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Evaluation of magnetic resonance imaging in staging of rectal cancer and its relationship with P16 expression

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Abstract. – OBJECTIVE: To explore the use of magnetic resonance imaging (MRI) in the staging of rectal cancer and its relationship with p16 expression.

PATIENTS AND METHODS: A total of 75 patients with rectal cancer treated in Oncology Department of our hospital from March 2013 to March 2017 were randomly included in this study. The entire pelvis was scanned by MRI, and clinicopathological staging was diagnosed. Subsequently, all patients underwent total mesorectal excision (TME). Histopathological gold standard [hematoxylin-eosin (HE) staining] was used to determine the stage. Immunohistochemistry (IHC) was adopted to detect the expression of p16 in cancer tissues and cancer-adjacent tissues. Compared with the results of the pathological examination, the accuracy of MRI diagnosis was analyzed. The relationship between p16 expression and MRI diagnostic materials was analyzed.

RESULTS: Compared with the results of the pathological examination, the total accuracy of MRI in the evaluation of T staging was 76.0% (57/75), and the excessive staging rate and insufficient staging rate were 8.0% (6/75) and 16.0% (12/75), respectively in the assessment of tumor T staging. IHC indicated that the positive expression rate of p16 in the tumor tissues was significantly lower than that in the tumor-adjacent tissues [34.67% (26/75) *vs.* 85.33% (64/75), $p<0.05$]. The chi-square test showed that the expression of p16 in the tumors was notably correlated with T staging, N staging, and myometrial invasion diagnosed with MRI.

CONCLUSIONS: P16 is significantly deficient in the rectal cancer tissues. MRI examination and identification are helpful for clinical diagnosis of rectal cancer staging. The combination of the two items may be helpful for the diagnosis of clinical rectal cancer staging and the establishment of reasonable treatment regimens.

Key Words
Magnetic resonance imaging, Rectal cancer, Clinical staging, p16.

Introduction

An epidemiological survey showed that rectal cancer is frequently observed in patients aged 50 to 55 years old (mainly in males) in China, and most of the lesions are located in the middle and lower rectum[1]. Influenced by the non-specificity of rectal cancer, patients' ignorance and other factors, most patients are clinically diagnosed with middle or advanced rectal cancer with poor prognosis[1]. The 5-year survival rate of patients with rectal cancer at stage III and IV is less than 40% and 12%, respectively[2]. Currently, surgical treatment, radiotherapy and chemotherapy are the commonly used treatment methods in clinical practice. Moreover, the postoperative local recurrence and distant metastasis often affect the surgical results and prognosis. Therefore, an accurate preoperative staging and a targeted establishment of individualized treatment regimens are of great significance to improve the effect of surgical treatment and reduce the postoperative recurrence rate[3]. The methods commonly used for preoperative staging of rectal cancer include spiral computed tomography (CT) and magnetic resonance imaging (MRI). MRI is characterized by high resolution of soft tissue and multi-layer imaging. It has unique advantages in preoperative staging diagnosis of rectal cancer. MRI cannot

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only clearly show the structure of rectal mucosa and muscularis, but also effectively display the location, size, and shape of the tumor, as well as its relationship with the surrounding tissues and organs[4].

P16 is a cell cycle regulatory protein with 148 amino acid residues and a relative molecular mass of about 16,000. It plays its role of inhibiting cell cycle and cell proliferation mainly by suppressing the activity of cell cycle-dependent kinase 4/6 (CDK4/6), so p16 is considered an important tumor-suppressing gene[5,6]. As a tumor-suppressing gene, p16 is found to be inactive in many tumors and is closely related to the occurrence and development of tumors[7]. The tumors with inactive p16 reported by now include oropharyngeal cancer, breast cancer, pancreatic cancer, esophageal cancer, lung cancer, gastric cancer, intestinal cancer, endometrial cancer, and thyroid cancer[8,9].

At present, there are many shortcomings in the diagnosis of rectal cancer staging using MRI alone. Therefore, this study was conducted to explore the relationship between p16, a molecular marker of rectal cancer, and MRI staging, thus providing a new idea for staging and diagnosis of rectal cancer in clinical practice.

