

Original Article

Experimental study on the expression of Id2 and E-cadherin in colorectal cancer tissues and its clinical significance

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Abstract: Recent studies have shown that there is close relationship of the occurrence and development of tumor with differentiation factors and other related factors, however, the exact mechanisms remain elusive. Here we demonstrated that the positive expression rate of Id2 in the colorectal cancer tissues was higher than that of the normal tissue adjacent to colorectal cancer, while the positive expression rate of E-cadherin in the colorectal cancer tissue was lower than that of the normal tissue adjacent to colorectal cancer. Moreover, correlation test showed that there was negative correlation between expression levels of Id2 and E-cadherin. The expression levels of Id2 and E-cadherin in colorectal cancer were correlated with the degree of tumor differentiation, TNM stage and lymph node metastasis, but uncorrelated with the age and sex. These findings suggest that there is close relationship between the expression levels of Id2 and E-cadherin in colorectal cancer and the occurrence and development of colorectal cancer. Thus this study is valuable in assessment and monitoring of clinical metastasis and prognosis of colorectal cancer.

Keywords: Colorectal cancer, inhibitors of differentiation 2, epithelial cadherin, human, clinicopathological characteristics

Introduction

Colorectal cancer is one of the most common gastrointestinal malignancies, and its incidence has increased in recent years. The occurrence and development of colorectal cancer have become a hotspot. The development of tumor is not only a process of constant cell proliferation, but also a process of constant cell differentiation suppression. The malignant degree of tumor cells is closely related to the degree of differentiation. When the inhibitory differentiation factors (Id), including Id1, Id2, Id3, Id4, were cloned since 1990 [1], it has been confirmed that there is an abnormal high expression level of Id protein in different types of tumor cells, and its biological behavior is associated with the high expression level of Id protein which regulates the differentiation of embryonic cells.

Differentiation inhibitory factor 2 (Id2) is a widely expressed regulatory factor of transcription in cell development process. As one member of Id protein family, it also has a helix-loop-helix structure. Earlier studies showed that Id2 was involved in the regulated process of the cell cycle, including development, maturity, growth, differentiation and death of cells. Later, it was found that ID2 showed diversity in the progressing of different types of tumor, such as infiltration, differentiation, invasion and metastasis of tumor [2-4]. At present, it is known that increased Id2 protein expression has been found in a variety of malignant tumor tissues, such as neuroblastoma [5], breast cancer [6], ovarian cancer [7], non-hodgkin's lymphoma [8] and pancreatic cancer [9]. Therefore, more and more attention has been paid to the occurrence and development of tumor.

Expression of Id2 and E-cadherin

Epithelial cadherin (E-cadherin) belongs to family members of calcium dependent adhesion protein, and its main function is to maintain the polarity of epithelial cells and the adhesion between the epithelial cells, which can effectively inhibit the invasion and metastasis of tumor cells [10]. In recent years, many studies found that there was close correlation of the invasion and metastasis of malignant tumor, especially the epithelial malignant tumor, with the expression level of E-cadherin and its dysfunction.

Therefore, in this study colorectal cancer tissues of patients were selected as the subjects to investigate and analyze the expression level of differentiation inhibitory factor-2 (Id-2) and epithelial cadherin (E-cadherin) in colorectal cancer tissues, and to investigate the relationship between clinicopathological parameters and Id-2 and E-cadherin expression in colorectal cancer as well as its significance, providing a new train of thoughts and theoretical basis for clinical prevention and treatment of colorectal cancer.

Materials and methods

Specimens of colorectal cancer

Between January 1, 2013 and September 1, 2014, 60 samples of primary colorectal cancer were collected from the Affiliated Hospital of Hebei Engineering University after surgical removal. The protocol was approved by Ethics Committee of the Medical College, Hebei University of Engineering, and the informed consent was obtained from each patient. Among the 60 cases, there were 46 males, and 14 females, aged 54 years on average (range, 33-75 years), with the median age of 55 years. According to the 7th version of tumor TNM staging system which was developed by the Cooperation of International Union against Cancer (UICC) and American Joint Committee on Cancer (AJCC), the stage of the colorectal cancer was divided into followings: stage I in 12 cases, stage II in 16 cases, stage III in 18 cases, and stage IV in 14 cases. According to histologic types of the WHO standard in 2000, there were 35 cases of high and moderate differentiation, 15 cases of low differentiation; there were 33 cases of lymph node metastasis, and 27 cases without lymph node metastasis. Cancer cells were not found in all broken end

tissues. All specimens were collected before chemotherapy and radiotherapy, and the diagnosis of colorectal cancer was confirmed pathologically.

