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Original Article

Expression of lncRNA-CCAT1, E-cadherin and N-cadherin in colorectal cancer and its clinical significance

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Abstract: Objective: To explore the expression and clinical significance of lncRNA-CCAT and EMT related molecule E-cadherin and N-cadherin in colorectal cancer. Methods: The expression of lncRNA-CCAT1, E-cadherin and N-cadherin in 37 colorectal cancer tissue and para-carcinoma tissue was detected using qRT-PCR method, and the correlation of expression level with clinical and pathological features was studied. Results: The expression of lncRNA-CCAT1 in tumor tissue was significantly higher than that in normal para-carcinoma tissue ($P < 0.001$), and the expression level of CCAT1was significantly correlated with local infiltration depth ($P < 0.001$), tumor staging ($P < 0.001$), vascular invasion ($P < 0.001$) and CA19-9 level ($P < 0.001$); but not related with age, gender, location of tumor, tumor differentiation level, size of primary lesion and level of CEA ($P > 0.05$). The expression of E-cadherin in tumor tissues was significantly lower than in normal para-carcinoma tissues ($P < 0.001$), and the expression of N-cadherin was significantly higher than that in normal para-carcinoma tissues. The decrease in expression of E-cadherin and increase in expression of N-cadherin were significantly correlated with local infiltration depth ($P < 0.001$), tumor staging ($P < 0.001$), vascular invasion ($P < 0.001$), tumor differentiation level ($P < 0.001$) and CA19-9 level ($P < 0.001$), however not related with age, gender, tumor location, size of primary lesion and CEA level ($P > 0.05$). Conclusion: CCAT1 plays an important role in the genesis, development, invasion and metastasis; it mediates the EMT process of colorectal cancer; and it's expected to be a new marker and treatment target in colorectal diagnosis and treatment.

Keywords: lncRNA, CCAT1, colorectal cancer, EMT, E-cadherin, N-cadherin

Introduction

Colorectal cancer is the most commonly seen human malignant cancers. The worldwide newly diagnosed colorectal cancer cases are around 1.2 million, and death number around 0.6 million. Its mortality rate is the third highest among all cancers [1]. In China, the prevalence of colorectal cancer is third highest among all population, which is higher in male than female, higher in urban areas than in rural areas; and the mortality rate is the fifth highest among all population, higher in males than in females, in urban areas than in rural area [2]. About 20% of patients experienced tumor metastasis at diagnosis and lost the best treatment opportunity. Surgery is the most promising treatment strategy for curing colorectal cancer, and the individualized comprehensive treatment regimen of surgery accompanied with chemotherapy, radiotherapy and biological target therapy greatly enhanced the prognosis and life quality of colorectal cancer patients, however still 50% of patients died of tumor recurrence or metastasis in the second year of operation. Based on the tertiary prevention principle of cancer, the first thing is to find out related carcinogenic factors and to avoid cancer risk factors, hence to prevent the happening of cancer [3]. To early discover, diagnose and treat cancer is helpful in realizing the secondary prevention of cancer, and helpful in preventing the development of de novo disease. To study the mechanisms of cancer genesis, development, invasion and metastasis] is helpful in realizing the tertiary prevention of cancer, i.e. to increase cure rate, decrease relapse rate, and improve end stage cancer patients' life quality.

Currently, CEA and CA19-9 are two colorectal cancer markers frequently used in clinical practice, however both the sensitivity and specificity

are low, hence, they have little significance in early diagnosis of colorectal cancer [4]. Research work on the mechanisms of tumor genesis, development, invasion and metastasis are abundant, and part of them are being applied in clinical practice, with good results. But tumor genesis, development, invasion and metastasis is a complicated multi-factor, multi-step and multi-mechanism biological process. All current studies are still limited, and thorough and wider researches are still expected.