Patients and Methods

Clinical Materials

A total of 75 patients with rectal cancer treated in Oncology Department of our hospital from March 2013 to March 2017 were randomly included in this study. The patients included 42 males and 33 females aged 34-79 years old with a median age of 60 years old. Inclusion criteria of clinical materials: patients without preoperative radiotherapy or chemotherapy but with complete MRI materials who received surgical treatment. Exclusion criteria: patients with the history of pelvic surgery; patients who were accompanied with or once suffered from other tumors and received pelvic radiotherapy and/or chemotherapy in the past. All patients underwent surgery within one week after MRI examination. The procedure was performed according to the principle of total mesorectal excision (TME). The surgical specimens were fixed for 48 h with formaldehyde solution, embedded in paraffin and cut into slices. They were stained with hematoxylin and eosin (HE) and observed under a microscope. This study was approved by the Ethics Committee of Jining No. 1 People's Hospital. Signed written informed consents were obtained from all participants before the study.

MRI Examination

MRI scanning: the 8-channel phased-array surface coil imaging was performed using a 3.0 T whole-body MR scanner (Siemens Magnetom Trio). Gradi-ent field strength: 40/40/45 mT/m, gradient switching rate: 200 mT/(m·ms). Scan sequence in turn: (1) T2 weighted image (T2WI) in the transverse plane (thickness of each layer: 6 mm; interlayer thickness: 0.6 mm); (2) T2WI in the coronal plane (thickness of each layer: 5 mm; interlayer thickness: 1 mm); (3) T2WI in the sagittal plane (thickness of each layer: 5 mm; interlayer thickness: 1 mm); (4) dynamically enhanced T1WI in the transverse plane with gadopentetate dimeglumine as the enhanced contrast agent (injection dose: 0.1 mL/kg; injection rate: 2 mL/s, bolus injection) for dynamically enhanced scanning on the transverse site (layer thickness: 4 mm; interlayer spacing: 1 mm). The total examination time was about 30 min, and the scope of scanning was the entire pelvic.

MRI Staging Criteria for Rectal Cancer

Stage T1: the tumor signal does not exceed the submucosa, and the tumor shows a relatively low signal compared with the high signal adjacent to the submucosa. Stage T2: the tumor signal invades the muscular layer, and the interface between the muscular layer and the submucosa disappears. Stage T3: the tumor signal penetrates the muscular layer and extends into the perirectal fat, and the interface between the muscular layer and the surrounding fat disappears. Stage T4: the tumor signal invades the surrounding structures or organs.

Two abdominal imaging diagnostic doctors analyzed the MRI images carefully before surgery, determined T stages, MRI signs of the tumor and the intestinal circumference invasion extent according to the standard and reached an agreement. After surgery, the results were compared with the pathological findings for analysis. The postoperative histopathological findings of the specimen were staged according to pathological staging using DUKES staging criteria[10].

Detection of the Level of p16 Using IHC

Seventy-five paraffin-embedded sections of surgically resected rectal cancer tissues and paired cancer-adjacent tissues were sliced from

the tumor center at the axial plane to ensure their correlations with preoperative MRI. The thickness of the sections of hepatocellular carcinoma (HCC) and cancer-adjacent tissues was 4 μm. P16 antibodies were purchased from Abcam (item No.: ab51243, Cambridge, MA, USA). IHC kits were purchased from Zhongshan Jinqiao Reagent (Guangzhou, China). All experimental procedures were performed according to the instructions provided.

The IHC results were reviewed and scored by an experienced pathologist. The IHC score was calculated according to the proportion of positive cells in the field of vision and staining intensity of positive cells: no positive cells=0 points, positive cells accounted for 1%-10%=1 point, 11%-50%=2 points, 51%-80%=3 points, 81%-100%=4 points; staining strength of positive cells: negative=0 points, weakly positive=1 point, moderately positive=2 points and strongly positive=3 points. The product of the two items was the IHC score of the lesion. The IHC score ranged from 0 to 12 points with the score of 0-1 defined as a negative expression and the score of 1-12 defined as a positive expression.

