High level of ezrin expression in colorectal cancer tissues is closely related to tumor malignancy

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AIM: To investigate the ezrin expression in normal colorectal mucosa and colorectal cancer tissues, and study the correlation between ezrin expression in colorectal cancer tissues and tumor invasion and metastasis.

METHODS: Eighty paraffin-embedded cancer tissue samples were selected from primary colorectal adenocarcinoma. Twenty-eight patients had well-differentiated, 22 had moderately differentiated and 30 had poorly differentiated adenocarcinoma. Forty-five patients and 35 patients had lymph node metastasis. Forty-five patients were of Dukes A to B stage, and 35 were of C to D stage. Another 22 paraffin-embedded tissue blocks of normal colorectal epithelium (> 5 cm away from the edge of the tumor) were selected as the control group. All patients with colorectal cancer were treated surgically and diagnosed histologically, without preoperative chemotherapy or radiotherapy. The immunohistochemistry was used to detect the ezrin expression in paraffin-embedded normal colorectal mucosa tissues and colorectal cancer tissue samples.

RESULTS: Ezrin expression in colorectal cancer was significantly higher than in normal colorectal mucosa (75.00% vs 9.09%, P < 0.01), and there was a close relationship between ezrin expression and the degree of tumor differentiation, lymph node metastasis and Dukes stage (88.46% vs 50.00%, P < 0.01; 94.28% vs 51.11%, P < 0.01; 94.28% vs 51.11%, P < 0.01).

CONCLUSION: Ezrin expression is obviously higher in colorectal cancer tissues than in normal colorectal mucosa tissues, and the high level of ezrin expression is closely related to the colorectal cancer invasion and metastasis process.

INTRODUCTION
Ezrin belongs to the ezrin/radixin/moesin (ERM) protein family, which act as membrane organizers and linkers between the plasma membrane and cytoskeleton[1,2]. Ezrin is mainly expressed on the cell surface to maintain the polarity of endothelial cells[3]. 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[4,5]. Overexpression of ezrin protein is correlated with the metastatic potential of several cancers[6,7], and a high level of ezrin protein expression can induce conversion of a variety of cell lines, as well as abnormal hyperplasia[8]. Tumor cell lines with stronger metastatic abilities are usually accompanied by overexpression of ezrin[9]. Through testing the expression of ezrin protein in normal colonic mucosa...
and colorectal cancer tissues, we aimed to establish the relationship between ezrin expression and clinical parameters, evaluate its molecular action mechanisms in the process of colorectal cancer carcinogenesis, invasion and metastasis, and provide the evidence for clinical prognosis and suitable adjuvant therapy.

MATERIALS AND METHODS

Patients and their pathological samples
The immunohistochemistry was performed in paraffin-embedded tissue samples. Eighty colorectal adenocarcinoma patients diagnosed by postoperative pathology were investigated. There were 44 male and 36 female patients, whose ages ranged from 31 to 80 years, with an average age of 55.5 years. Histologically, 28 patients had well-differentiated, 22 had moderately differentiated, and 30 had poorly differentiated adenocarcinoma. Forty-five patients were without and 35 patients had lymph node metastasis. Forty-five patients were of Dukes A to B stage, and 35 were of C to D stage. Another 22 paraffin-embedded tissue blocks of normal colorectal epithelium (> 5 cm away from the edge of the tumor) was the control group.

Drugs and reagents
Mouse anti-human ezrin mAb was purchased from Fujian Maixin Biotechnology Development Co. Ltd, and SP kit DAB from Beijing Zhong Shan Jinqiao Biotechnology Development Co. Ltd. Experiments were performed following the instructions of the manufacturers. PBS (0.01 mmol/L) was used to replace the first antibody as a negative control, while the normal colorectal mucosa was a positive control.

Result judgment
Each stained slide was assessed and given a score according to the classification standard of Mathew et al.\[11\]: score 0, no expression; score 1, < 50% of cells staining positive expression or less; score 2, ≥ 50% of cells staining positive expression. Score 0-1 was recorded as negative, and score 2 recorded as positive.

Statistical analyses
SPSS for Windows version 11.0 was used for statistical analyses. The \( \chi^2 \) test was used in the analysis of the relationship between ezrin and colorectal cancer clinicopathological parameters. \( P \leq 0.05 \) was considered as a significant difference.

RESULTS

The positive expression of ezrin in colorectal cancer was significantly higher than that in normal colorectal mucosa (Figure 1A-E). The positive rate of ezrin protein in normal colorectal mucosa was 9.09% (2/22) and 75.00% (60/80) in colorectal cancer tissues. There were significant differences between the two groups (75.00% vs 9.09%, \( P < 0.01 \)), as shown in Table 1.

| Group                      | \( n \) | Positive expression (%) |
|----------------------------|--------|-------------------------|
| Normal colorectal mucosa   | 22     | 2 (9.09)\(^a\)          |
| Colorectal cancer tissues  | 80     | 60 (75.00)              |

\( ^a \)P < 0.01 vs colorectal cancer tissues.