Experimental groups

All the tissues were divided into two groups, cancer group (the central cancer tissue), and normal group (the normal tissues adjacent to the tumor, 5 cm away from the cancer tissues).

Histopathological features of tissues

All specimens were fixed with 4% paraformaldehyde solution, and then embedded in the paraffin. Sections of 4 μm in thickness were cut with a microtome and mounted on the glass slides. When the sections were dry, they were stained with Hematoxylin and eosin (H&E). The histopathological structures in the cancer group and normal group were observed under a light microscope.

Immunohistochemistry analysis

Immunohistochemistry SP technique was applied to observe and analyze the expression of Id-2 and E-cadherin in cancer group and normal group.

The above two groups of tissues were fixed with 4% paraformaldehyde solution, and then embedded in the paraffin. Sections of 4 μm in thickness were cut with a microtome and mounted on the glass slides. Using PBS instead of the primary antibody as the negative control, the sections were treated with 0.25% Triton X-100 for 10 min. After blocking with 5% normal goat serum for 20 min at 37°C, the rabbit anti-human monoclonal antibodies Id2 and E-cadherin (1:100) (Zhongshan Bio-Tech, Beijing, China) were incubated with the cells at 4°C overnight. Then the sections were incubated with goat anti-rabbit IgG (1:100; Boster Corporation, Wuhan, China) for 20 min at 37°C and stained with DAB (5 mg/mL; Sigma) for 5 min at room temperature. The sections were washed with PBS for 5 min three times and observed under a light microscope.

The judgement method of Id2 staining results

If there were tan or brown granules within cytoplasm of tumor cells, it was judged as positive

