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RESEARCH ARTICLE

Expression and Significance of Ezrin and E-cadherin in Colorectal Carcinoma

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Abstract

Background: Colorectal carcinoma (CRC) is the third most common human cancer and the fourth most common cause of cancer deaths. Ezrin may be involved in several functions including cell adhesion, motility and cell survival. There is increasing evidence that it regulates tumor progression and metastasis. Also E-cadherin dysfunction is proposed to contribute to cancer progression, facilitating proliferation, invasion, and possibly metastasis. **Aim**: To evaluate the expression of Ezrin and E-cadherin in colorectal carcinoma and its relation to clinicopathological parameters. **Material and Methods:** The expressions of Ezrin and E-cadherin were detected by immunohistochemical staining (streptavidine-peroxidase method) in 50 cases of colorectal carcinoma and their adjacent normal colorectal mucosa. **Results:** The expression of Ezrin in colorectal carcinoma was significantly higher than that in normal colorectal mucosa (74% *versus* 20%, $P < 0.001$).The expression of E-cadherin in colorectal carcinoma was significantly lower than that in adjacent normal colorectal mucosa (32% *versus* 100%, $P < 0.001$). Ezrin and E-cadherin expression were associated with decreased tumor differentiation, advanced Dukes stage and lymph node metastasis ($P<0.05$). Ezrin expression was associated with invasion depth ($P=0.009$).The expressions of E-cadherin and Ezrin were negative related in colorectal cancer ($P<0.001$). **Conclusion**: Ezrin and E-cadherin are associated with the tumorigenesis and development of colorectal cancer, and play an important role in invasion and metastasis processes. Combined detection of the expression of E-cadherin and Ezrin may serve as an important prognostic predictor for colorectal cancer.

INTRODUCTION

Colorectal carcinoma is the third most common human cancer and the fourth most common cause of cancer deaths, with one million new cases diagnosed annually and more than half a million cases die from this disease, accounting for 8% of all cancer-related deaths worldwide [1]. In Egypt, colon cancer is a surprisingly young-age disease and has a more unfavorable outcome. More than one third of Egyptian colorectal carcinomas affect young people before 40 years and 60% affect people younger than 50 years, whereas only 15% of patients were older than 60 years in a multicenter study in Egypt [2]. Egyptian colorectal carcinomas usually present at an advanced stage, with low prevalence of colorectal polyps, and high proportion of rectal cancer [2,3].

Ezrin belongs to the Ezrin/radixin/moesin (ERM) protein family, which acts as membrane organizers and linkers between cell surface receptor, especially adhesion molecule and actin cytoskeleton and have traditionally been known as molecules involved in maintaining the integrity and morphology of cells [4]. These proteins also have been widely shown to regulate the migration of the cells. They have also been suggested to be candidate molecules in directional cell movement, including that of cancer cells [5]. Ezrin is mainly expressed at the top of the

cell surface to participate and to maintain the polarity of epithelial cells [6]. Recent studies have found that, through regulating adhesion molecules and signal transduction pathways, Ezrin is involved in cell-cell and cell-matrix interactions, and might play an important role in the process of tumor cell invasion and metastasis [7]. Overexpression of Ezrin protein is correlated with the metastatic potential of several cancers [8-10].

In recent years, research has shown that epithelial-mesenchymal transition (EMT) offers a relevant point for research. EMT refers to the process in which an epithelial cell disengages from its parent tissue in exchange for morphology and adhesion marker profiles, consistent with a mesenchymal cell. It is regarded as the first necessary step of invasion and metastasis [11]. The vast majority of known signaling pathways have been implicated in the regulation of EMT [12]. Loss of E-cadherin expression is considered to be a hallmark of EMT. E-cadherin, a key transmembrane molecule, cell adhesion protein, is a member of a family of functionally related transmembrane glycoproteins that mediate Ca2+-dependent intercellular adhesion. The adhesion is mediated via interaction with adjacent cells through their N-terminal ectodomains. The cytoplasmic terminal tail of E-cadherin links specifically to β-catenin that binds directly to cytoskeletal actin, which helps to assemble epithelial cell sheets. A loss of its expression or function diminishes cell-cell contacts, which leads to impaired intercellular signaling. E-cadherin expression was shown to be frequently downregulated in many different types of tumors, where it accompanies the invasiveness and metastatic behavior of malignant cells [13].

