Colonoscopy Combined with Me-Nbi to Observe the Canceration Characteristics of Colon Polyps and the Correlation of RHOC Protein Expression

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ABSTRACT

The current study was carried out to analyze the characteristics of colon polyps canceration observed by colonoscopy combined with ME-NBI (Magnifying Endoscopy combined with Narrow-Band Imaging) and its correlation with RhoC (Ras homolog gene family, member C) protein expression. For this purpose, A total of 300 patients with colorectal polyps and cancerous lesions (192 colorectal polyps and 200 cancerous lesions) who were treated in the digestive endoscopy room of the hospital and underwent colonoscopy were selected, and they were divided into polyp group and malignant lesion according to the diagnosis results. groups, 150 cases in each group. There were 75 patients with non-adenomatous polyps and 75 patients with adenomatous polyps in the polyp group; 75 patients with high-grade neoplasia and cancerous changes in the malignant group. The microvascular structure and surface structure of the lesions were observed by colonoscopy, and the correlation between microvascular morphological characteristics and RhoC protein expression was analyzed. Results showed that the probability of positive RhoC protein expression in the polyp group was significantly lower than that in the malignant transformation group, and the difference was statistically significant (P<0.05). In the malignant transformation group, the positive rate of RhoC expression in mucosal and submucosal superficial infiltration of 150 patients with colon polyp carcinoma was lower than that in submucosal deep infiltration, and the difference was statistically significant (P<0.05). NICE (National Institute for Clinical Excellence) type 2 was diagnosed as colorectal superficial submucosal The sensitivity, specificity, and accuracy of colorectal submucosal invasion were 73.1%, 84.6%, and 83.2%, respectively; the sensitivity, specificity, and accuracy of NICE type 3 in diagnosing colorectal submucosal invasion were 74.6%, 96.8%, and 92.7%, respectively. . Type 2 and type 3 lesions with cancerous features in NICE classification were correlated with the expression of RhoC protein (P<0.05). In conclusion, NICE classification under colonoscopy combined with magnifying colonoscopy has a good effect on colorectal lesions. Differential diagnostic value, RhoC protein is highly expressed in colon cancer and is closely related to the occurrence of colon cancer and the depth of lesion invasion. With the progression of colorectal adenomas, the expression of RhoC protein in the lesions gradually increased.

Introduction

Colorectal cancer (CRC) is a common malignant tumor in the digestive system, with a significant increase in morbidity and mortality. Gastric cancer is one of the most common cancers in the world, ranking fifth and fourth in morbidity and mortality, respectively. According to the latest cancer statistics, 1.09 million gastric cancers are diagnosed worldwide each year and 770,000 people die from it each year. It is the sixth most common cancer and the third most common cause of death for all cancer types (1-3). The 5-year survival rate of early gastric cancer patients is over 95%. Therefore, early detection of GASTRIC cancer is crucial for a good prognosis (4-5).

With the development and progress of endoscopy for gastric tumor diagnosis, the diagnostic level of endoscopy equipment for gastric tumors has been improved. Endoscopic imaging has evolved from white light endoscopy to the use of new electronic imaging techniques that utilize a technique that alters white light with the push of a but-

ton. With the addition of magnification, endoscopists are now able to assess mucosal surface structures in greater detail. In particular, the development of image-enhanced endoscopes, such as narrow-band imaging (ME-NBI), has significantly improved the qualitative and quantitative diagnosis of gastrointestinal tumors. The core of endoscope ME-NBI technology is mainly reflected in that it can not only accurately observe the morphology of digestive tract mucosal epithelium, such as epithelial glandular concave structure. At the same time, it can better assist endoscopists to distinguish gastrointestinal epithelium, such as Barrett's intraesophageal metaplasia epithelium, irregular changes in the concave of early gastrointestinal tumors and changes in the shape and density of blood vessels in gastrointestinal inflammation, thus improving the accuracy of endoscopic diagnosis and treatment. Narrow-band imaging (ME-NBI) is an imaging technology that uses blue and green light of a specific wavelength to penetrate into the skin surface (6-8). Two selective narrowband wavelengths, 415 nm and 540 nm, were generated by the white light source filter