Statistical Analysis

The experimental results were analyzed using GraphPad Prism software (Version 5.01, GraphPad Software Inc., La Jolla, CA, USA). Chi-square test was used to analyze the difference of p16 expression in the rectal cancer tissues and the cancer-adjacent tissues, and to compare the correlations of p16 expression with MRI parameters. $p<0.05$ suggested that there was a significant difference.

Results

MRI Materials of Patients With Rectal Cancer

The MRI images of all the enrolled patients and the HE-stained pathological image samples were selected for analysis. The confirmation and staging of MRI for rectal cancer primarily depended on the difference in signal intensity of tumors, mucosa and submucosa, muscular layer, perirectal fat and mesorectum on T2WI. The mesorectum has a high signal on T2WI, surrounding the low-signal inherent muscular layer. The tumor has a moderate signal, and the rectal fascia has a thin, line-like low signal surrounding the high-signal mesorectum (Figure 1).

Comparison Between Histopathological Findings and Preoperative Evaluation of T Staging With MRI

The total accuracy of MRI in the evaluation of T staging was 76.0% (57/75), and the excessive staging rate and insufficient staging rate were 8.0% (6/75) and 16.0% (12/75), respectively in the assessment of tumor T staging (Table I).

Comparison Between Histopathological Findings and Preoperative Evaluation of N Staging With MRI

The total accuracy of MRI in the evaluation of N staging was 80.0% (60/75), and the excessive staging rate and insufficient staging rate were 8.0% (6/75) and 12.0% (9/75), respectively in the assessment of tumor T staging (Table II).

P16 Expression in the Rectal Cancer Tissues and Cancer-Adjacent Tissues Detected Using IHC

Figure 2 showed that p16 was expressed in both tumor tissues and tumor-adjacent tissues and located in the cytoplasm. The positive cells are expressed with brown cytoplasm. The positive expression rate of p16 in tumor tissues was significantly lower than that in tumor-adjacent tissues [34.67% (26/75) *vs.* 85.33% (64/75)]. The difference was statistically significant ($p<0.05$) (Table III).

Relationship Between MRI Staging and p16 Expression in Rectal Cancer

The analysis of the pathological data of preoperative MRI diagnosis and postoperative p16 expression in rectal cancer specimens showed that the expression of p16 in the tumors was significantly correlated with T staging, N staging, and myometrial invasion diagnosed with MRI ($p<0.05$) (Table IV).

