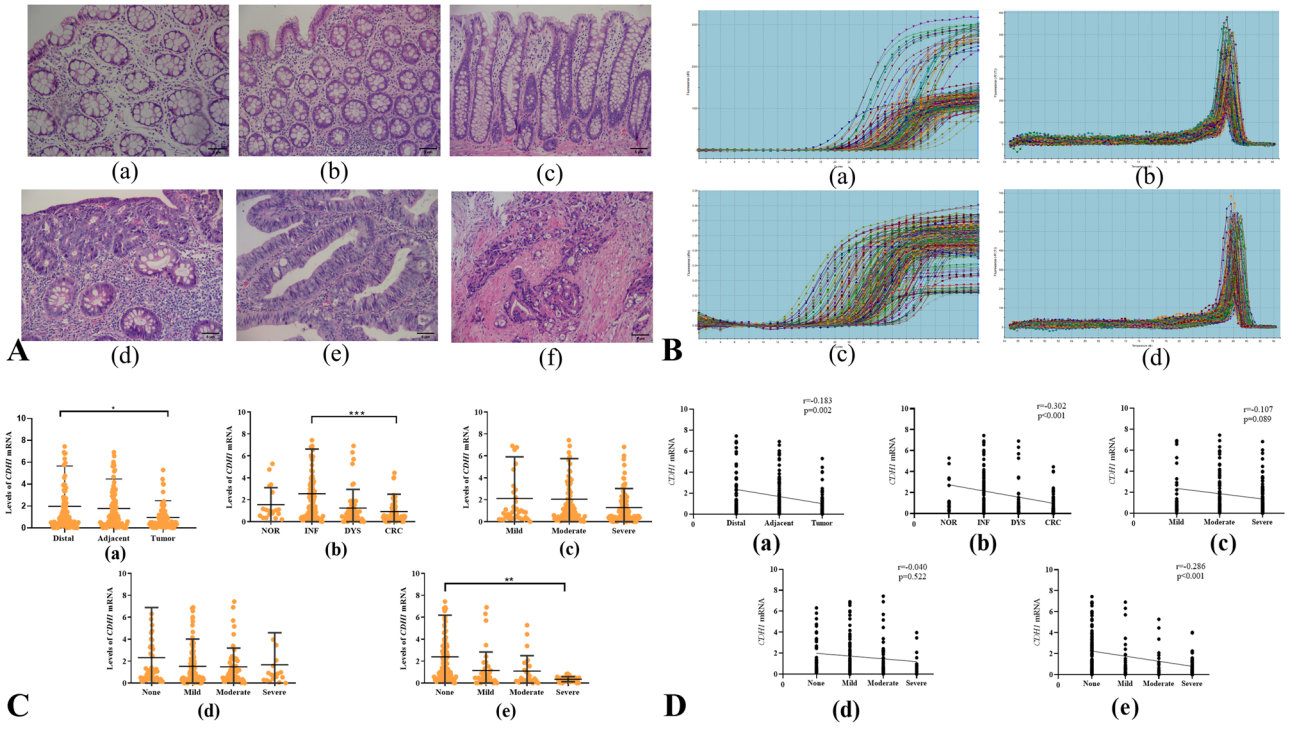

Fig. 2 A. Histopathological features of colorectal mucosa (hematoxylin-eosin staining 200×) (**a**) normal colorectal mucosa; (**b**) colorectal mucosa with moderate inflammation; (**c**) colorectal mucosa with moderate inflammation and mild dysplasia; (**d**) colorectal mucosa with severe inflammation and severe dysplasia; (**e**) adenocarcinoma; (**f**) signet-ring cell carcinoma. B. Amplification and dissociation curve of mRNA: (**a**) and (**c**) show the amplification curve of β-actin and *CDH1* mRNA; (**b**) and (**d**) show the dissociation curve of β-actin and *CDH1* mRNA. C. *CDH1* mRNA expression in (**a**) tumor, adjacent, and distal tissue; (**b**) histopathological group; (**c**) mucosal inflammation; (**d**) inflammatory activity; (**e**) mucosal dysplasia. D. Correlation analysis: (**a**) tumor, adjacent, and distal tissue; (**b**) histopathological group; (**c**) mucosal inflammation; (**d**) inflammatory activity; (**e**)mucosal dysplasia. **Annotations**: NOR: normal tissue group; INF: inflammatory group; DYS: dysplasia group; CRC: colorectal group. **P* < 0.05, ***P* < 0.01, ****P* < 0.001

