ORIGINAL RESEARCH

The Expression and Clinical Significance of Ezrin and MMP-9 in Colorectal Cancer Tissue

Qingfei Zhang, MM; Zhiqian Ye, BM; Yuanchan Zhang, MM; Fabiao Liao, MM

ABSTRACT

Objective • Colorectal cancer is a malignant tumor with high mortality, but is hard to detect at its early stage. Recent studies highlighted the crucial roles of Ezrin protein and MMP-9 in the development and malignancy of colorectal cancer, but Ezrin protein and MMP-9 in early diagnosis of colorectal cancer require further investigation. Therefore, we aimed to investigate their roles in the occurrence and metastasis of colorectal cancer, and to analyze their clinical significance in diagnosing and treating colorectal cancer.

Method • The diagnosis of collected colorectal cancer tissue and adjacent tissue samples from colorectal cancer patients confirmed by clinical symptoms was performed using Hematoxylin Eosin staining. The expression levels of Ezrin and MMP-9 in 50 colorectal cancer tissue and 50 cases adjacent colorectal cancer tissue were detected by the immuno-histochemical MaxVision method. The relationship between the positive expression rate of Ezrin and MMP-9 in colorectal cancer tissue and clinical pathological factors was analyzed, and the correlation between Ezrin and MMP-9 was examined.

Results • The positive expression rate of Ezrin in colorectal cancer tissue (78%) was significantly higher compared to adjacent non-cancerous tissues (6.0%) ($P < .05$). There was no significant correlation of gender/age and Ezrin/MMP-9 expressions ($P > .05$). The expression level of Ezrin exhibited statistically significant differences in the pathological factors including tumor diameter, depth of invasion, degree of differentiation, presence or absence of lymph node metastasis, and distant metastasis ($P < .05$). Additionally, the positive expression rate of MMP-9 in colorectal cancer tissue (76%) was markedly elevated compared to adjacent tissues (8.0%) ($P < .05$). The expression level of MMP-9 showed statistically significant differences in the pathological factors including tumor diameter, depth of invasion, degree of differentiation, presence or absence of lymph node metastasis, and distant metastasis ($P<0.05$). In addition, the expression of Ezrin and MMP-9 in colorectal cancer tissue showed a significant positive correlation (r=0.637, $P < .01$).

Conclusion • Ezrin and MMP-9 may synergistically participate in the occurrence, invasion, and metastasis of colorectal cancer. The combined assessment of Ezrin and MMP-9 expression levels in colorectal cancer patients holds significant potential for clinical diagnosis and personalized therapeutic applications. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

From a worldwide perspective, colorectal cancer ranks third in terms of incidence rate and mortality among all malignant tumors. It is widely considered a high-incidence malignant tumor. Due to the improvement of living standards in China, as well as the changes in people's diet and dietary structure, the incidence rate of rectal cancer has continued to rise in recent years, and the age of rectal cancer patients continues to tend to be younger. It is reported that the mortality of colorectal cancer declined overall from 2011-2020, but increased in individuals younger than 50 years and in Native Americans younger than 65 years. Despite continued overall declines, colorectal cancer is rapidly shifting to diagnosis at a younger age, at a more advanced stage, and in the left colon/rectum.[1] What's worse, most patients have already reached the stage of progression by the time they seek medical treatment because of the lack of specific clinical manifestations in early colorectal cancer. Despite the continuous progress of tumor surgery,

radiotherapy, chemotherapy, immunity, and other treatment methods, the 5-year survival rate of colorectal cancer patients has significantly improved. However, the sequelae of surgical treatment and the side effects of radiotherapy and chemotherapy still make the prognosis of rectal cancer patients unsatisfactory. The anterior resection that is commonly used as the surgical approach against colorectal cancer is known to cause bowel injury and dysfunction.[2] Likewise, the radiotherapy and chemotherapy might increase the risk of serious gastrointestinal complications or late small bowel obstruction.[3] Therefore, searching for novel targets for the early diagnosis of colorectal cancer at the very beginning is as crucial as developing new therapeutic approach in improving the overall prognosis of patients.