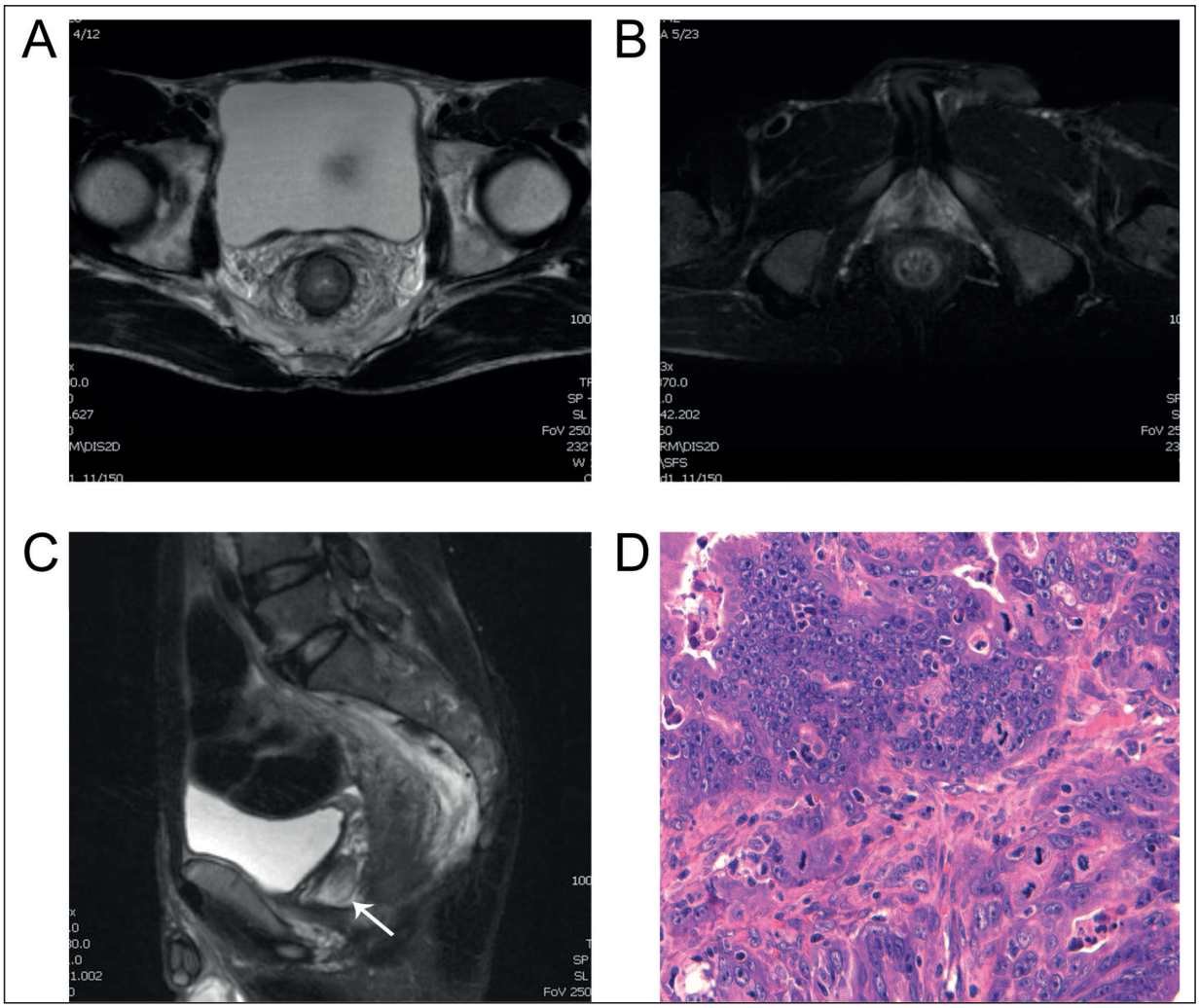

Figure 1. MRI materials of patients with rectal cancer. Male patients aged 51 years old. ***A*-B**, T2WI and T1WI images show significant thickening of the rectal wall. T2WI shows that the surrounding of the tube wall exhibits low signal which is considered to be space occupying lesion. **C**, shows bilateral levator ani muscle (arrow) at the coronal site of MRI. D, indicates poorly differentiated gland cancer through pathological diagnosis.