in front of the endoscope xenon arc lamp. The length of the wavelength is proportional to the depth of penetration. 415 nm wavelength only highlights the brown superficial mucosa of capillaries, while 540 nm wavelength penetrates into the lower and submucosa, giving them a blue-green tint. Me-NBI can increase the contrast between the vascular system and mucosal superficial tissue structure, and improve tumor visibility by enhancing the contrast between vascularized lesions and normal mucosa. Visible light wavelength is short and tissue permeability is low, making it an ideal choice for observing mucosal surface structure. These two short wavelengths were consistent with the peak absorption zones of oxyhemoglobin, and mucosal capillaries were observed to be significantly low in signal compared to surrounding tissues. To establish qualitative and quantitative diagnosis of esophageal tumor in capillary loops in esophageal epithelium (9-11). Me-NBI is a new and powerful optical imaging technique that significantly improves endoscopic diagnosis when using narrowband filters. It enables better visualization of microsurface and microvascular patterns on the mucosal surface and advanced evaluation of abnormal lesions, such as dysplasia and cancer (12-14). Therefore, ME-NBI has been widely used in the diagnosis of early gastric cancer (EGC). During ME-NBI, irregular MV or MS with DL can be diagnosed as early gastric cancer.

There is a close correlation between the prognosis and the stage of colorectal cancer. Therefore, good diagnosis and treatment can significantly improve the prognosis of colorectal cancer patients. The vast majority of colorectal cancer is formed from the adenoma or adenoma stage. If changes in the microstructure of colon polyps and cancerous lesions can be observed with the help of colonoscopic narrow-band technology, the possibility of malignant changes and the depth of lesion invasion can be predicted. It can not only improve the accuracy of biopsy and achieve the goal of early diagnosis but also provide the basis for further treatment of lesions by endoscope or surgery.

This study analyzed the correlation between colon polyp cancer characteristics and RhoC protein expression observed by endoscopic ME-NBI technology.

Materials and Methods

General Information

A total of 300 patients with colorectal polyps and cancer (192 colorectal polyps and 200 cancerous lesions) who received colonoscopies in the digestive endoscopy department from 2019 to 2021 were selected. According to the diagnosis results, they were divided into the polyp group and malignant transformation group, 150 cases in each group. In the polyp group, there were 75 non-adenomatous polyps and 75 adenomatous polyps. In the malignant transformation group, there were 75 cases of high-grade neoplasia and 75 cases of cancer. In the polyp group, there were 87 males and 63 females, aged 24-70 years, with an average age of (38.62 ± 2.65) years. There were 81 males and 69 females in the malignant transformation group, aged 27-69 years, with an average age of (37.91 ± 3.48) years. Immunohistochemical staining was performed on the diseased tissues to test the RhoC protein. The correlation between NB1 microvessel and histological results was compared, and the feasibility of simultaneous observation of mucosal microvessel formation by endoscope was ana-

lyzed. There was no difference in general data between the two groups, which was comparable. This study was approved by the hospital ethics committee.

Inclusion and exclusion criteria
Inclusion criteria
(1) All patients underwent colonoscopy and pathological analysis. (2) All patients underwent ME-NBI magnification endoscopy, and the images were clear. (3)RhoC protein was detected by immunohistochemistry in pathological tissues.

Exclusion criteria
(1) There are colorectal resection patients; (2)Patients with a history of inflammatory bowel disease; (3)Patients with familial polyp disease; (4) Serrated adenoma; (3) Patients who did not want to participate in the study.

Instruments and Equipment
Cf-hq290z colonoscopy (Olympus Corporation, Japan) was used; LEICA ASP300S automatic tissue dehydrator (LEICA, Germany); LEICA RM2245 semi-automatic rotary slicer (LEICA, Germany); OLYMPUS BX43F Biological microscope (OLYMPUS Corporation, Japan). Rabbit Anti-human RhoC monoclonal antibody (Beijing Zhongshan Jinqiao Biotechnology Co., LTD.); Chemiluminescence immunoassay was used for detection using SP kit (Wuhan Boshide Biotechnology Company). Me-NBI system (EVIS LUCERA ELITE). The Olympus Medical Systems LTD. (Tokyo, Japan) used in this study consists of an enlarged GASTROINTESTINAL video endoscope (GIF-H260Z or -H290Z), an image processor (CV-290), and a light source (CLV-290). Soft black hood (MAJ1990; Olympus Medical Systems, Inc.) and masking balloons (Fujifilm Systems, Tokyo, Japan) mounted on video endoscopes.