Long non-coding RNAs (lncRNAs) were first described in rat full length cDNA sequence library. The first isolated lncRNAs are non-coding RNAs (nc RNAs) close to protein coding genes. They are newly discovered RNAs which make up 80% of non-coding RNAs. They mainly exist in high eukaryote nuclei or cytoplasm, with conservative secondary structure, and are RNA side products of polymerase II. Previously they are thought to be the "noise" or "dark matter" of gene transcriptome with no biological function [5]. However more and more studies showed that lncRNAs almost participate in every stage of cell life, including cell proliferation, differentiation, apoptosis, epigenetic processes (DNA methylation, histone modification, chromatin remodeling, and miRNA regulation etc.), and gene expression at pre-transcription, transcription and post-transcription level [6]. LncRNA is relatively stable, and can be detected from patients' peripheral blood, urine and stool. Studies in China and abroad show lncRNAs have close relationship with tumor genesis and development, they are involved in the whole process of tumor genesis, development, invasion and metastasis. What's more, lncRNAs, with space, time and tissue specificity, are promising tumor markers or gene therapy targets, thus playing an important role in diagnosis and individualized treatment. Colon Cancer Associated Transcript 1 (CCAT1) is a type of newly discovered lncRNA with RDA, cDNA cloning, and RACE, it probably participates in the genesis, development, invasion and metastasis of colorectal cancer, and is of high research significance.

EMT is a process of epithelial cells transforming into mesenchymal cells in specific physical or pathological conditions [7]. The main morphological features of EMT are: losing typical intercellular junction structure of epithelial cells, cytoskeleton remodeling, and changing shape from polygon shape to fusiform fibrocyte form. After EMT, cells are isolated, apoptosis resistant, and their motor ability enhanced. The main molecular features of EMT are: the loss of expression and function of epithelial markers such as E-cadherin and occluding, and over expression of mesenchymal cell markers like N-cadherin and vimentin [8, 9]. According to the different biological process EMT participated, it can be divided into three categories: EMT in embryogenesis and organ development, EMT in trauma restoration and organ fibrosis, and EMT in tumor [10].

In our study, we detected lncRNA-CCAT1, E-cadherin and N-cadherin expression with qRT-PCR method, and analyzed its correlation with clinical pathology, hoping to discover a new diagnostic and treatment approach of colorectal cancer.

Materials and methods

Data

37 colorectal cancer samples from Department of General Surgery, The Second Affiliated Hospital of Soochow dating from January 2013 to September 2013 were obtained, and a piece of para-carcinoma tissue 8 cm from tumor tissue was also obtained in each case. After surgical samples were obtained, aseptic sampling was done within 10 minutes, blood and remaining impurities was washed away with aseptic precooling PBS, and immersed in Trizol. Samples were preserved in liquid nitrogen. In our study, there were 21 male patients, 16 female patients, with the age ranging from 43 to 85 years old, the mean age was 62.95 ± 10.63 years old. Dukes staging: stage A (5 cases), stage B (17 cases), stage C (15 cases), and stage D (0 cases). Tumor location: colon cancer (18 cases), rectal cancer (19 cases). All cases underwent pathological confirmation, and no cases had done chemo or radio therapy before surgery. The study was approved by hospital's ethical committee, and informed consent was obtained from all participants.

Materials

Trizol Reagent was purchased from Introgen, and RT-PCR primers and kits were purchased from GenePharma of Soochow; related primers were designed and synthesized by Sangon

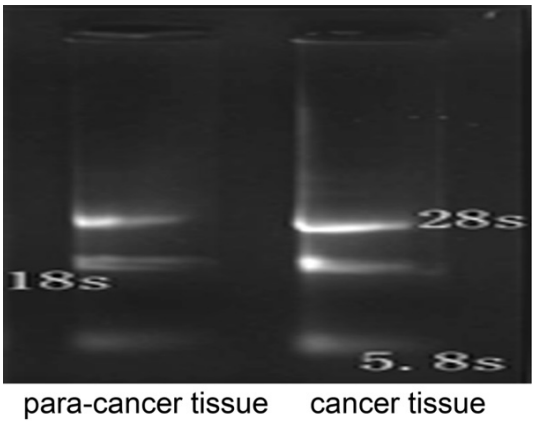

Figure 1. The total RNA quality by agarose gel electrophoresis.