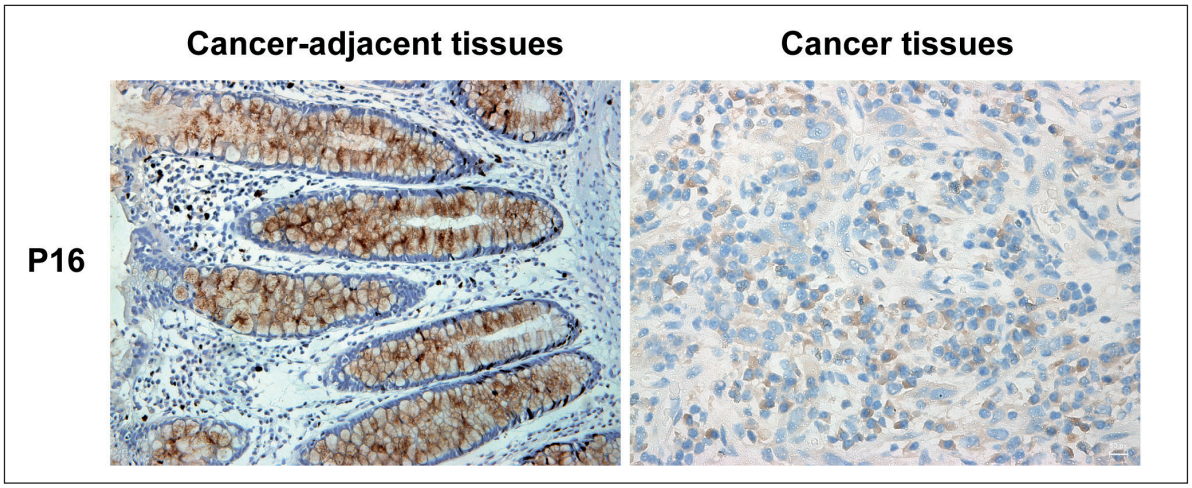

Figure 2. Difference in p16 expression in the rectal cancer tissues and cancer-adjacent tissues (×400 magnification).

Table I. Comparison of MRI staging diagnosis with postoperative pathological T staging results (n).

Pathological staging	n	MRI staging				
		T1	T2	T3	T4	Accuracy
T1	10	8	2	0	0	80.0%
T2	21	4	15	2	0	71.43%
T3	35	2	4	27	2	77.14%
T4	9	0	0	2	7	77.78%
Total	75	14	21	31	9	76.0%

Table II. Comparison of MRI staging diagnosis with postoperative pathological N staging results (n).

Pathological staging	n	MRI staging			
		N0	N1	N2	Accuracy
N0	18	15	3	0	83.33%
N1	46	5	38	3	82.61%
N2	11	1	3	7	63.64%
Total	75				80.0%

Table III. Difference in p16 expression in the rectal cancer tissues and cancer-adjacent tissues.

Group	n	Survivin	
		Positive	Negative
Tumor tissues	75	26 (34.67%)	49 (65.33%)
Tumor-adjacent tissues	75	64 (85.33%)	11 (14.67%)
χ^2-value		7.47	
p-value		0.011	

Table IV. Relationship between MRI staging and p16 expression in rectal cancer.

MRI diagnosis		n (75)	Expression of p16 in the tumor		χ^2-value	*p*-value
			Positive (26)	Negative (49)		
T stage	T1	14	4	10	6.14	0.015
	T2	21	7	14		
	T3	31	12	19		
	T4	9	3	6		
N stage	N0	21	15	6	5.14	0.021
	N1	44	11	33		
	N2	10	2	8		
Invasion of muscular layer	Shallow invasion of muscular layer	44	20	24	4.26	0.038
	Deep invasion of muscular layer	31	6	25		

Discussion

Rectal cancer is a common malignant tumor in the gastrointestinal tract, accounting for 65%-75% of colon cancers, and the accurate preoperative staging of rectal cancer is instructive in the selection of treatment regimen[11]. In the past, the conventional pelvic sequence was commonly used for MRI examination of rectal cancer in Chinese studies. In recent years, attention has been gradually paid to the application of high-resolution MRI in the staging of rectal cancer[12]. MRI can clearly show the extent of tumor invasion and surgical anatomical structure of mesorectum, making the accuracy of T staging reach 70%-80%[13]. Under MRI scanning, the fat around the rectum forms a good natural contrast. The T2WI can clearly show the anatomical structure of the pelvis and can provide accurate signs of the tumor. T2WI is superior to T1WI in displaying the invasion of the lesion and adjacent tissues[14].