Check Methods
All patients were given polyethylene glycol electrolytes for bowel preparation prior to endoscopy. The white light mode was used to enter the ileocecal region, identify the structure of the appendiceal foramen, ileocecal flap and y-type ecal fold, understand the ileocecal flap structure, and enter the distal small intestine to prove that the ileocecal region had been reached by the endoscopic approach. The whole colon was observed by descoping, and the morphology of microvessels of polypoid lesions was closely observed based on ME-NBI combined with magnifying colonoscopy, based on which the NICE classification was defined. Resection of endoscopic submucosal colitis (EMR) and endoscopic submucosal dissection (ESD) were performed to treat the lesion. All specimens were cured by 4% formaldehyde and embedded in conventional paraffin wax. The adopted slides were disposed of with APES preventer. In addition, rabbit anti-human RhoC monoclonal antibody was detected by SP method. Primary antibody was replaced with phosphate buffered aline (PBS) as negative for comparison. All operation procedures are strictly based on reagent instructions.

Observation and evaluation indicators
(1) Pathological diagnosis. According to the relevant standards of the World Health Organization (WHO), two pathologists WHO did not know the information of endos-

copy were diagnosed successively: the cancers with severe dysplasia and those not reaching the mucosal muscular were called high-grade intraepithelial neoplasia, including those confined to the mucosa but infiltrated by the lamina propria. When the submucosal infiltration depth <1000um, it was submucosal shallow infiltration, which was the indication of endoscopic treatment; when the submucosal infiltration depth > 1000um, it was submucosal deep infiltration. (2) ME-NBI image microvascular shape evaluation. The NICE classification was adopted: The DIAGNOSTIC criteria of NICE classification were as follows: Type i: the lesions were bright in color visible to the naked eye, similar to or brighter than the background mucosa, indicating that the structures were white punctured structures with only a few filamentous vessels or no vessels at all, suggesting hyperplastic polyps. Type ii: the color of the lesion is brown and the surface structure is oval. There are brown thickened vessels around, branching, tubular, neoplastic lesions suggest.

Statistical methods

SPSS26.0 software was used to conduct statistical research on the data. The statistical and descriptive methods of counting data were frequency and percentage, etc. $X2$ or Fisher was used to test the comparison between multiple groups, and Spearman was used to analyze the correlation. $P<0.05$ was considered as statistically significant difference.

Results

Comparison of RhoC protein expression between the two groups

The probability of positive RhoC protein expression in the polyp group was significantly lower than that in the malignant transformation group, and the difference was statistically significant ($X2 =32.543$, $P<0.05$). In the malignant transformation group, the positive rate of RhoC

protein expression in mucosal and submucosal superficial infiltration was lower than that in submucosal deep infiltration. The difference was statistically significant ($X2 =5.059$, $P<0.05$). The probability of positive RhoC protein expression in the polyp group was significantly lower than that in the malignant group. The difference was statistically significant ($\chi^2 =32.543$, $P<0.05$), as shown in Table 1.

Comparison of positive Expression of RhoC protein at different infiltration depths in cancer patients in malignant group

The positive expression of RhoC protein in 75 colon polyp cancer patients in malignant group was related to the infiltration depth of cancer, and the positive expression rate of RhoC protein in mucosal and submucosal infiltration was higher than that in muscle and serous layer. The difference was statistically significant $x2=4.823$, $P<0.05$), as shown in Table 2.

In the glandular mucosa of the fundus without inflammation, the glandular ducts are regularly arranged in a concave position perpendicular to the mucosal surface. When this was assessed with ME-NBI, the crypt opening was observed as a brown spot and the surrounding MCE as WZ. Thin columns around the middle of WZ show a brown mesh pattern. Inflammatory changes caused by helicobacter pylori (HP) infection gradually distort the glandular ducts. The crypt opening becomes oval or unclear, while the rounded WZ becomes oval. As inflammation progresses, the oval WZ gradually becomes tubular, with unclear or coiled capillaries in the middle. When the fundus glands of the stomach are absent, their appearance resembles the mucosa of the pyloric gland. Efficacy of NICE typing in the diagnosis of colorectal lesions 242 lesions in 300 patients were NICE typed. The NICE type 1 diagnosis was a hyperplastic polyp in 91% (62/68). Type 2 was diagnosed as adenoma or intramucosal carcinoma, accounting for 86% (84/98). Submucosal deep invasive carcinoma was diagnosed as type 3 in 90% (36/40). The

Table 1. Positive comparison of RhoC protein expression between the two groups.