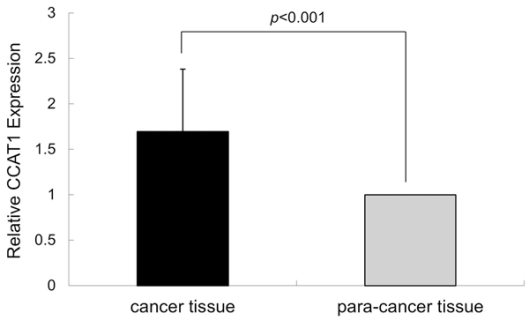

Figure 2. The comparison between cancer tissue and para-cancer tissue expression in CCAT1.

Biotech (Shanghai) Co., Ltd. Real-time PCR amplifier was purchased from BIO-RAD of United States.

Tissue total RNA extraction

100 mg colorectal tissue was processed with 1 ml of Trizol, and mashed with homogenizer. According to lab procedure, tissue was isolated with chloroform, precipitated with isopropanol, washed with 75% ethanol and dissolved in DECP water, procedure was done on ice. After extraction, RNA concentration was measured with ultraviolet spectrophotometry. RNA samples underwent 1% agarose gel electrophoresis, and 28 s, 18 s, 5.8 s bands width and brightness were observed to determine whether RNA degraded. Qualified samples were preserved in -80°C fridge.

RT-PCR reaction

RT-PCR primers and kits were purchased from GenePharma of Soochow, and 20 μl reaction system was prepared according to instruction: RT-PCR primer (10 μM) 0.2) 0.2 μl, RNA 1 μg, 5× reaction buffer 4 μl, RNAse inhibitor (20 μ/μl) 1 μl, dNTP (10 mM) 2 μl, MMLV reverse Transcriptase (200 μ/μl) 1 ul, and add nuclease free water till 20 μl. The reaction condition was: 70°C 5 min, 37°C 5 min, 42°C 60 min. After reaction, synthesized cDNA was preserved in -70°C environment.

qRT-PCR

Primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. Upstream primer of lncRNA-CCAT1 is: 5'-AGAAACACTATC-ACCTACGC-3', downstream primer: 5'-CTTAAC-AGGGCATTGCTAATCT-3'; Upstream primer of E-cadherin: 5'-CGCATTGCCACATACACTCT-3', downstrean primer: 5'-TTGGCTGAGGATGGTGTA-AG-3'; upstream primer of N-cadherin: 5'-AGTCA-ACTGCAACCGTGTGT-3' downstrean primer: 5'-A GCGTTCCTGTTCCACTCAT-3'; internal GAPDH upstream primer: 5'-TGTTGCCATCAATGACCCC-TT-3', downstream primer: 5'-CTCCACGACGTA-CTCAGCG-3'. Prepare 20 μl SYBR Green qRT-PCR kit (Soochow GenePharma Co. Ltd.) reaction system: cDNA (500 ng/20 μl): 1 μl, primer (10 pmol/L): 0.6 μl, 2×SYBR Green PCR Master Mix 9 μl, RNase-free water 8.4 μl. Reaction condition: 95°C 2 min heating, 95°C 15 s denaturing, 55°C 30 s annealing, 68°C 30 s extension, 40 cycles. All reactions had 3 duplicates, CCAT1, E-cadherin and N-cadherin expression in colorectal tissue was calculated with $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data were analyzed by SPSS 17.0, and presented as means ± standard deviation. Comparison between expression of lncRNA-CCAT1, E-cadherin and N-cadherin was analyzed with independent sample t-test; correlations between lncRNA-CCAT1, E-cadherin and N-cadherin expression and clinical features were calculated by Spearman's correlation analysis. $P < 0.05$ was considered statistically significant.

Results

Total RNA quality

A260/A280 value of total RNA extracted from samples was between 1.80-2.00, A260/A230

Table 1. Correlation of CCAT1 expression in colorectal cancer and para-cancer tissues with clinical features