Some studies have revealed an association of Ezrin with E-cadherin and β catenin through hepatocyte growth factor (HGF) induced phosphorylation of Ezrin, resulting in reduced cell–cell adhesiveness and an increase in cell motility and invasiveness [4]. Therefore, Ezrin regulates cell–cell and cell–matrix adhesion by interacting with E-cadherin and β catenin. These findings demonstrate the importance of the association between HGF/met, the cadherin/catenin complex, and Ezrin proteins in the acquisition of a tumor invasive phenotype [14]. Also Ezrin is important for localization of E-cadherin to the plasma membrane, so that, E-cadherin and β-catenin downregulation is linked to Ezrin overexpression [15].

This study aimed to evaluate the expression of Ezrin and E-cadherin in colorectal carcinoma and its relation to clinicopathological parameters.

MATERIAL AND METHODS

Tissue specimens

The immunohistochemistry was performed on paraffin-embedded tissue samples. Fifty colorectal adenocarcinoma diagnosed by postoperative pathology were obtained from the archive of Pathology Department, Faculty of Medicine, Zagazig University in the period 2011-2013. None of the patients had received chemotherapy or radiation therapy before surgery. Clinicopathologic data were based on the original pathologic reports and the patients' clinical records. There were 22 male and 28 female patients, whose ages ranged from 31 to 70 years, 30 were <55years and 20 were ≥ 55years. Histologically, 23 patients had well-differentiated, 17 had moderately differentiated, and 10 had poorly differentiated adenocarcinoma. Twenty one patients had lymph node metastasis and 29 patients had no nodal metastasis. Twenty nine patients were of Dukes A to B stage, and 21 were of C to D stage. Regarding the depth of invasion, thirty one were T1andT2 and 19 were T3and T4. Ten patients had distant metastasis. Ethical approval and patient consent were obtained.

Four-micron thick sections were transferred to adhesive slides from representative formalin fixed, paraffin-embedded blocks. The sections were deparaffinized in xylene and dehydrated through a series of graded alcohols. Antigen retrieval was performed by boiling tissue sections in citrate buffer, pH 6.0 in a microwave oven, for 10 minutes. To block endogenous peroxidase activity, the sections were incubated with 0.3% hydrogen peroxide in methanol for 30 minutes after cooling to room temperature. Primary mouse monoclonal antibodies for both Ezrin (3C12, 1:100, Lab vision, Fremont, CA, U.S.A.) and E-cadherin (dilution 1:25, clone 36B5, Novocastra, U.K.) were added for 30 minutes at room temperature. A biotinylated secondary antibody was applied to sections for 30 minutes at room temperature. After incubation, the reaction product was visualized using diaminobenzidine. Finally, the sections were counterstained with Mayer's hematoxylin.

Positive and negative control:

Each block contained an internal positive control for both Ezrin and E-cadherin consisting of normal non-neoplastic colorectal mucosa. Negative control sections were treated with phosphate-buffered saline (PBS) instead of primary antibody.

Scoring system

The stained slides were examined by two pathologists (SA, TI) blindly and independently without knowing the original histologic diagnosis. In the evaluation of immunohistochemical staining, brown membranous or cytoplasmic staining was accepted as Ezrin immunoreactivity, each stained slide was assessed and given a score according to the classification standard of Mathew et al [16]: score 0, no expression; score 1, < 50% of cells staining positive expression; score 2, ≥ 50% of cells staining positive expression. Score 0-1 was recorded as negative, and score 2 recorded as positive.

For E-cadherin, the scoring was determined as previous studies [17,18]. Preserved expression of E-cadherin was defined where tumor cells were stained as strongly and homogeneously as normal epithelial cells. Heterogeneous staining, weaker staining, staining with altered cellular distribution (cytoplasmic) or completely negative staining of E-cadherin was considered as reduced expression.

Statistical analysis

Data were represented as number and percentage. The differences were compared for statistical significance by chi-square test. Difference was considered significant at $p<0.05$. The statistical analysis was performed using (SPSS 16.0 for Windows; SPSS Inc. Chicago, Illinois, USA).