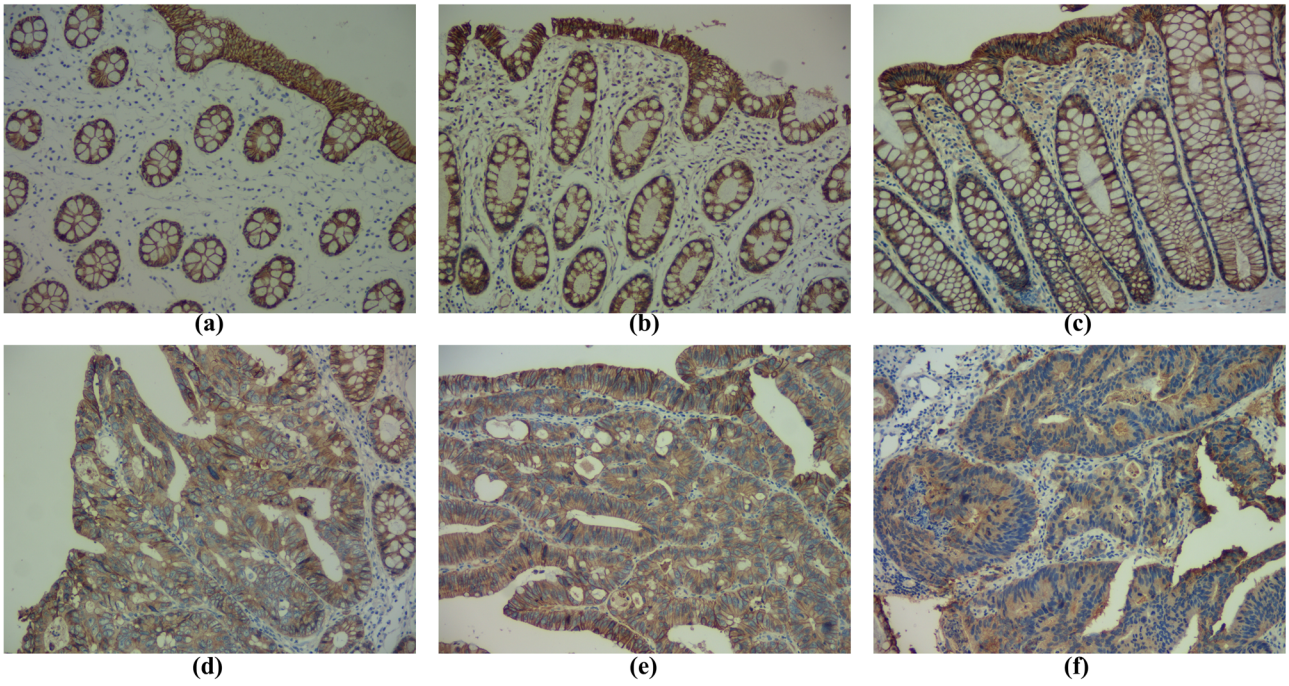

Fig. 3 E-cadherin expression in colorectal mucosa(x200). (**a**) normal mucosa; (**b**) mucosa with mild inflammation; (**c**) mucosa with mild dysplasia; (**d**) mucosa with severe dysplasia. (**e**) carcinoma mucosa, E-cadherin expressed both on membrane and cytoplasm; (**f**) carcinoma mucosa, loss of E-cadherin on membrane

Table 4 Location of E-cadherin in colorectal mucosa

Genotype	Epithelial		*P*	Crypt		*P*
	Cytoplasm	Both		Cytoplasm	Both	
Histopathological groups						
NOR	0(0.0%)	32(100.0%)	**< 0.001**	0(0.0%)	32(100.0%) *	**< 0.001**
INF	0(0.0%)	115(100.0%) *		4(3.3%)	117(96.7%) *	
DYS	0(0.0%)	60(100.0%) *		3(4.5%)	63(95.5%) *	
CRC	55(71.4%)	22(28.6%)		64(73.7%)	23(26.7%)	
Tissue district						
Carcinoma	49(53.8%)	42(46.2%)	**< 0.001**	58(58.6%)	41(41.4%)	**< 0.001**
Adjacent	5(5.7%)	83(94.3%) #		10(10.2%)	88(89.8%) #	
Distal	1(1.0%)	98(99.0%) #		2(2.0%)	97(98.0%)#△	