Clinical data	Case (n)	CCAT1 ($2^{-\Delta\Delta Ct}$) ($\overline{X} \pm S$)	*P* value
Gender			0.493
Male	20	1.635 ± 0.683	
Female	17	1.761 ± 0.701	
Age			0.161
< 60	10	1.964 ± 0.724	
≥ 60	27	1.596 ± 0.656	
Tumor size			0.976
< 5 cm	19	1.646 ± 0.523	
≥ 5 cm	18	1.748 ± 0.836	
Differentiation level			0.051
Median to high	32	1.603 ± 0.637	
Low	5	2.292 ± 0.756	
Local invasion depth			0.0002
T1/T2	9	1.040 ± 0.342	
T3/T4	28	1.906 ± 0.634	
Dukes staging			0.0001
A/B	22	1.340 ± 0.438	
C/D	15	2.217 ± 0.656	
Vascular invasion			0.0001
No	27	1.479 ± 0.559	
Yes	10	2.281 ± 0.673	
Tumor location			0.915
Colon	18	1.642 ± 0.534	
Rectus	19	1.747 ± 0.815	
CEA (ng/ml)			0.063
< 6.50	15	1.420 ± 0.576	
≥ 6.50	22	1.884 ± 0.702	
CA19-9 (U/ml)			0.0006
< 37.00	25	1.422 ± 0.505	
≥ 37.00	12	2.265 ± 0.676	

value was between 1.90-2.10, 1% agarose gel electrophoresis showed clear 28 S and 18 S bands, and the width of 28 S band was twice the width of 18 S band, indicating that RNA was qualified, and can be used in the following procedure (**Figure 1**).

CCAT1 expression in colorectal cancer and para-carcinoma tissues

CCAT1 expression was significantly higher in colorectal cancer tissues than in para-carcinoma tissues, the relative expression level ($2^{-\Delta\Delta Ct}$) was 1.696 ± 0.685, and the expression in corresponding para-carcinoma tissue was 1.000 ± 0.000. (t = 6.715, P < 0.001, **Figure 2**).

Correlation of CCAT1 expression with clinical and pathology features

As shown in **Tables 1** and **2**, the CCAT1 expression level increased as tumor invasion depth increased. The relative expression of CCAT1 in T3/T4 was significantly higher than that in t1/t2 (P < 0.001). According to Dukes staging, CCAT1 expression increased as clinical stage increased: expression in C/D stage was significantly higher than that in A/B stage (P < 0.001). Considering CCAT1 expression level in patients with and without vascular invasion, we found that CCAT1 expression was significantly higher in patients with vascular invasion than those who are without (P < 0.001). In patients group with CA19-9 was ≥ 37.00 U/ml, CCAT1 expression was significantly higher than those with CA19-9 was < 37.00 U/ml (P < 0.001). The expression of CCAT1 was not correlated with patients' gender, age, size of tumor, differentiation level of tumor and CEA level (P > 0.05).

Expression of E-cadherin, N-cadherin in colorectal cancer and para-carcinoma tissue

The expression of E-cadherin was significantly lower than that in para-carcinoma tissues (0.526 ± 0.117 vs 1.000 ± 0.000, t = 4.623, P < 0.001, **Figure 3**). The expression of N-cadherin was significantly higher than that in para-carcinoma tissue (t = 5.117, P < 0.001, **Figure 4**).

Expression of E-cadherin, N-cadherin with clinical and pathology features

As tumor local invasion depth increases, the expression of E-cadherin decreases and N-cadherin increases. At T3/T4 level, the relative expression level of E-cadherin was significantly lower than that in T1/T2 (P < 0.001); At T3/T4 level, the relative expression level of N-cadherin was significantly higher than that in T1/T2 (P < 0.001); According to Dukes staging, as clinical stage advances, E-cadherin expression decreases and N-cadherin expression

Table 2. Correlations between lncRNA, E-cadherin, N-cadherin and clinical features

	CCAT1		E-cadherin		N-cadherin	
	Correlation	P	Correlation	P	Correlation	P
Gender	0.228	0.262	0.422	0.307	0.375	0.517
Age	0.561	0.674	0.664	0.521	0.486	0.388
Tumor size	0.279	0.098	0.571	0.129	0.334	0.248
Differentiation	0.607	0.188	0.774	0.286	0.687	0.122
Local invasion	0.445	0.005	0.432	0.011	0.226	0.003
Dukes staging	0.318	0.023	0.524	0.001	0.537	0.038
Vascular invasion	0.087	0.002	0.408	0.001	0.069	0.001
Tumor location	0.117	0.359	0.219	0.406	0.367	0.348
CEA (ng/ml)	0.042	0.116	0.174	0.552	0.471	0.295
CA19-9 (U/ml)	0.561	0.008	0.198	0.001	0.582	0.001