The occurrence and development of colorectal cancer are caused by multiple factors, including adhesion molecules, protein-degrading enzymes, various angiogenic factors, bone proteins, cell growth factors, and signaling pathways. Especially, the intrinsic factors including genetic abnormalities such as *TP53*, heterogeneity of tumor metastasis-initiating cells, and epithelial-mesenchymal transition play vital roles in the pathogenesis of colorectal cancer.[4] Also, its pathogenesis includes multiple stages, including gene mutations, inhibition of apoptosis, uncontrolled cell proliferation and differentiation, malignant cell transformation, invasion, and metastasis. Therefore, in-depth research on the occurrence and development mechanisms of various biological protein molecules in colorectal cancer has clinical value, providing new tumor markers for the diagnosis and prognosis of early colorectal cancer. At the same time, this is also conducive to providing new theoretical support for molecular targeted therapy of colorectal cancer for clinical treatment.

Ezrin protein connects the cell membrane to the cytoskeleton and is a member of the ERM protein family. It is a key molecule that transmits extracellular signals to skeletal proteins, mediating communication between cells and the matrix between cells. It participates in cell movement and also in cell growth. MMP-9 is a member of the proteolytic enzyme family and plays a crucial role in activating growth factors and maintaining normal tissue structure stability in the human body. Research has found that both Ezrin and MMP-9 could promote excessive cell proliferation and enhance cell invasiveness and metastasis. As a membrane-cytoskeleton linking protein, Ezrin acts by regulating cell shape, enhancing cell adhesion to surrounding tissues, mediating signaling pathways, and influencing the tumor microenvironment to support tumor cell migration.[5] MMP-9, due to its proteolytic cleavage activity in the extracellular environment, participates in degrading the extracellular matrix, modifying cell interactions, cleaving cell surface proteins, and altering the extracellular protein landscape. Particularly, MMP-9's involvement in basement membrane degradation, rich in Type IV Collagen, is crucial for tumor invasion and metastasis during tumor development.[6] Moreover, studies have proved that Ezrin and MMP-9 might be predictive markers in the development rectal cancer, and also have the property in responding therapeutic effect in cancer chemotherapy or radiotherapy.[7-9] However, whether the changes of Ezrin and MMP-9 at the early stage of colorectal cancer benefit the early diagnosis remains further investigations. Therefore, we speculate that Ezrin and MMP-9 expressions in the tumor tissue are correlated with the progression of colorectal cancer, and they may have synergistic effects in the occurrence, infiltration, and metastasis of tumors.

The expression and significance of Ezrin and MMP-9 in different tumor tissues have been studied domestically and internationally. However, there were few reports on the correlation of Ezrin and MMP-9 in the tumor tissue and the invasion, metastasis, and early prediction of rectal cancer. In the present study, we detected the expression of Ezrin and MMP-9 in colorectal cancer tissues through immuno-histochemical MAXVISION and analyzed the correlation between colorectal cancer and clinical pathological factors. We aimed to explore the role of Ezrin and MMP-9 in the occurrence and development of colorectal cancer, so as to provide a theoretical basis and prognosis judgment for the early diagnosis and personalized treatment approach.

MATERIALS AND METHODS

General materials

Tissue samples were collected from 50 colorectal cancer patients stored in the Pathology Department of Longgang District People's Hospital and Longgang Central Hospital in Shenzhen from February 2018 to June 2022.

Bias control 1) Implementing random to ensure the sample is representative of the target population. 2) Clearly defining the inclusion/exclusion criteria for participant selection to reduce selection bias. 3) Reporting the sample size calculation and justification to demonstrate sufficient statistical power. 4) The use of blinding or masking procedures, where relevant, to reduce experimental bias during data collection and analysis.

Inclusion criteria 1) colorectal cancer patients that were confirmed by clinical symptoms such as weight loss, abdominal pain, changes in bowel habits and rectal bleeding, colonoscopy, imaging tests such as CT scans or MRI, pathological examination of tissue samples, and blood tests for tumor markers like CEA and CA19-9; 2) Patients who have not undergone chemotherapy, radiation therapy, or other special treatment for tumors before surgery, and have no family history of colorectal cancer or primary colorectal cancer.