Annotation: NOR: normal tissue group; INF: inflammatory group; DYS: dysplasia group; CRC: colorectal group. *Compared with CRC group, $P < 0.05$; #Compared with carcinoma group, $P < 0.05$; △ Compared with adjacent group, $P < 0.05$

than the CRC group (mean: 2.55 vs. 0.93, $P < 0.001$). Similarly, intestinal mucosal tissues without dysplasia had elevated *CDH1* mRNA levels compared to those with severe dysplasia (mean: 2.40 vs. 0.96, $P = 0.004$). Correlation analysis (Fig. 2D) indicated an inverse relationship between *CDH1* mRNA expression and both the severity of intestinal mucosal malignancy (Spearman's r = -0.302, $P < 0.001$) and the degree of dysplasia (Spearman's r = -0.286, $P < 0.001$). Additionally, *CDH1* mRNA expression decreased as the tissue approached the cancerous site (Spearman's r = -0.183, $P = 0.002$).

E-cadherin expression and CRC

The expression of E-cadherin in intestinal mucosa is depicted in Fig. 3, with localization and quantitative evaluations presented in Tables 4 and 5. In the CRC group, there was marked a loss of E-cadherin expression on the cytomembrane of both epithelial and crypt cells ($P < 0.05$). The proportion of cytoplasmic E-cadherin expression in the epithelial zone and crypt was substantially higher in cancerous tissue compared to adjacent cancerous and distal normal tissue ($P < 0.001$). In addition, the proportion of cytoplasmic E-cadherin expression in the cancer-adjacent tissue was significantly greater than in distal normal tissue ($P = 0.032$). Semiquantitative evaluation revealed that E-cadherin expression in

Table 5 Semiquantitative results of E-cadherin

Group	(+) (*n*, %)	(++) (*n*, %)	(+++) (*n*, %)	*P*
Histopathological groups				
NOR*	2(6.1%)	7(21.2%)	24(72.7%)	< 0.001
INF*	3(2.5%)	12(9.8%)	107(87.7%)	
DYS*	3(4.5%)	4(6.1%)	59(89.4%)	
CRC	13(15.1%)	34(39.5%)	39(45.3%)	
Tissue district				
Carcinoma	13(13.1%)	30(30.3%)	56(56.6%)	< 0.001
Adjacent#	2(2.0%)	16(16.2%)	81(81.8%)	
Distal#	6(5.5%)	11(10.1%)	92(84.4%)	

Annotation: NOR: normal tissue group; INF: inflammatory group; DYS: dysplasia group; CRC: colorectal group. *Compared with CRC group, $P < 0.05$; # Compared with carcinoma, $P < 0.05$

the CRC group was significantly lower than in the NOR, INF, and DYS groups *(*$P = 0.012$, < 0.001, < 0.001), and E-cadherin levels in cancerous tissue were lower than in cancer-adjacent and distal normal tissue *(*$P < 0.001$). Furthermore, correlation analysis suggested a negative correlation between E-cadherin expression and both intestinal malignancy (Spearman's r=-0.296, $P < 0.001$) as well as the degree the intestinal mucosal dysplasia (Spearman's r=-0.317, $P < 0.001$).

The influence of *CDH1* SNP on its transcription and translation

To investigate the impact oof the *CDH1*-160 gene polymorphism on its mRNA expression, we conducted a comparative analysis of *CDH1* mRNA levels in cancer tissue, cancer-adjacent tissue, and distal normal tissues from patients with different genotypes (Fig. 4). The findings revealed that patients with the C/A genotype exhibited significantly higher *CDH1* mRNA expression levels in cancer tissues compared to those with the A/A genotype (mean: 2.63 vs. 0.36, $P = 0.032$). Despite this, correlation analysis indicated no linear relationship between alterations in the *CDH1*-160 genotype and mRNA transcript levels. Furthermore, the impact of *CDH1* polymorphism on E-cadherin expression was explored (Tables 6 and 7). The results indicated that patients with either C/A or A/A genotypes showed ectopic expression of epithelial E-cadherin protein in their intestinal cancer tissues. Nonetheless, no significant effect of the polymorphism at this locus on the overall expression of E-cadherin was observed.

Statistical power analysis

To examine the statistical power of the study, post-hoc power analysis was conducted (Supplementary file 1). Post-hoc power analysis revealed that among the 25 statistically significant results, 15 exhibited strong power (> 80%), 8 had moderate power (50–80%), and 2 showed weak power (< 50%) (Supplementary File 1). This indicates that the majority of our findings (92%, 23/25) were supported by sufficient statistical power to detect clinically meaningful effects.