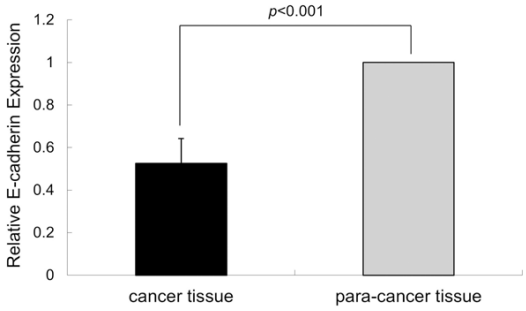

Figure 3. The comparison between cancer tissue and para-cancer tissue expression in E-cadherin.

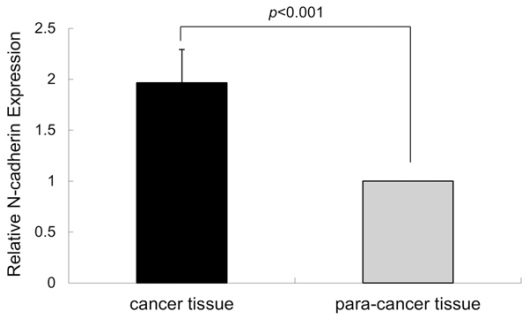

Figure 4. The comparison between cancer tissue and para-cancer tissue expression in N-cadherin.

increases; in C/D stage, expression level of E-cadherin was significantly lower than that in A/B stage (P < 0.001); in C/D stage, expression level of N-cadherin was significantly higher than that in A/B stage, (P < 0.001). Expression of E-cadherin in patients with vascular invasion was significantly lower than those without (P < 0.001); Expression of N-cadherin in patients with vascular invasion was significantly higher than those without (P < 0.001). The expression of E-cadherin in patients with low differentiation level was significantly lower in those with median or high differentiation level (P < 0.001). The expression of N-cadherin in patients with low differentiation level was significantly higher in those with median or high differentiation level (P < 0.001); The expression of E-cadherin in patients with CA19-9 ≥ 37.00 U/ml was significantly lower than those with CA19-9 < 37.00 U/ml (P < 0.001); The expression of N-cadherin in patients with CA19-9 ≥ 37.00 U/ml was significantly higher than those with CA19-9 < 37.00 U/m (P < 0.001). E-cadherin and N-cadherin expression was not related to patients' gender, age, tumor size and CEA level (P > 0.05) (**Tables 2**-**4**).

Discussion

In the past, people focused on the role of coding RNA and ignored the role of non-coding RNA. Recently as the rapid development of biological technologies, especially the emergence of high throughput sequencing and gene microarray, large amount of important non-coding RNA has been discovered, such as miRNA and lncRNA. LncRNA was first discovered by Okazaki [11] in 2002 from mouse whole length cDNA sequence. Since 2007, a lot of tumor related lncRNAs were discovered, and caused an lncRNAs feaver. It became another research focus in gene study field following miRNA. The study of lncRNA now is still a tip of the iceberg, and lots of biological functions are yet unknown and needs exploring.

Colorectal colonscopy associated with tissue pathology study is the golden standard of colorectal cancer, however it's traumatic and has its limitations. Thus developing new colon cancer biomarkers of high specificity and sensitivity is another clinical project. Some studies in China and abroad showed that lncRNAs played key roles in tumor genesis, development, invasion and metastasis, similar to cancer-promoting genes or cancer suppressor genes. Up to

Table 3. E-cadherin expression in colorectal cancer and para-cancer tissues with clinical features