Exclusion criteria. 1) Incomplete clinical and pathological data, poor compliance, and inability to determine immunohistochemical results; 2) comorbid metabolic, immunological, or hematological disorders that could interfere with the study.

Reagents

In order to determine the expression levels of Ezrin and MMP-9 in the tumor tissues, we purchased the antibodies for immunohistochemistry assay. Recombinant Rabbit Monoclonal

Antibody Ezrin Antibody (SAB, USA); Mouse monoclonal antibody MMP-9 (HUABIO); DAB Color Reagent Kit (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China); Ready to use rapid immunohistochemistry MaxVision reagent kit (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China).

Immuno-histochemical staining

The clinical and pathological data of colorectal cancer patients were retrieved from the hospital's medical record system. Patients who did not meet the eligibility criteria were excluded. Subsequently, 50 suitable colorectal cancer tissue samples were obtained from the pathology department. All tissue specimens were collected with the verbal consent of the patients, and the study utilized archived clinical samples, for which the requirement for written informed consent was waived. The research protocol was reviewed and approved by the hospital's ethics committee.

Colorectal cancer tissue and adjacent tissue (normal colorectal mucosal tissue>5cm from the edge of the tumor lesion) stored in the pathology department was collected and cut into small pieces with a thickness of approximately 2-3 mm. After fixation, dehydration, waxing, embedding, and making paraffin sections, the diagnosis was confirmed by hematoxylin-eosin staining. Two experienced pathologists, blinded to the study design, conducted the histopathological examinations of the tissue samples. This approach aimed to eliminate potential subjective bias and enhance the objectivity, accuracy, and reliability of the pathological diagnoses.

Immuno-histochemical staining was performed by setting up a control in the experiment, using PBS buffer instead of the first antibody as a negative control and using Ezrin and MMP-9 positive sections as positive controls. In brief, the tissue samples are collected, fixed, embedded, and sectioned; antigen retrieval is performed under high temperature and pressure using citrate buffer (pH=6.0) to expose the target proteins. The primary antibodies against Ezrin and MMP-9 (1:500) are incubated to the tissue samples at 4°C overnight. After PBS washing, the corresponding secondary antibody (1:1000) is added and incubated at room temperature for 40 min, the target protein expression and localization is observed and imaged under a microscope.

The film was read by two pathologists using a double-blind reading method, and each film was randomly observed with 5 high-power fields. Positive expression of Ezrin protein and MMP-9 was determined in the cell membrane and cytoplasm based on the number and intensity of chromogenic cells, as well as brown-yellow particles. 0 point: Provide the score for non-cell chromogenic individuals in proportion to the number of chromogenic cells; 1 point: The proportion of cell coloration (50%); 2 points: those with a cell color ratio ≥ 50%; Scoring based on the strength of color expression: no cell coloration; 1 point: light yellow; 2 points: brownish yellow.

Statistical analysis

All parameters were processed and statistically analyzed using Statistic Package for Social Science (SPSS) 26.0 software (IBM, Armonk, NY, USA). Since our study involves categorical and ordinal variables, χ^2 tests, and Spearman correlation analysis performed the comparison among different groups. $P < .05$ indicates that the difference is statistically significant; the minimum sample size is three.

RESULTS

The expression of Ezrin protein and MMP-9 in colorectal cancer tissues and adjacent tissues.

The positive rate of Ezrin in 50 colorectal cancer tissues was 78% (39/50), while the positive rate in 50 adjacent tissues was 6.0% (3/50). The positive rate of MMP-9 in 50 colorectal cancer tissues was 72% (36/50), while the positive rate in 50 adjacent tissues was 8.0% (4/50). There was a significant difference in the expression of Ezrin and MMP-9 in colorectal cancer tissue and adjacent tissues (χ^2=53.202, $P < .001$; χ^2=42.667, $P < .001$). These results showed that the expressions of Ezrin and MMP-9 protein were significantly increased in the colorectal cancer tissues, indicating that the elevated Ezrin and MMP-9 expressions are closely related to tumor progression.