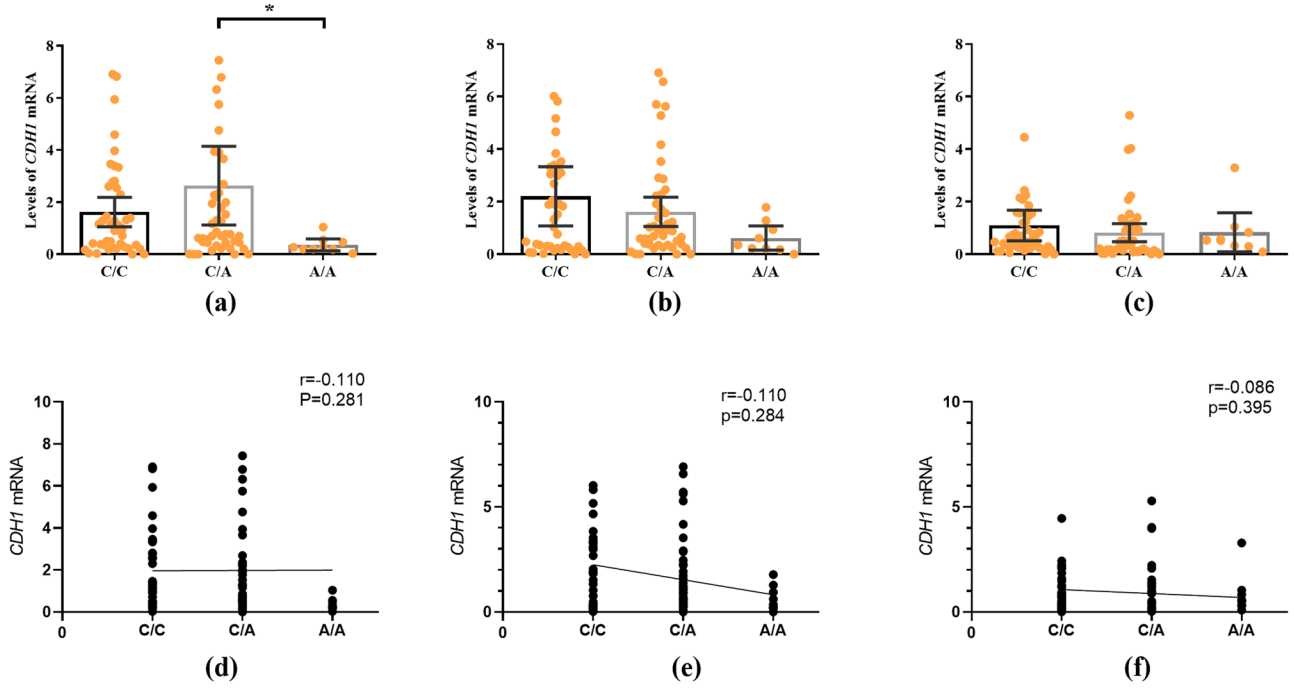

Fig. 4 The relationship between genetic variant of *CDH1*-160 and its mRNA expression. (**a**)-(**c**) show the expression of *CDH1* mRNA among different genotype in distal normal tissue, adjacent tissue, and tumor tissue. (**d**)-(**f**) show the result of correlation analysis in distal normal tissue, adjacent tissue, and tumor tissue. **Annotations**: *$P < 0.05$

Table 6 *CDH1* genetic variant and E-cadherin location

Genotype	Epithelial		*P*	Crypt		*P*
	Cytoplasm	Both		Cytoplasm	Both	
Distal						
C/C	-	39(48.8%)	-	1(100.0%)	39(47.6%)	0.297
C/A + A/A	-	41(51.2%)		0(0.0%)	43(54.2%)	
Adjacent						
C/C	3(75.0%)	30(44.8%)	0.239	5(62.5%)	32(45.1%)	0.349
C/A + A/A	1(25.0%)	37(55.2%)		3(37.5%)	39(54.9%)	
Carcinoma						
C/C	15(37.5%)	24(66.7%)	**0.011**	23(46.9%)	18(54.5%)	0.499
C/A + A/A	25(62.5%)	12(33.3%)		26(53.1%)	15(45.5%)	