Expression of Id2 and E-cadherin

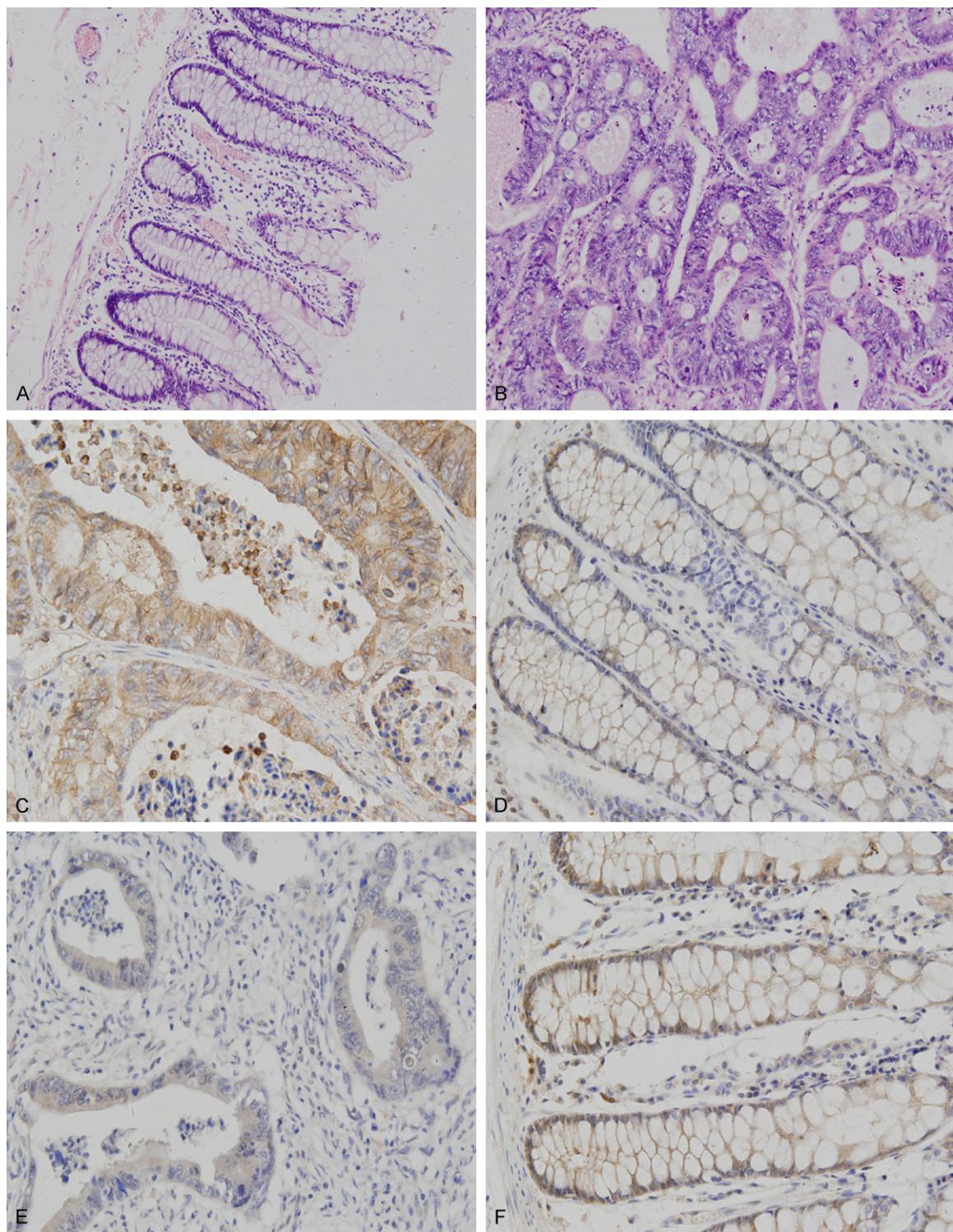


Figure 1. The observation of tissues under a light microscope ($\times 400$). A. The observation of normal colorectal tissues stained by H&E staining; B. The observation of colorectal cancer tissues stained by H&E staining; C. The observation of Id2 protein expression in normal colorectal tissues detected by immunohistochemistry; D. The observation of Id2 protein expression in colorectal cancer tissues detected by immunohistochemistry; E. The observation of E-cadherin protein expression in normal colorectal tissues detected by immunohistochemistry; F. The observation of E-cadherin protein expression in colorectal cancer tissues detected by immunohistochemistry.

Expression of Id2 and E-cadherin

Table 1. The protein Expression of Id2

Group	N	Id2				Rate of positive (%)	χ^2	P
		-	+	++	+++			
Normal colorectal tissue	60	31	19	6	4	48.33	6.806	0.009

Table 2. The protein Expression of E-cadherin

Group	N	E-cadherin			Rate of positive (%)	χ^2	P
		-	\pm	+			
Normal colorectal tissue	60	7	10	43	71.67	7.761	0.005
Colorectal cancer tissue	60	20	12	28	46.67		

Table 3. The correlation between Id2 and E-cadherin

Id2	E-cadherin		Total
	+	-	
+	8	21	29
-	20	11	31
Total	28	32	60

$r_s = -0.370$, $P=0.004$.

expression. Under 400 \times magnification, five fields were randomly selected for each section. According to the cell staining intensity and the proportion of positive cells, the result was judged using semi-quantitative integration, and the average was calculated. Compared with the background color, the staining intensity was scored: colorless, zero point; light yellow, 1 point; tan, 2 points; dark brown, three points. The proportion of positive cells was scored: no positive cells, zero point; $\leq 25\%$, 1 point; $26\% \sim 50\%$, 2 points; $51\% \sim 75\%$, 3 points; $\geq 76\%$, 4 points. After the multiplication of both scores, 0 point indicated negative (-), 1~3 points weak positive (+), 4~8 points positive (+ +), and 9~12 points strong positive (+ + +).

The judgement method of E-cadherin dyeing results

If there were tan or brown granules located in cell membrane of tumor cells, it was judged as positive expression. Under 400 \times magnification, five fields were randomly selected for each section. The proportion of positive cells was scored: <10%, negative expression (-); 10%~90%, lower expression (+/-); >90%, normal expression (+) (-) and (\pm) cases were included in the abnormal cases.