Group	Cases	RhoC protein expression	
		Positive	**Negative**
Polyps	150	21	129
Nasal endoscopy	150	78	82
χ^2		32.543	
P		<0.05	

Table 2. Comparison of RhoC protein expression at different infiltration depths in 75 patients with colon polyp cancer in the malignant transformation group.

Infiltrating depth	Cases	Negative	Positive	Positive rate (%)
Mucosa and submucosa	11	7	4	36.4
Muscle layer and serous layer	64	10	54	84.4
χ^2				4.823
P				<0.05

Table 3. Effectiveness analysis of NICE typing in the diagnosis of colorectal lesions (%).

NICE	Sensitivity,	Specificity	Accuracy
2	73.1	84.6	83.2
3	74.6	96.8	92.7

sensitivity, specificity and accuracy of NICE type 2 in diagnosing submucosal infiltration of colorectal were 73.1%, 84.6% and 83.2%, respectively. The sensitivity, specificity and accuracy of NICE type 3 in diagnosing colorectal submucosal infiltration were 74.6%, 96.8% and 92.7%, respectively, as shown in Table 3.

Correlation between RhoC protein positive expression and NICE typing under endoscopy

Polypoid lesions of type 2 and type 3 in NICE typing were characterized by canceration. Endoscopy showed that the positive expression of RhoC protein in type 2 lesions was 33.3% (15/45). The positive expression of RhoC protein in type 3 lesions was 84.2% (32/38). The characteristics of type 2 and type 3 polypoid lesions were positively correlated with RhoC protein expression.

Discussion

Colorectal cancer is a kind of malignant tumor with a high incidence in clinical practice. The morbidity and mortality of colorectal cancer in China rank among the top in the world. 9 Prognosis of colorectal cancer is closely related to early diagnosis and treatment. Colonoscopy is regarded as the main method of early diagnosis and treatment. Due to the rapid development of endoscopic diagnosis and treatment, several endoscopic classification systems for colorectal tumors have been constructed. However, there is no clear evidence on which types are most accurate in the diagnosis and treatment of precolorectal cancer. It is therefore impossible to assess precisely which subtypes have the most advantage. Currently, there are not much literature about NICE typing, and the accuracy of NICE typing in predicting pathological types of lesions is not outstanding. Chemical staining should be combined for further analysis. In NICE classification, the sensitivity, specificity and accuracy of type 2 in the diagnosis of submucosal superficial infiltration of colorectal were 73.1%, 84.6% and 83.2%, respectively, which were lower than those of type 3. In addition, pathologic findings corresponding to type 2 structures include adenoma, intramucosal carcinoma, and submucosal invasive carcinoma. Such results are obviously not conducive to making reasonable endoscopic diagnosis and treatment decisions, and it is of great clinical significance to subclassify the type 2 structure in the NICE classification. RhoC protein, as a member of the Rho family, is called a "molecular switch". A number of studies have shown that the RhoC protein not only plays an important role in regulating cytoskeleton, cell movement, proliferation, apoptosis, transcription, transformation and tumor cell invasion and metastasis but also is closely related to tumor angiogenesis. It was found that the expression of RhoC protein in superficial invasive carcinoma and deep invasive carcinoma tissue lesions was 33.3% and 84.2%, respectively, and the difference between them was statistically significant. Therefore, NICE classification of lesions by endoscopy, combined with pathology and RhoC protein expression, can better analyze the depth of lesion invasion.

Conclusion

Me-NBI is applied to the non-cancerous gastric mucosa. As normal gastric mucosa shows various mucosal patterns due to differences in glandular area and inflammatory changes, understanding these typical images is very important for the ME-NBI diagnosis of gastric cancer. NICE classification is widely used in clinical practice because it is independent of ME and has high accuracy in the diagnosis of non-neoplastic lesions and deep submucosal invasive carcinoma. However, this classification is difficult to distinguish between low-grade and high-grade intraepithelial neoplasia and superficial submucosal invasive carcinoma. Therefore, EMR/ESD and surgical decision-making are influenced. In addition, the diagnostic efficacy of TYPE 2 in the NICE classification was lower than that of type 3. RhoC protein is highly expressed in colon cancer and is closely related to the occurrence and invasion of colon cancer. With the progression of colorectal adenoma, the expression of RhoC protein in the lesion site increased gradually, and the type 2 structure appeared in the NICE classification. RhoC protein expression increased gradually when the lesion progressed to submucosal deep infiltration.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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