The relationship between the expression of Ezrin and MMP-9 and clinical pathological factors in colorectal cancer

It is reported that early-onset of colorectal cancer had adverse histopathological features including poor differentiation, perineural invasion, lympho-vascular invasion, and mucinous and/or signet cell morphology.[10] Therefore, we conducted correlation analyses to determine whether elevated Ezrin and MMP-9 expressions are related to the clinical pathological factors in colorectal cancer. It is reported that gender and age did not influence the predictive value of Ezrin or MMP-9 expressions in different cancers.[11,12] Here, we found that there was no statistically significant difference in the expression of Ezrin and MMP-9 regarding gender and age, as previously reported ($P > .05$). However, there were statistically significant correlations in the expression of Ezrin and MMP-9 in tumor diameter, depth of invasion, clinical staging, lymph node metastasis, and degree of differentiation (Tables 1 and 2). These results indicated that Ezrin and MMP-9 expressions are positively related to the occurrence of tumor metastasis and cancer progression.

Overall, our findings highlighted that Ezrin and MMP-9 might be effective biomarkers in the early diagnosis and metastasis of colorectal cancer, indicating their clinical potential in diagnosing and treating colorectal cancer.

DISCUSSION

In our study, we found that the positive expression rate of Ezrin in colorectal cancer tissue was 78%, significantly different from its positive expression rate of 6.0% in adjacent tissues. Besides, clinical analysis of pathological factors in colorectal cancer showed that Ezrin protein was closely related to the

Table 1. The relationship between Ezrin expression and clinical pathological factors in colorectal cancer tissue.

Clinical pathological factors	Number of cases	Number of cases of Ezrin expression		χ^2	*P* value
		+	-		
Gender				0.242	.623
male	26	21	5		
female	24	18	6		
Age (Years old)				0.055	.815
≥50	28	22	6		
<50	22	17	5		
Tumor size (cm)				5.453	.020
≥5	31	28	3		
<5	19	11	8		
Invasion depth				6.336	.012
Infiltrating serosa	28	26	2		
Without reaching the serosa	22	13	9		
Lymph node metastasis				4.056	.044
Yes	27	24	3		
No	23	15	8		
Distant metastasis				7.203	.007
Yes	29	27	2		
No	21	12	9		
Degree of differentiation				4.222	.040
High/Medium	37	32	5		
Low	13	7	6		

Table 2. The relationship between MMP-9 expression and clinical pathological factors in colorectal cancer tissue.

Clinical pathological factors	Number of cases	Number of cases of MMP-9 expression		χ^2	*P* value
		+	-		
Gender				0.651	.450
male	26	20	6		
female	24	16	8		
Age (Years old)				0.284	.594
≥50	28	21	7		
<50	22	15	7		
Tumor size (cm)				5.702	.017
≥5	31	26	5		
<5	19	10	9		
Invasion depth				5.937	.015
Infiltrating serosa	28	24	4		
Without reaching the serosa	22	12	10		
Lymph node metastasis				5.062	.024
Yes	27	23	4		
No	23	13	10		
Distant metastasis				3.964	.046
Yes	29	24	5		
No	21	12	9		
Degree of differentiation				5.259	.022
High/Medium	37	21	6		
Low	13	5	8		

diameter, depth of invasion, degree of differentiation, presence of lymph nodes, and presence of distant metastasis of the tumor and was not related to gender and age. Our findings were similar to those reported in relevant literature at home and abroad.[13] Through the study of this experiment, we concluded that Ezrin played a crucial role in the occurrence, infiltration, and metastasis of colorectal cancer. It might be helpful to promote the occurrence, infiltration, and metastasis of colorectal cancer by preventing tumor cell apoptosis, enhancing tumor cell motility, and regulating downstream signal transduction of adhesion molecules and factor connections related to tumor metastasis.

Ezrin is mainly distributed in the microvilli, small protrusions, and membrane folds of cells that connect the actin microfilament skeleton. It maintains cell morphology, polarity, adhesiveness, mitosis, cell movement, signal transmission, communication between cells and the matrix, and promoting cell growth.[14] In recent years, research has found that Ezrin is also involved in regulating the rearrangement, aggregation, and separation of the cytoskeleton,

Figure 1. Positive expression of Ezrin in colorectal cancer tissue.