Statistical analysis

All data were analyzed with SPSS 15.0 for Windows. Comparison between groups was detected by chi-square test. $P<0.05$ indicated significant difference. Pearson correlation test was applied to detect the expression correlation between Id2 and E-cadherin, and the significant level was set as 0.01 (2-tailed).

Results

Histopathological structure features

The normal tissue: mucosa surface was smooth and glossy, without villi. The epithelia were monolayer and columnar, which were composed of columnar cells and considerable goblet cells. Lamina propria contained many single tubular glands and solitary lymph nodules, and glandular tubes had the same size and lined up in order. The gland was composed of columnar cells and considerable goblet cells. Lamina muscularis mucosa was comprised of lamellar inner ring and outside microtubule smooth muscles (**Figure 1A**).

Colorectal cancer tissues: well and moderately differentiated tissues were composed of glandular tubes of different size, while poorly differentiated tissues were composed of a small quantity of glandular tubes, cell trabs and cancer nests. There were poor cell differentiation, pleomorphism and different size. Cells showed many layers of fake, and cell nuclei were large with karyokinesis and few cytoplasm (**Figure 1B**).

Expression of Id2 protein in different colorectal tissues

The positive expression rate of Id2 in the normal group and cancer group was 48.33% and 71.67%, respectively, that's to say, the positive expression rate of Id2 in the normal group was higher compared to the cancer group, with significant difference ($P<0.05$) (**Figure 1C, 1D** and **Table 1**).

Expression of E-cadherin protein in different colorectal tissues

The positive expression rate of E-cadherin in the normal group and cancer group was 71.67%

Expression of Id2 and E-cadherin

Table 4. The relationship between the clinical pathologic character and the expression of Id2

Clinical pathologic character	N	Id2					Rate of positive (%)	χ^2	P
		-	+	++	+++				
Age									
<55	29	9	11	5	4		68.97	0.202	0.653
≥55	31	8	11	8	4		74.19		
Gender									
Man	44	12	16	11	5		72.73	0.091	0.762
Woman	16	5	6	2	3		68.75		
Degree of differentiation									
Well and Moderately	35	13	14	6	2		62.86	4.374	0.036
Poorly	25	4	8	7	6		84.00		
Stage of TNM									
I-II	28	12	9	4	3		57.14	5.454	0.020
III-IV	32	5	13	9	5		84.38		
Lymph node metastasis									
Yes	34	14	12	5	3		58.82	6.374	0.012
No	26	3	10	8	5		88.46		

Table 5. The relationship between the clinical pathologic character and the expression of E-cadherin

Clinical pathologic character	N	E-cadherin					Rate of positive (%)	χ^2	P
		-	±	+					
Age									
<55	29	11	5	13			44.83	0.076	0.782
≥55	31	9	7	15			48.39		
Gender									
Man	44	14	9	21			47.73	0.075	0.785
Woman	16	6	3	7			43.75		
Degree of differentiation									
Well and moderately	35	12	2	21			60.00	6.000	0.014
Poorly	25	8	10	7			28.00		
Stage of TNM									
I-II	28	7	4	17			60.71	4.163	0.041
III-IV	32	13	8	11			34.38		
Lymph node metastasis									
Yes	34	9	5	20			58.82	4.659	0.031
No	26	11	7	8			30.77		

Correlation between Id2 and E-cadherin expression levels

Pearson correlation test showed that there was negative correlation between Id2 and E-cadherin expression levels ($r=-0.370$, $P=0.004$) (Table 3).

The relationship between the positive expression rate of Id2 in the cancer group and the clinicopathological characteristics

The positive expression rate of Id2 (62.86%) in the poorly differentiated group was significantly higher than that in the moderately and well differentiated groups (36.00%), and that in the lymph node metastasis group (73.07%) was significantly higher compared to no lymph node metastasis group (41.12%). The positive expression rate of Id2 in III~IV stage group (64.29%) was significantly higher than that in I~II stage group (37.50%). The Id2 expression levels in colorectal cancer tissues were not significantly associated with gender and age of the patients ($P>0.05$) (Table 4).