Table 7 *CDH1* genetic variant and E-cadherin expression

Genotype	(+) (*n*, %)	(++) (*n*, %)	(+++) (*n*, %)	*P*
Distal				
C/C	3(60.0%)	6(66.7%)	31(44.9%)	0.406
C/A + A/A	2(40.0%)	3(33.3%)	38(55.1%)	
Adjacent				
C/C	2(100.0%)	8(61.5%)	27(41.5%)	0.127
C/A + A/A	0(0.0%)	5(38.5%)	38(58.5%)	
Carcinoma				
C/C	5(45.5%)	10(41.7%)	26(55.3%)	0.525
C/A + A/A	6(54.5%)	14(58.3%)	21(44.7%)	

Discussion

CRC is one of the most prevalent malignant tumors, posing a significant threat to human health. Despite the growing utilization of various screening tests and diagnostic techniques, the early detection rate of CRC remains relatively low, and its mortality rate is high. E-cadherin, a crucial factor in inhibiting tumor invasion and metastasis, plays a vital role in maintaining normal tissue morphology. The absence of E-cadherin impairs cellular adhesion, leading to the detachment and metastasis of tumor cells. In this study, we focused on the *CDH1* gene and its encoded protein E-cadherin to investigate the effect of SNP at the rs16260 locus on the pathogenesis of CRC, as well as its impact on transcription and translation.

Previous studies on *CDH1*-160 polymorphisms have primarily concentrated on gastric, breast, and bladder cancers. In the Kurdish population, the A allele at this locus is a potential risk gene for breast cancer, associated with high-grade, stage IV, and metastatic tumors [13–14]. Conversely, the A allele of *CDH1*-160 has been found to reduce the risk of bladder cancer in European and Asian populations [15]. Additionally, the A/A genotype at this locus has been linked to an increased risk of gastric cancer [16]. However, research on *CDH1*-160 gene polymorphism in colorectal cancer is limited and presents conflicting conclusions. A meta-analysis suggested that the *CDH1*-160 C/A polymorphism might be a protective factor for CRC in the Caucasian population, hospitalized patients, and for distal CRC [17]. Contrarily, Mohammad et al. reported no significant association between the *CDH1*-160 C > A polymorphism and the development of gastrointestinal tumors [18]. In our study, we identified the *CDH1*-160 SNP as a risk factor for CRC. Patients with the C/A or A/A genotypes and the A allele demonstrated a lower risk of progressing to advanced-stage CRC and developing lymph node metastasis compared to patients with the C/C genotype and the C allele. Therefore, we propose that the *CDH1*-160 C > A polymorphism may act as a protective factor for CRC, potentially inhibiting lymph node metastasis and suppressing tumor progression.

We then further analyzed the effect of *CDH1*-160 SNP on its transcriptional and translational levels. At the transcriptional level, *CDH1* mRNA expression in cancer tissues from patients with the C/A genotype was significantly higher than in those with the A/A genotype. At the level of E-cadherin expression, cytomembrane E-cadherin was significantly absent in cancer tissues from patients with the C/A genotype, suggesting that the *CDH1* C > A polymorphism may regulate E-cadherin expression by affecting its transcriptional activity, thereby influencing CRC development and progression. Similarly, Valli et al. [19] found evidence that the *CDH1*-160 polymorphism can regulate its mRNA expression in gastric cancer, while Zhang et al. [20] suggested that intron mutations, gene methylation, and SNPs may affect *CDH1* expression. Another *in virto* study confirmed that the transversion of C to A in the − 160 promoter of *CDH1* gene causes decreasing of transcriptional efficiency compared with the C allele [21]. SNP can influence the non-coding mRNA structure and regulate E-cadherin promoter. It was reported that the A to C change alters the locally dynamic and metastable structure of the sense-oriented paRNA (S-paRNA) [22], thereby influencing the ability of the isomiR-4543 and Argonaute 1(AGO1) to interact with the S-paRNA [23]. Thus, we speculate that the *CDH1*-160 SNP can regulate E-cadherin expression to some degree by affecting

transcriptional activity, and the A allele at this locus may serve as a protective factor for CRC.

The mRNA is the intermediate stage of the gene expression process, regulated by genes and capable of affecting protein expression. In this study, we found that *CDH1* mRNA expression was significantly lower in cancerous tissues compared to distal normal tissues, with expression decreasing as the tissues got closer to the cancerous site. Additionally, *CDH1* mRNA expression was inversely correlated with intestinal mucosal malignancy and the degree of mucosal dysplasia. This finding aligns with Arıkan et al., who observed a progressive decrease in mRNA expression in tumor tissues as the clinical stage of the tumor advanced (I-IV) [24]. In addition, *CDH1* mRNA levels were found to be strongly positively correlated with E-cadherin protein in eight of nine lineages of carcinoma cell lines, which upregulate in the early stage of tumor in colon or endometrial carcinoma [25]. Therefore, *CDH1* mRNA expression may be utilized as a molecular marker to assess tumor progression in CRC, with its decrease potentially indicative of carcinogenesis in the intestinal mucosa and tumor metastasis to some extent.