The results of this study showed that the total accuracy of MRI for T staging of rectal cancer was 76.0%. The accuracy in the diagnosis of stage T1, T2, T3, and T4 was 80.0%, 71.43%, 77.14%, and 77.78%, respectively. In addition, the overall accuracy in the diagnosis of lymph node metastases also reached 80.0%, of which the accuracy in the diagnosis of N0, N1, and N2 was 83.33%, 82.61%, and 63.64%, respectively. The results are identical with those of previous studies. In this group, there were 18 cases of T stages mistakenly diagnosed with MRI, including 2, 6, 8, and 2 cases of misdiagnosis of T1, T2, T3, and T4, respectively. The retrospective image analysis showed that the main cause was that MRI had a certain limit on the judgment of staging critical surface (submucosal and muscular layer, muscular layer and perienteral fat, tumor and other organs of pelvic cavity). Most rectal cancers are in stage T2 or stage T3, and the major problem in MRI staging is the identification of stage T2 and the criticality of stage T3. Previous studies have shown that the criteria for the determination of stage T3 at least need to clarify that there is no muscular layer of the rectal wall between the edge of the tumor and the extracellular fat. The relatively reliable sign is the intrusion of tumor nodules into the perienteral fat, while the spinescent or fine abnormal cable signal shadows around the diseased intestine cannot be taken as the basis of intestinal invasion of tumors[15]. Local fibrosis or inflammation can lead to incomplete continuity of the muscular layer or similar signs of tumor infiltration in the thin strips of the surrounding adipose tissues, which is a major cause of wrong staging of T2 and T3[16].

Currently, there are no uniform diagnostic criteria for the evaluation of lymph node metastases with MRI. In this study, the identification was conducted mainly relying on the size and shape of lymph nodes as well as the signal intensity. In the results, the long diameter (>3 mm) of the lymph nodes around the intestine was judged as positive lymph node metastases. A total of 15 cases were mistakenly diagnosed with lymph node metastases, including 3, 8, and 4 cases of misdiagnoses of N0, N1, and N2, respectively, which might be related to the increased reactivity of inflammatory lymph nodes combined with cancer.

Recent studies have proved that the inactivity of p16 (a cancer-suppressing gene) commonly exists in human malignant tumors. The inactivity of p16 is mainly caused by the suppression of epigenetic expression which is resulted from deficiency or mutation of genes and the methylation of gene promoter[17]. However, in some tumor tissues, the expression of p16 is significantly increased, which may be the result of the activation of p16 expression by oncogenic factors. The deficiency of p16 homozygosity will lead to complete disappearance of p16 in the cells[18]. However, if the deficient allele can be expressed normally in the cells with deficient p16 heterozygosity, the content of p16 in the cells will be normal. The further mutation of the allele or methylation modification of the promoter can also lead to a massively reduced expression level of p16 in the cells[19]. Moreover, studies have also showed that the methylation in p16 promoter region may also be involved in the occurrence, evolution, and metastasis of rectal cancer. Lam et al[20] used quantitative methylation-specific polymerase chain reaction (PCR) technique to study the methylation status of p16 promoter in 50 cases of colorectal cancer specimens and normal tissue specimens and found that there are 20 (40%) cases with abnormal methylation of p16 promoter. Moreover, hypermethylation is closely related to the clinical stage of colorectal cancer, lymph node metastasis, and the size of the tumor. In addition, the methylation of p16 promoter in colorectal tumors is more prone to liver metastases and peritoneal metastases.

In this work, the expression of p16 in 75 postoperative rectal cancer tissues was analyzed. The results showed that the positive rate of p16 was 34.67% (26/75), which was significantly lower than that in the cancer-adjacent tissues [85.33% (64/75), $p<0.05$]. It suggests that the expression of p16 in rectal cancer is notably decreased. Further,

the correlations of the expression levels of p16 with MRI diagnostic materials were analyzed. It was found that the expression of p16 was significantly correlated with T staging, N staging, and invasion of muscular layer diagnosed using MRI ($p<0.05$). With the increase in the depth of T staging, N staging, and invasion, the positive rate of p16 was reduced.

Conclusions

We showed in this study that p16 was notably deficient in rectal cancer tissues. MRI examination and identification are helpful for the clinical diagnosis of rectal cancer staging, and the MRI diagnostic results are notably correlated with the expression of p16 in the tumors. It suggests that the combination of MRI with the examination of p16 may be helpful for clinical diagnosis of rectal cancer and the establishment of reasonable treatment regimens.

Conflict of interest

The authors declared no conflict of interest.

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