The relationship between the positive expression rate of E-cadherin in the cancer group and the clinicopathological characteristics

and 46.67%, respectively, and the positive expression rate of E-cadherin in the normal group was lower than that of the cancer group, with significant difference ($P<0.05$) (Figure 1E, 1F and Table 2).

The positive expression rate of E-cadherin (71.43%) in moderately and well differentiated groups was significantly higher than that in the poorly differentiated group (40.00%), and that in the no lymph node metastasis group

(69.23%) was significantly higher compared to lymph node metastasis group (41.18%). The positive expression rate of E-cadherin in I~II stage group (71.43%) was significantly higher than that in III~IV stage group (43.75%). The E-cadherin expression level in colorectal cancer tissues was not significantly associated with gender and age of the patients ($P>0.05$) (Table 5).

Discussion

Studies have shown that Id2 protein is closely related to tumor differentiation degree, tumor infiltration, lymph node metastasis and prognosis of tumor, etc. The study of Cooper et al. [11] has shown that the higher protein expression level of Id2 contributed to more aggressiveness of tumor cells. Itahana [12] found that the Id2 gene was transferred into epithelial cells of SCp2 rats, and cell proliferation was significantly slowed down. Id2 mRNA expression was detected in breast cancer cells by Northern imprinting, and it was found that the expression level of Id2 was increased in breast cancer cell lines of low invasion and high differentiation. The results of clinicopathological examinations showed that the protein expression levels of Id2 in breast carcinoma *in situ* were significantly higher than those in invasive ductal carcinoma of the breast. The higher the histological grade, the lower the protein expression level of Id2 was. For instance, the protein expression level of Id2 in grade I infiltrating ductal cancer was significantly higher than that in grade III. Schindl [13] detected the protein expression level of Id2 in early stage of cervical cancer tissues by the immunohistochemical technique, and found that the protein expression level of Id2 was uncorrelated with the prognosis of cervical cancer. The positive expression rate of Id2 in colorectal cancer tissues was obviously higher than that of normal tissues. The expression level of Id2 was related to the degree of differentiation, TNM stage and lymph node metastasis, but unrelated to age and gender of the patients.

E-cadherin, a single-span transmembrane glycoprotein of five repeats and cytoplasmic domain, is expressed primarily in epithelial cells. It is a kind of calcium dependent transmembrane glycoproteins and plays an important role in normal physiologic processes, such

as development, cell polarity, cellular adhesion, and tissue morphology [14, 15]. Because it can mediate adhesion between cells, the decrease or loss of E-cadherin can reduce the adhesion between cells and change the configuration of cells [14, 16]. E-cadherin is a well-characterized tumor suppressor, well-known for its important function in epithelial mesenchymal transition (EMT). During EMT, down-regulation of E-cadherin expression leads to loss of epithelial characteristics and acquisition of a mesenchymal phenotype, which promotes cell proliferation, motility and invasiveness and contributes to cancer progression [17]. Several studies have provided consistent evidence for the function of E-cadherin as a tumor suppressor [18]. Down-regulated expression, loss or change of the position of E-cadherin is observed in the development of various types of tumor such as hepatocellular carcinoma [19, 20], breast cancer [21], ovarian cancer [22], etc. This study revealed that the expression level of E-cadherin in colorectal cancer tissues was decreased, and the down-regulated E-cadherin expression was related to low differentiation of tumor, lymph node metastasis and TNM stage. It was suggested that the down-regulated E-cadherin expression in colorectal cancer tissues promoted invasion and metastasis of colorectal cancer cells and led to the development of colorectal cancer.

This study also revealed that there was negative relationship between the expression level of Id2 and E-cadherin in colorectal cancer, and the higher expression level of Id2 and lower expression level of E-cadherin promoted the occurrence and development of colorectal cancer. However, it is not clear about the interaction and influence between the two at present, and further research is still needed.

In summary, the excessive expression of Id2 and the down-regulated expression of E-cadherin play an important role in the occurrence and development of colorectal cancer. Therefore, the expression level of Id2 and E-cadherin can be considered as an indicator to assess and monitor the clinical metastasis and prognosis of colorectal cancer.

Disclosure of conflict of interest

None.

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