Table 2 Relationship between ezrin expression in colorectal cancer tissues and clinicopathological parameters \( n (%) \)

| Clinicopathological parameters | \( n \) | Ezrin positive expression (%) |
|-------------------------------|--------|-----------------------------|
| Well-differentiated           | 28     | 14 (50.00)\(^b\)           |
| Moderately and poorly differentiated | 52 | 46 (88.46)                  |
| Lymph node metastasis         | 35     | 33 (94.28)\(^d\)          |
| Without lymph node metastasis | 45     | 27 (51.11)\(^c\)          |
| Dukes A to B stage            | 45     | 27 (51.11)\(^c\)          |
| Dukes C to D stage            | 35     | 33 (94.28)\(^d\)          |

There was a close relationship between ezrin expression and the degree of tumor differentiation, lymph node metastasis and Dukes stage. There were significant differences between the well-differentiated and the moderately and poorly differentiated groups (\( P < 0.01 \)); lymph node metastasis group vs group without lymph node metastasis (\( P < 0.01 \)); Dukes A to B stage vs Dukes C to D stage (\( P < 0.01 \)).

DISCUSSION

Ezrin protein expression in specific cell membrane regions is mainly involved in the connection between the epithelial cell cytoskeleton and the cell membrane, through membrane surface signaling molecules and some transmembrane signal transduction pathway. It participates in the regulation of cell survival, adhesion, proliferation and migration processes. Recent studies have found that ezrin protein may play an important role in the tumorigenesis, development, invasion and metastasis process, probably through regulating adhesion molecules and participating in cell signal transduction, and other channels in the tumor.\[12-15\]. Ezrin protein is an indispensable factor for tumor cell metastasis of osteosarcoma\[16\], breast cancer\[17\], nasopharyngeal carcinoma\[18\], and prostate cancer\[19\]. In addition, in malignant tumor tissues, there are also changes in subcellular localization of ezrin expression. Moilanen et al.\[20\] found that ezrin expression in normal ovarian epithelial cells is a kind of cell polarity expression, and that ezrin expression in malignant ovarian tumor cells is more diffusive, with a different degree of tumor cell differentiation, and the location and intensity of ezrin expression in cells is quite different. Therefore, we speculate that ezrin subcellular localization in normal
cells forms the foundation of various physiological functions and cell structure. Abnormal ezrin expression or distribution will also lead to abnormal cell structure and physiological function, and accordingly, these abnormal changes participate in the occurrence, development, invasion and metastasis of malignant tumors.

The role of ezrin in tumor progression is very important and deserves much attention. Recent studies have found that ezrin is a key factor in Fas-mediated apoptosis[23], in the P-gp1-mediated multidrug resistance of cancers, and in cannibalism of metastatic tumors[24]. The active ezrin C-terminal is connected with the actin cytoskeleton, and the N-terminal is connected with cell adhesion molecules such as E-cadherin, and CD44[25,26], etc. Ezrin participates in regulating cell-cell and cell-extracellular matrix adhesion, thus influencing tumor cell invasion and other biological behavior[27-30]. CD44 is a cellular membrane receptor which can specially recognize hyaluronic acid and collagen, and regulate cell-cell and cell-extracellular matrix adhesion. Some studies have found that ezrin, CD44 and CD44 variants could make up a compound that is co-expressed in the tumor cells[31]. Pujuguet et al[32] have found that ezrin can regulate E-cadherin expression in the cell membrane through Rho protein, thereby regulating cell adhesion. At the same time, ezrin also has regulating function in the E-cadherin membrane localization, and activated ezrin can make the E-cadherin protein aggregate in the cell, thereby undermining the cell-to-cell contact and intercellular adhesive ability, and the overexpression of ezrin in the tissues also has the same function of weakening the intercellular adhesion[33]. Through activation of RhoA and the MAPK pathway, ezrin can promote the cell adhesion plaque formation, thereby promoting the adhesive function between the tumor cells and other cells, as well as stoma cells[34]. Therefore, we believe, through participation in the formation of the cell adhesion plaque, cytoskeletal connections and cell surface compartments assembly, and other biological functions, ezrin protein mediates and regulates cell-cell and cell-extracellular matrix adhesion, and is also involved in the malignant tumor invasion and metastasis process. This study showed that, the overexpression of ezrin in colorectal cancer tissues may be involved in cancer invasion and metastasis. The studies on the correlation between ezrin protein and cancer might help us further reveal the tumor invasion and metastasis mechanism, and find the targets for inhibiting tumor metastasis, or indicators that forecasts the prognosis of patients with tumors.

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