Clinical data	Case (n)	E-cadherin ($2^{-\Delta\Delta Ct}$) ($\bar{X} \pm S$)	*P* value
Gender			0.514
Male	20	0.441 ± 0.093	
Female	17	0.415 ± 0.115	
Age			0.322
< 60	10	0.356 ± 0.121	
≥ 60	27	0.427 ± 0.103	
Tumor size			0.274
< 5 cm	19	0.514 ± 0.133	
≥ 5 cm	18	0.611 ± 0.104	
Differentiation level			0.0003
Median to high	32	0.452 ± 0.106	
Low	5	0.224 ± 0.119	
Local invasion depth			0.0001
T1/T2	9	0.325 ± 0.042	
T3/T4	28	0.198 ± 0.025	
Dukes staging			0.0002
A/B	22	0.304 ± 0.101	
C/D	15	0.218 ± 0.126	
Vascular invasion			0.0001
No	27	0.377 ± 0.089	
Yes	10	0.298 ± 0.073	
Tumor location			0.896
Colon	18	0.614 ± 0.119	
Rectus	19	0.599 ± 0.203	
CEA (ng/ml)			0.086
< 6.50	15	0.622 ± 0.077	
≥ 6.50	22	0.583 ± 0.083	
CA19-9 (U/ml)			0.0004
< 37.00	25	0.378 ± 0.110	
≥ 37.00	12	0.226 ± 0.088	

now, discovered colon cancer related lncRNAs include CCAT1 [12], CCAT1-L [13], MALAT1 [14], GAS5 [15], HOTAIR [16], and CCAT2 [17] etc.

Colon Cancer Associated Transcript 1 (CCAT1) is a newly discovered long non-coding RNA discovered with RDA, cDNA cloning and RACE technology, and the whole length was 2628 nt, located near the well-known cancer gene c-Myc, or on 8q24 region. Much of colon cancer and prostate cancer genetic information was hidden in this region, and many cancer related single nucleotide polymorphisms (SNPs) occurred [18, 19]. Xiang [9] et al discovered a new lncRNA which is specifically expressed in colon cancer, located in "gene desert" region (-8q24 region) and 515 kb upstream of MYC gene by whole length sequencing of colon cancer. It overlaps with CCAT1 in spatial structure, the length is 5200 nt, and was named CCAT1-L. CCAT1-L can regulate MYC through regulating chromatin's higher structure, and promotes cancer genesis and development. Some researches show that CCAT1 is a potential tumor promotion gene. Alaiyanl et al [12] studied 10 samples of normal colon mucosa, 18 samples of colorectal adenomatous polyps, 22 samples of primary colon cancer, 16 normal para-carcinoma mucosa tissue, 20 samples of para-carcinoma lymph nodes, 8 samples of liver metastasis colon cancer and 19 samples of peritoneum metastasis colon cancer. The result showed that, compared to normal colon mucosa tissues, the expression of CCAT1 in other tissues all elevated in varying degrees, and the expression of CCAT1 was elevated in all colon adenoma to cancer process. Yang et al [20] studied 20 gastric cancer samples and para-carcinoma tissue, the result showed expression of CCAT1 and c-Myc was significantly higher in gastric cancer tissues than that in corresponding para-carcinoma tissues; Elevated expression level of CCAT1 in gastric cancer cell lines AGS and MKN45 promotes cancer cell proliferation and migration; and c-Myc can directly bind to E-box component of CCAT1 promoter region, and activates CCAT1, promotes the development of gastric cancer. Liu et al. [21] analyzed the CCAT1 expression level in ovarian cancer cell SKOV3.ip1, and it was 2.6 fold of that in SKOV3 with gene microarray, and 6.7 fold with qRT-PCR. The invasion ability of SKOV3.ip1 is much stronger than that of SKOV, and after processing SKOV3.ip1 CCAT1 with siRNA, its invasion ability decreases. Thus they predict CCAT1 potentially enhances the invasion ability of SKOV3.ip1, but the detailed mechanism is to be studied.

Invasion and metastasis is the basic biological nature of malignant cancer cells, and is the lethal factor of most cancer patients. Up to now, the mechanism of invasion and metasta-

Table 4. N-cadherin expression in colorectal cancer and para-cancer tissues with clinical features