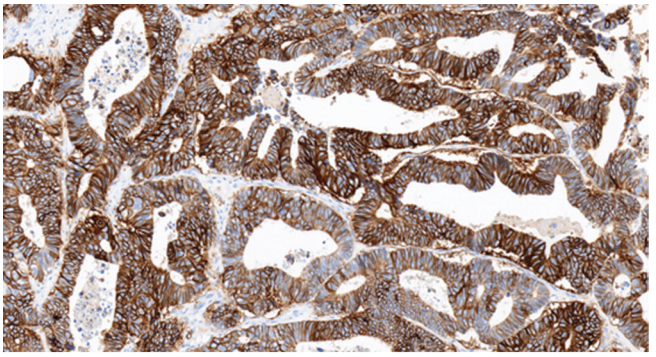
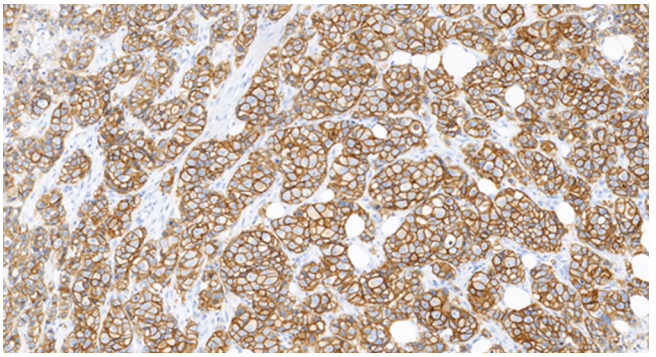


Figure 2. Positive expression of MMP-9 in colorectal cancer tissue.



interfering with normal signal transduction, increasing the generation of filamentous and plate-like pseudopodia, enhancing the motility, invasiveness, and metastasis of tumor cells, ultimately leading to the invasiveness and metastasis of tumors.[5,15,16] It is reported that compared with normal tissues, the mRNA or protein expression of Ezrin in breast cancer,[17] liver cancer, prostate cancer[18] and other malignant tumor tissues was significantly enhanced. The proportion of Ezrin-positive expression in tumor cells gradually increases with the invasion of blood vessels, lymph node metastasis, and remote metastasis of tumor cells.[19] Compared with non-metastatic colorectal cancer tissues, Ezrin showed a significant increase in positive expression rate in tumors with distant lymph node metastasis, indicating that Ezrin may be associated with poor prognosis in colorectal cancer patients.

MMP-9 is the gelatinase with the highest relative molecular weight in the MMP family, involved in the degradation and reconstruction of the extracellular matrix. When it interacts with metal zinc ions, it is called a matrix factor. The extracellular matrix components can be reconstructed under normal physiological conditions to regulate intercellular adhesion, affect the activity of extracellular components and other proteins, and enable potential biological functions to take effect.[6,20] At the same time, the extracellular matrix can also directly or indirectly participate in embryonic development, tissue remodeling, and wound healing processes. Repairing damage is a normal physiological function that can play a role by participating in

tumor invasion, metastasis, and neovascularization, as well as mediating the tumor microenvironment.[21,22] MMP-9 has been found to be a potential biomarker for giant cell tumors of bone, small cell lung cancer, cervical cancer,[23] breast cancer, and other cancers, and can be used as a biomarker to carry out exploration work in the field of diagnosis, efficacy monitoring, disease progress monitoring, etc. We found that the positive proportion of MMP-9 in rectal cancer tissue reached 72%, much higher than in adjacent cancer tissue at 8%. In addition, MMP-9 was closely related to tumor diameter, depth of invasion, degree of differentiation, lymph node status, distant metastasis, etc., but it was not related to the age and gender of onset of colorectal cancer. The results indicated that MMP-9 played an important role in the occurrence, infiltration, and metastasis of colorectal cancer. MMP-9 can promote the development, infiltration, and metastasis of colorectal cancer by blocking cell apoptosis, enhancing tumor angiogenesis, and enhancing cell invasion and migration ability.[22,24] In colorectal cancer tissue, the positive expression rate of MMP-9 was significantly higher in cases with distant metastasis than in cases without metastasis, suggesting that MMP-9 can potentially evaluate the occurrence and development of colorectal cancer and the prognosis of patients.