Finally, we examined the qualitative localization of E-cadherin in intestinal mucosa and found that E-cadherin was obviously absent on the cytomembrane in the epithelial cells of cancerous tissue and the proportion of E-cadherin cytomembrane absent in epithelial cells was greater the closer to the tumor site. Meanwhile, the semiquantitative measurement suggested that E-cadherin expression gradually decreased in the malignant evolution of intestinal mucosa, which is inversely correlated with the malignancy of intestinal mucosa. Plenty of studies have demonstrated that E-cadherin reduced or was absent in gastric cancer, bladder cancer, and breast cancers [9–11]. A recent meta-analysis on CRC revealed that the low expression of E-cadherin was associated with several adverse clinicopathological features such as the high risk of later TNM stage, deep infiltration, lymph node metastasis, lymphatic invasion, distal metastasis, low differentiation, and late Dukes' stage [26]. In our study, it is interesting to note that E-cadherin still existed in cytoplasm instead of being completely lost in the cancerous tissue, therefore, we hypothesize that it is the loss of E-cadherin on cytomembrane that is the key factor in the promotion of cancer cell metastasis. Similarly, RIYAD et al. found that a lack of cytomembrane expression and increased cytoplasmic expression of E-cadherin predicted poor of survival prognosis [27]. The shedding of tumor cells from the primary tumor tissue and their entry into the circulation then colonization of the targeted organ are the two main steps for tumor cell metastasis [28]. Cellular experiments suggested that E-cadherin activation inhibits tumor cell metastasis at multiple stages, including the accumulation of cancer cells from the primary tumor and the extravasation of tumor cells from the vasculature, involving changes in cell-cell adhesion, local invasion, intravasation, cell survival, and extravasation [29]. The regulatory mechanism of CDH1 in tumor growth and metaplasia involves multiple signaling pathways. In vitro experiments have demonstrated that E-cadherin-positive cancer stem cells exhibit higher proliferative potential, which may be attributed to elevated NANOG expression. This upregulation consequently leads to increased expression of cyclin D1 and B1 [30]. Notably, the KRAS/MEK pathway has been shown to repress PLCδ1 expression, thereby promoting E-cadherin expression, and suppressing malignancy in colorectal cancer cells [31].

However, there are also some limitations to this study. Firstly, all subjects included were CRC patients and no healthy subjects were recruited for comparison, which likely resulted in some selection bias. In addition, this study is a single center trial and the sample size is relatively small, which may result in some bias to some extent. The two underpowered results should be interpreted with caution, as they may reflect type II errors (false negatives) or require replication in larger cohorts. Therefore, a large multicenter study should be performed to further verify the finding.

Conclusion

The A allele of the *CDH1*-160 may be a protective gene against CRC, and the C > A polymorphism may regulate its transcription activity and expression of E-cadherin. The decrease of the *CDH1* mRNA transcription level and the absence of E-cadherin on cytomembrane may promote carcinogenesis of the intestinal mucosal and accelerate metastasis of cancer cells. Deficiency of cytomembrane expression of E-cadherin protein may have some early warning signs for malignant lesions of the gut mucosa.

Abbreviations

CI	Confidence intervals
CRC	Colorectal cancer
DYS	Dysplasia group
INF	Chronic inflammation group
NOR	Normal control group
OR	Odds ratios
SNP	Single nucleotide polymorphism

Supplementary Information

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Supplementary Material 1

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None.

Author contributions
Ling Hu conceived and designed the study, supervised experiments, and revised the manuscript. Yunbo Wu analyzed the data and wrote the manuscript. Ying Yu, Yunkai Dai, and Danyan Li performed the experiment and collected the samples. Jianyu Wu, Zijing Zhang helped to collect the samples. Huaigeng Pai, Weijing Chen, Ruliu Li helped revise the manuscript and supervised the study. All authors approved the final submitted manuscript.

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Data availability
The datasets used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was established according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine [ethics approval No. (2019) 140]. Written informed consent was obtained from all participants.

Consent for publication
None.

Competing interests
The authors declare no competing interests.

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