Clinical data	Case (n)	N-cadherin ($2^{-\Delta\Delta Ct}$) ($\overline{X} \pm S$)	*P* value
Gender			0.585
Male	20	1.987 ± 0.554	
Female	17	2.178 ± 0.647	
Age			0.224
< 60	10	2.224 ± 0.675	
≥ 60	27	2.198 ± 0.776	
Tumor size			0.868
< 5 cm	19	1.976 ± 0.623	
≥ 5 cm	18	2.157 ± 0.763	
Differentiation level			0.0001
Median to high	32	2.194 ± 0.706	
Low	5	2.449 ± 0.619	
Local invasion depth			0.0001
T1/T2	9	1.855 ± 0.409	
T3/T4	28	2.119 ± 0.523	
Dukes staging			0.0002
A/B	22	1.683 ± 0.308	
C/D	15	1.887 ± 0.517	
Vascular invasion			0.0001
No	27	1.883 ± 0.554	
Yes	10	2.286 ± 0.608	
Tumor location			0.981
Colon	18	1.963 ± 0.319	
Rectus	19	1.999 ± 0.405	
CEA (ng/ml)			0.178
< 6.50	15	1.885 ± 0.307	
≥ 6.50	22	1.991 ± 0.514	
CA19-9 (U/ml)			0.0005
< 37.00	25	2.107 ± 0.319	
≥ 37.00	12	2.528 ± 0.482	

sis is not fully understood, but studies showed that tumor invasion and metastasis is a multi-factor and multi-stage process, depending on the interaction between cancer cells and internal environment factors which promotes cancer cell growth, invasion, metastasis and angiogenesis [22]. E-cadherin is a Ca^{2+} dependant adhesion molecule, with the same molecule affinity, and can stabilize cell-cell connection [23]. Vertebrates epithelial cells' intercellular connection is largely dependent on tight junction on the apical side and adheren junction and desomosome on the side [24]. Tight junction is composed by junctional adhesion molecule (JAM), claudin and occluding, and they are connected to actin cytoskeleton with ZO-1, ZO-2 and ZO-3. AJ is composed by transmembrane adhesion molecule nectin and E-cadherin, and they are connected to cytoskeleton with afadin and catenin. EMT will cause deficits in E-cadherin, claudin, occluding expression in epithelial cells, and destroy cell polarity. EMT will also promote overexpression of some resolvase like MMP which participates in extra-cellular matrix (collagen, laminin and fibronectin etc.) and basal membrane degrading and destruction, destroys the tissue barrier of cancer cell invasion, and making way for cancer cells detach from primary tumor to invade and metastasize. Conventionally, EMT is thought to happen in the beginning phase of tumor metastasis. Apart from giving cancer cell the ability of migration and invasion, EMT gives cancer cells with stem cell features, and enables cancer stem cells (CSCs) growth [25, 26]. CSCs are migration cells, which are the basis of tumor infiltration, metastasis and invasion. Because EMT are involved in some transcription factor blocking cell growth, the process often produces some cells which are difficult to proliferate, and mesenchymal- epithelial transition (MET) is helpful in cancer cell's invasion into secondary tissue or organ matrix and forming a metastatic tumor. In addition, EMT can block tumor aging and apoptosis, and help tumor cells escape from immune surveillance, thus survive in human body [27]. Up to now, tumor EMT regulation mechanism was not clear, according to researches, TGF-β, Wnt/β-catenin, Notch, Hedgehog, IL-6/STAT3 and NF-κB can induce EMT process. Important transcription factors in EMT are: Snail1, Snail2, Twist1, Twist2, ZEB1 and ZEB2, etc. [28, 29]. In addition, many non-coding RNA such as microRNA and long non-coding RNA participate in EMT regulation too [30].

In our study, we tested CCAT1, E-cadherin and N-cadherin expression in 37 colorectal cancer

tissues and para-carcinoma tissues with qRT-PCR, and analyzed its correlation with clinical and pathology features. The result shows: CCAT1 expression was upregulated in colorectal cancer tissues, and the expression level was significantly related with local infiltration depth, cancer staging, vascular invasion, CA19-9 level; expression of E-cadherin was significantly lower in cancer tissue than normal para-carcinoma tissues, expression of N-cadherin was significantly higher in cancer tissue than normal para-carcinoma tissues. The low expression of E-cadherin and high expression of N-cadherin was significantly related with local infiltration depth, tumor staging, vascular invasion, tumor differentiation level an CA19-9. Thus we predict CCAT1 may play a cancer promoting gene- like role, participate in the genesis, development, invasion and metastasis process of colon cancer, and mediate the EMT process of colorectal cancer. It is a possible tumor marker and target of gene therapy of colorectal cancer.

Disclosure of conflict of interest

None.

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