RESULTS

Ezrin immunostaining

Ezrin protein was expressed focally in normal colonic mucosa (figure 1a). The staining pattern of Ezrin proteins in tumor cells was seen in cell membrane, and in the cytoplasm (more in metastatic and tumors of high grade), figure 1 (b, c and d). The positive expression of Ezrin in colorectal cancer was significantly higher than that in normal colorectal mucosa (figure 1). The positive rate of Ezrin protein in normal colorectal mucosa was 20% (10/50) and 74% (37/50) in colorectal cancer tissues. There were significant differences between the two groups (74.00% *versus* 20%, $P < 0.001$), as shown in table1.

Relationship between Ezrin expression in colorectal cancer tissues and clinicopathological parameters Figure 1 (b, c, d)

There was a statistically significant relationship between Ezrin expression and the degree of tumor differentiation, lymph node metastasis, advanced Dukes stage, increased depth of invasion and distant metastasis (88.9% *versus* 56.5%, $P = 0.009$; 90.5% *versus* 62.1%, $P =0.024$; 90.5% *versus* 62.1%, $P =0.024$; 94.7% *versus* 61.3%, $P =0.009$ and 100% *versus* 67.5%, $P =0.036$, respectively), as shown in table 2. No significant relationship was found between Ezrin expression and age or sex (80% *versus* 70%, $P =0.43$ and 82.1 % *versus* 63.6%, $P =0.139$, respectively), table (2).

E-cadherin immunostaining

E-cadherin expression was exclusively membranous in normal colonic mucosa (figure 1a). Preserved E-cadherin expression with staining intensity and cellular localization similar to those seen in normal colonic mucosa was found in 32% of cases. Reduced E-cadherin expression (heterogenous, cytoplasmic or negative staining) was evident in 34 out of 50 (68%) of cases. The positive expression of E-cadherin in normal colorectal mucosa was significantly higher than that in colorectal cancer tissue. The positive rate of E-cadherin protein in normal colorectal mucosa was 100% (50/50) and 32% (16/50) in colorectal cancer tissues. There were significant differences between the two groups (100% *versus* 32%, $P < 0.001$), as shown in table 1.

Relationship between E-cadherin expression in colorectal cancer tissues and clinicopathological parameters Figure 2 (b, c, d)

Reduced E-cadherin expression was significantly related to decreasing tumor differentiation, nodal metastasis and advanced Dukes stage (81.5% *versus* 52.2%, $P = 0.027$; 85.7% *versus* 55.2%, $P =0.022$; and 85.7% *versus* 55.2%, $P =0.022$, respectively), as shown in table 2.

No significant relationship was found between reduced E-cadherin expression and age, sex, depth of invasion or distant metastasis (73.3% versus 60%, $P =0.322$; 68.2 % versus 67.9%, $P =0.98$; 84.2% versus 58.1%, $P =0.054$; and 80% versus 65%, $P =0.363$, respectively), table (2).

Relationship between Ezrin expression and E-cadherin expression in colorectal cancer tissue:

There was a significant negative relationship between Ezrin expression and E-cadherin expression, Ezrin-positive patients showed a higher percentage of reduced E-cadherin than Ezrin-negative ones (86.5% versus 15.4%, $P < 0.001$, table 3).

Table 1: Ezrin and E-cadherin expression in normal colorectal tissue in relation to colorectal cancer tissue

Type of colorectal tissue	N	Ezrin		*P*	E-cadherin		*P*
		Negative	Positive		Preserved	Reduced	
Normal colorectal tissue	50	40 (80%)	10 (20%)	<0.001	50 (100%)	0(0.0%)	<0.001
Colorectal cancer tissue	50	13 (26%)	37 (74%)		16 (32%)	34 (68%)	

Table 2: Ezrin and E-cadherin expression in colorectal cancer tissue in relation to clinicopathologic parameters