Immunohistochemistry was performed to further observe the expression of Ezrin and MMP-9 in colorectal cancer tissue. The results indicated that Ezrin and MMP-9 might exert synergistic effects in the occurrence, infiltration, and metastasis of colorectal cancer. However, the specific mechanism is not yet clear and needs to be further investigated. We speculate that its mechanism of occurrence is that the overexpression of Ezrin promotes the expression of MMP-9, leading to the degradation of IV collagen in the extracellular matrix, thereby creating a microenvironment that promotes tumor infiltration and metastasis. CD44 molecule, as a mediator between cells and extracellular matrix, adheres between cells and interferes with normal information transmission pathways, causing excessive proliferation of tumor cells, increased angiogenesis, and enhanced motility and invasiveness of tumor cells. Therefore, Ezrin and MMP-9 can serve as tumor markers for colorectal cancer. The combined detection of Ezrin and MMP-9 helps to enhance the diagnostic accuracy of early colorectal cancer, evaluate patient prognosis and tumor progression, and may provide a theoretical basis for molecular targeted therapy for colorectal cancer. In general, Ezrin and MMP-9 exhibit excessively high expression levels in rectal cancer tissue. The positive expression rates of both also showed a positive correlation with the increase in diameter, depth of infiltration, degree of differentiation, presence of lymph node metastasis, and distant metastasis of colorectal cancer tumors.

Our findings suggest that the combined and early detection of Ezrin and MMP-9 expression levels in colorectal cancer has considerable clinical diagnostic and therapeutic value. However, there are limitations in the present study. Several limitations of this study should be acknowledged. First, the inclusion of only colorectal cancer patients from China may limit the generalizability of the results to more diverse populations. The relatively small sample size also raises concerns about the statistical power to detect meaningful effects, which could impact the reliability and interpretation of the findings. Additionally, the cross-sectional study design prevents us from drawing causal conclusions about the relationship between Ezrin, MMP-9, and colorectal cancer progression. Future longitudinal studies would be needed to better elucidate the temporal and predictive nature of these biomarkers. Furthermore, the data collection methods, such as reliance on medical records and self-reported patient information, introduce the potential for measurement errors or biases. The relatively short timeframe of the study also limits the ability to evaluate long-term outcomes and survival. These limitations should be considered when interpreting the results of this study. Addressing these methodological issues through larger, more representative samples, longitudinal designs, and objective data collection methods would strengthen the validity and generalizability of future research in this area. Therefore, further studies may focus on expanding the sample size and include a more diverse population from various regions to enhance the generalizability of the findings. First, expanding the sample size and including a more diverse population from various geographic regions would enhance the generalizability of the results. Additionally, future studies should aim to elucidate the molecular mechanisms linking the expression and regulation of Ezrin and MMP-9 in the context of colorectal cancer development and metastasis. Exploring the roles of these biomarkers in other cancer types would also be valuable, as they may have broader clinical utility beyond colorectal malignancies.

In summary, Ezrin and MMP-9 may cooperate and exert synergistic effects in the occurrence, infiltration, and metastasis of colorectal cancer. Evaluating the efficacy of targeting Ezrin and MMP-9 in therapeutic interventions, either individually or in combination, could provide important insights into their potential as novel treatment strategies for colorectal cancer.

ETHICAL COMPLIANCE

This study was approved by the ethics committee of Longgang District People's Hospital and Longgang Central Hospital.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report that are relevant to this article.

AUTHOR CONTRIBUTIONS

QZ and ZY designed the study and performed the experiments, ZY and YZ collected the data, YZ and FL analyzed the data, and QZ prepared the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the Longgang District of Shenzhen 2020 Annual Medical and Health Science and Technology Project Fund (LGKCYLWS2020056); Project name: Ezrin and MMP - 9 expression in colorectal cancer and correlation analysis.

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