Parameters	Total	Ezrin		*P*	E-cadherin		*P*
		Negative 13 (26%)	Positive 37 (74%)		Preserved 16 (32%)	Reduced 34 (68%)	
Age							
<55	30	9 (30)	21 (70)	0.43	8(26.7)	22(73.3)	0.322
≥ 55	20	4 (20)	16 (80)		8(40)	12(60)	
Gender							
Male	22(44)	8 (36.4)	14 (63.6)	0.139	7 (31.8)	15 (68.2)	0.98
Female	28(56)	5 (17.9)	23 (82.1)		9 (32.1)	19 (67.9)	
Tumor differentiation							
Well	23(46)	10 (43.5)	13 (56.5)	0.009	11 (47.8)	12 (52.2)	0.027
Moderate &poor	27(54)	3 (11.1)	24 (88.9)		5 (18.5)	22 (81.5)	
Nodal metastasis							
With	21(42)	2 (9.5)	19 (90.5)	0.024	3 (14.3)	18 (85.7)	0.022
Without	29(58)	11 (37.9)	18 (62.1)		13 (44.8)	16 (55.2)	
Depth of invasion							
T1,2	31(62)	12 (38.7)	19(61.3)	0.009	13 (41.9)	18 (58.1)	0.054
T3,4	19(38)	1 (5.3)	18(94.7)		3 (15.8)	16 (84.2)	
Dukes stage							
A&B	29(58)	11 (37.9)	18 (62.1)	0.024	13 (44.8)	16 (55.2)	0.022
C&D	21(42)	2 (9.5)	19 (90.5)		3 (14.3)	18 (85.7)	
Distant metastasis							
With	10(20)	0 (0.0)	10 (100)	0.036	2 (20)	80 (80)	0.363
Without	40(80)	13 (32.5)	27 (67.5)		14 (35)	26 (65)	

Table 3: Association between Ezrin and E-cadherin immunoexpression in the study group

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E-cadherin / Ezrin	Preserved (N=16)	Reduced (N=34)
Positive (N=37)	5(13.5%)	32(86.5%)
Negative (N=13)	11(84.6%)	2(15.4%)
$(X^2) = 22.4$	*P* value < 0.001	

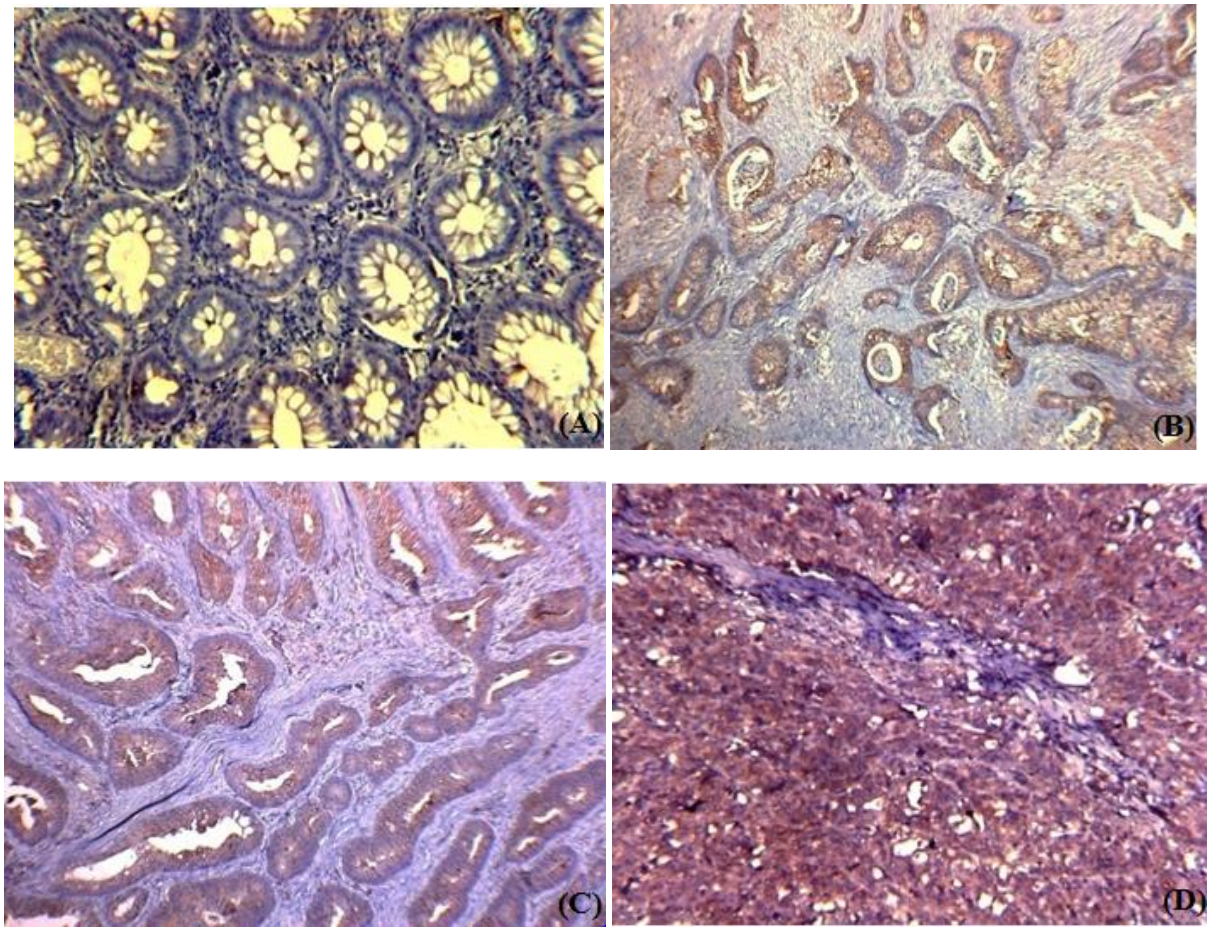
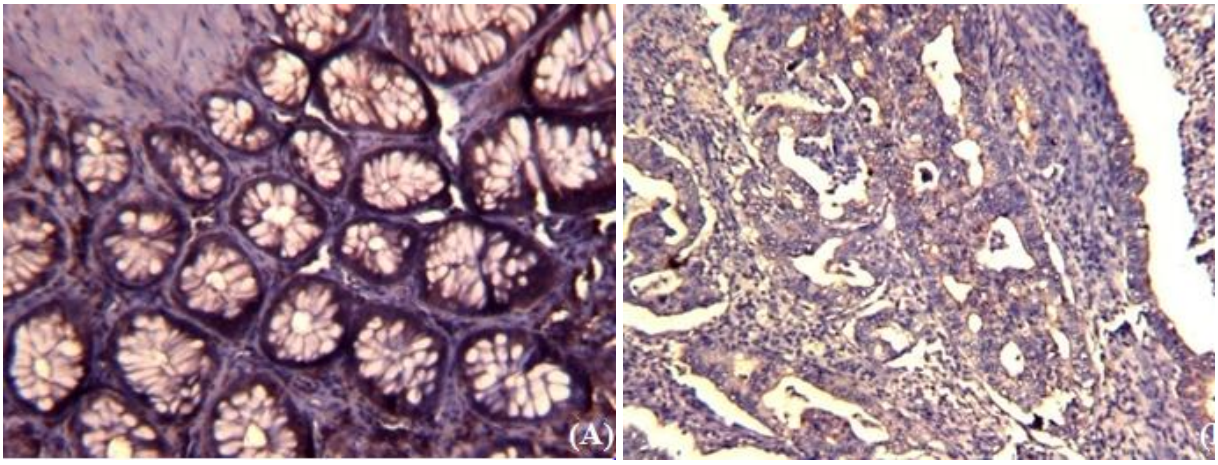


Figure 1. Immunohistochemical staining patterns for Ezrin; (A) Normal colorectal epithelium showing focal membranous expression *(ABC, DAB chromogen X200)*; (B) Membranous expression of Ezrin in colorectal adenocarcinoma low grade *(ABC, DAB chromogen X100)*; (C) Cytoplasmic expression of Ezrin in colorectal adenocarcinoma with nodal metastasis *(ABC, DAB chromogen X100)*; (D) Intense cytoplasmic expression of Ezrin in poorly differentiated colorectal adenocarcinoma *(ABC, DAB chromogen X200)*



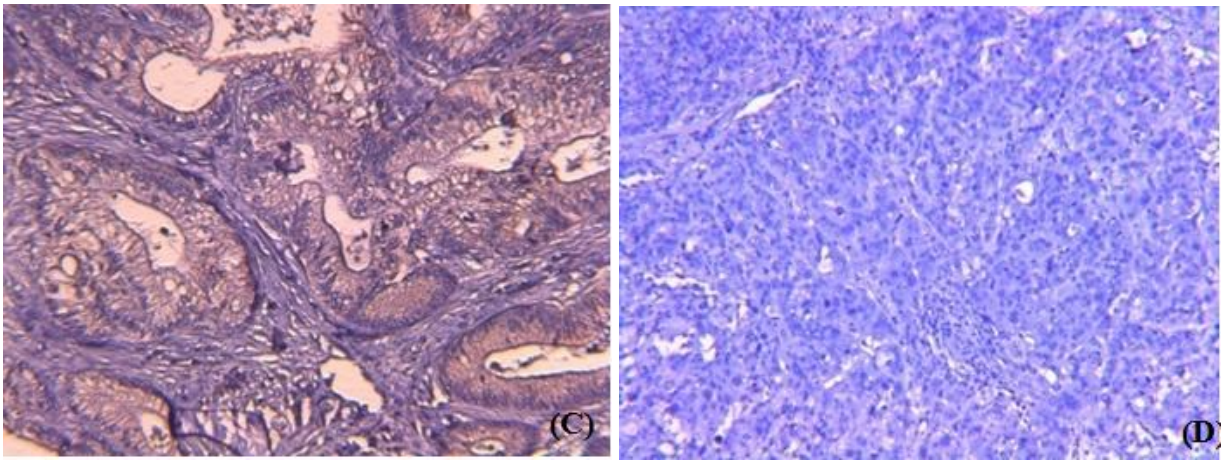


Figure 2. Immunohistochemical staining patterns for E-cadherin; (A) Normal colorectal epithelium showing strong membranous expression; (B) Reduced E-cadherin expression in colorectal adenocarcinoma; (C) Reduced membranous and aberrant cytoplasmic expression of E-cadherin in colorectal adenocarcinoma; (D) Negative expression of E-cadherin in colorectal adenocarcinoma. ***Magnification of all samples (ABC, DAB chromogen X200)***

DISCUSSION

Ezrin is known to be a component of cell-surface structures that, together with ERM proteins, are involved in cell adhesion to the extracellular matrix, as well as in cell-cell interactions [7]. One of the functions of Ezrin is to participate in the formation of cell-surface complexes, such as E-cadherin and integrin that mediate cell-cell and cell-extracellular matrix attachments [13]. E-cadherin is one of the key proteins in epithelial-mesenchymal transition (EMT), a process crucial for the metastatic process [19]. There is a possibility that Ezrin affects cadherin switching and EMT induction through signaling modifications [20].

Pujuguet et al. showed that Ezrin plays a role in the transition from polarized epithelial cell form to a more spreading form by regulating the transport of E-cadherin to the plasma membrane. This mechanism between Ezrin and E-cadherin may be important in its emerging role in tumor progression [21].

In our study, we examined the expression of Ezrin in colorectal cancer tissue, and the relationship between the overexpression of Ezrin protein and clinicopathologic parameters in colorectal carcinoma. We found that the expression of Ezrin in colorectal cancer tissue was membranous and/or cytoplasmic and was significantly higher than that in adjacent normal colorectal mucosa. Similar findings were demonstrated by previous studies [7,22-26]. Köbel et al. and Choi also reported that Ezrin was overexpressed in cancer cells, and it is localized at the apical membrane in normal cells; however, it is distributed throughout the cytoplasmic or membranous portion in cancer cells [27,28]. However, Elzagheid et al. and Lin and Chen showed that Ezrin expression in the colorectal cells was typically cytoplasmic [29,30]. These findings may be related to the poor survival outcomes of patients of the study group.

This study demonstrated that overexpression of Ezrin was significantly related to nodal metastasis and advanced Dukes stage, in accordance with previous studies [23,25,26,31-33].

In our study, we found that overexpression of Ezrin was significantly related to decreased tumor differentiation and presence of distant metastasis. Some previous studies reported similar relation between Ezrin expression and tumor grade [23,26,31,32,34]. While, Ge et al. and Ying et al. showed that there was no relationship between the expression of Ezrin and differentiation of malignancy and its expression in metastatic group was significantly higher than that in non-metastatic group [22,24]. This may be attributed to different statistical cut off values.

As regards the depth of invasion, our study revealed that there was a significant association between increasing the depth of invasion and the overexpression of Ezrin, this agrees with the results obtained by Wei et al. [31], but not in agreement with Ge et al. and Zhang, who could not find similar correlation [22,34].

Patara et al. found a significant relation between Ezrin expression and differentiation degree, lymph node invasion and metastasis at diagnosis and concluded that higher expression of Ezrin correlates with tumor aggressiveness and worse prognosis for colorectal cancer [32].

In our work, there was no relationship between the expression of Ezrin and age or sex, comparable to data recorded by previous studies [22,24,26,34].

Regarding the results of E-cadherin, we found that normal epithelial cells were strongly and homogeneously stained in the membrane, while tumor cells were stained mainly in the membrane and occasionally in the cytoplasm with a significant difference in expression. The loss of expression of E-cadherin in cell membranes, often with relocation to the cytoplasm, has been observed in many malignancies, and is an indicator of abnormal cadherin function [31,35,36]. Genetic alterations, transcriptional changes or protein trafficking could disturb E-cadherin functions. A possible explanation for reduced level of E-cadherin is that proper formation of cell-cell junctions could be disrupted by Src kinase. Elevated Src expression disturbs E-cadherin regulation in colon cancer cells [37].

The present study revealed that reduced E-cadherin expression was associated with loss of differentiation as has been previously reported [38,39]. Zhang, in his study found that The positive rate of E-cadherin was not associated with the degree of differentiation [34], while, Bezdekova et al. showed that there were no differences between protein expression in tumors and normal mucosa and that E-cadherin expression in adenocarcinomas was detected to be mostly strong in grade II and grade III tumors. They explain these findings by suggesting that E-cadherin presence in colorectal carcinoma, either caused no alteration of adherent junctions or that intercellular signaling may be at least partly E-cadherin independent, and the activation of catenin can be mediated directly via Snail-1 and epidermal growth factor receptor [40].

Decreased level of membranous E-cadherin could have an impact on invasiveness of colorectal cancer cells. Tumor cell release as a result of disruption of adherent junctions plays a crucial role in metastasis [36].

In our study, we found that reduced E-cadherin expression was significantly related to nodal metastasis, advanced Dukes stage and distant metastasis, this is consistent with other previous studies [31,38,39,41]. However, some studies failed to find a correlation between E-cadherin loss and nodal metastasis, Dukes stage, and distant metastasis [34,36]. This variability may be due to different scoring systems or different statistical cut off points.

In this work, no relationship was detected between the expression of E-cadherin and age, sex or depth of infiltration, comparable with data recorded by previous studies [18, 34].

In this study, the expressions of E-cadherin and Ezrin were negative related in colorectal cancer; this is consistent with other results [31]. Also, Zhang, in his study found a significant negative correlation between Ezrin and E-cadherin and suggested that the former may down-regulate the expression of the latter to promote the development and progression of colorectal cancer [34].

CONCLUSIONS

Ezrin and E-cadherin are associated with the tumorigenesis and development of the colorectal cancer, and play an important role in invasion and metastasis processes. Combined detection of the expression of E-cadherin and Ezrin may serve as an important prognostic predictor for colorectal cancer. The studies on the correlation between Ezrin protein, E-cadherin and cancer might help us further reveal the tumor invasion and metastasis mechanisms, and find targets for inhibiting tumor metastasis, or indicators that forecasts the prognosis of patients with tumors.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SA, TI, AH: Provision of study material, design, collection, tissue processing techniques and assembly of data; SA, TI: Examination and histologic grading of the pathologic specimens. AH: Sharing in identification of the normal tissues and statistical analysis of results. SA wrote the manuscript; TI and AH critically revised the manuscript. All authors have read and approved the final manuscript.

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