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ORIGINAL ARTICLE

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직장 카르시노이드 종양에서 Ki-67 발현의 예후적 중요성

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Prognostic Significance of Ki-67 Expression in Rectal Carcinoid Tumors

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Background/Aims: Rectal carcinoid tumors can be resected with endoscopy, and it is important to assess their prognostic factors. We evaluated the potential of Ki-67 expression as a prognostic factor in rectal carcinoid tumors.
Methods: We retrospectively reviewed the medical records of 37 patients with rectal carcinoid tumors who got endoscopic resection from January 2001 to January 2011 at Inje University Seoul Paik Hospital. We analyzed their endoscopic and histologic findings, Ki-67 expression, clinical outcome, and prognosis.
Results: The mean age (±SD) of the patients was 56.3±10.7 years, and the male : female ratio was 3.6 : 1. The mean tumor size was 0.5±0.4 cm, 33 patients showed grade 1 tumors (89.2%) and the average Ki-67 expression was 0.7±1.2%. Thirty five patients underwent endoscopic mucosal resection, and two required endoscopic submucosal dissection. Eight patients had positive margins after resection, but no cases of lymphovascular invasion were identified. The median follow-up duration was 21.4±25.4 months, and no recurrences were observed.
Conclusions: In low grade rectal carcinoid tumors which are lack of central depression on colonoscopy, the expression of a molecular marker of malignant potential, Ki-67, was low. Therefore, endoscopic resection seemed to be a safe and effective treatment for these tumors. **(Korean J Gastroenterol 2013;61:82-87)**

Key Words: Rectum; Carcinoid tumor; Ki-67; Endoscopy; Therapeutics

INTRODUCTION

Carcinoid tumors can occur in nearly any gastrointestinal (GI) tissue, and historically they have been reported most frequently in the appendix. However, the bronchus/lung (27.9%), ileum (14.9%) and rectum (13.6%) are now the most commonly reported sites.[1] Rectal carcinoid tumors account for 1.1-1.3% of all rectal tumors, their metastatic incidence is 3.9%, and compared to other tumors they are less invasive and less progressive.[2] Rectal carcinoid tumors are often incidentally diagnosed during screening lower endoscopy for colorectal cancer or other unrelated indications.[3] They are most commonly found in the mid-rectum and can have a wide range of sizes. Rectal carcinoid tumors that are less than 1 cm in diameter rarely metastasize (3-5%), and they can be managed with local endoscopic resection.[4-7] If the carcinoid tumor is over 2 cm, a full oncologic resection should be performed.[2] The treatment of rectal carcinoid tumors between 1-2 cm in diameter is controversial. In these cases it is critical to assess prognostic factors such as World Health

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Organization (WHO) classification, tumor/nodes/metastasis (TNM) stage, histologic grade, size of the tumor, depth of invasion, lymphovascular invasion, and mitotic bodies on histologic examination.

Ki-67, a marker of cellular proliferation, has recently been introduced as a promising prognostic factor for rectal carcinoid tumors.[8] In this study, we evaluated the role of Ki-67 expression as a prognostic factor and predictive biomarker in low grade rectal carcinoid tumors.

SUBJECTS AND METHODS

1. Patients

We conducted cross sectional study on human participants with the approval of the Institution Ethics Committee of Seoul Paik Hospital. We retrospectively reviewed the medical records of 37 patients with rectal carcinoid tumors who were treated with endoscopic resection between January 1, 2001 and January 1, 2011 at Inje University Seoul Paik Hospital. For each patient we analyzed the endoscopic and histologic findings, clinical outcome, and prognosis. In regards to treatment strategy, 35 patients underwent endoscopic mucosal resection (EMR), and two patients required endoscopic submucosal dissection (ESD). Follow-up colonoscopic endoscopic examinations were performed 3-6 months after therapeutic resection and every 1-2 years thereafter to monitor for local recurrence.

2. Immunohistochemical staining

All rectal carcinoid tumor specimens were reviewed by one expert pathologist and classified based on WHO criteria (Table 1).[9,10] The tissue specimens were then stained for Ki-67 expression by the following method. Paraffin blocks were cut into 4 μm thick sections and peeled off by xylene. Each paraffin block and section was mounted on a silanized slide. Blocking was performed by incubation with citrate buffer (10 mM, pH 6.0) for 10 minutes, and the buffer was washed off with cold water. On dewaxed and rehydrated slides, we performed endogenous peroxidase blocking by incubation with 3% hydrogen peroxide for five minutes. After incubation with Ki-67 monoclonal antibodies (NCL-L-Ki-67-MMI dilution 1 : 250; Novocastra, Newcastle Upon Tyne, UK) for 50 minutes at room temperature, we washed the slides with Tris buffered saline (TBS). We then incubated the slides with Dextran coupled with peroxidase molecules and goat secondary antibodies against rabbit and mouse immunoglobulins (REAL envision/HRP, Rabbit/Mouse envision; Dako, Glostrup, Denmark), which minimized the effect of endogenous biotin interruption by excluding avidin-biotin. The secondary antibody solution was washed off with TBS. Sections were then stained with diamino-benzidine for five minutes, followed by hematoxyline staining immediately before viewing.[11]

Table 1. Grading of Gastroenteropancreatic NET according to Proliferative Activity by WHO Classification

Grade	Criteria
G1 (low grade)	< 2 mitoses/10 HPF and < 3% Ki-67 index
G2 (intermediate grade)	2-20 mitoses/10 HPF or 3-20% Ki-67 index
G3 (high grade)	> 20 mitoses/10 HPF or > 20% Ki-67 index

NET, neuroendocrine tumor; WHO, World Health Organization; G, grade.

3. Determination of staining results

Tumor cells were observed by light microscopy at 200× magnification. Ki-67 positivity was calculated as the percentage of the total neoplastic cells within 1 mm^2 that had positive nuclear staining. Stromal and endothelial cells were excluded from the analysis.

4. Statistical analysis

All statistical calculations were performed with PASW Statistics version 18.0 (IBM, Armonk, NY, USA). Patient age, gender, tumor size, and Ki-67 expression were reported as means±SD. Variables were grouped by grade 1 or grade 2 proliferative activity and Ki-67 expression $< 0.7\%$ or $\geq 0.7\%$. Analysis was performed with an independent samples t-test.

RESULTS

1. Patient characteristics and outcomes

The mean age of the patients was 56.3±10.7 years (range 37-79 years), and there were 29 males (78.4%) and 8 females (21.6%). Mean rectal carcinoid tumor size was 0.5±0.4 cm (range 0.2-1.5 cm). Tumor size of 31 patients was <1 cm, of 6 patients was ≥ 1 cm and ≤ 1.5 cm. The baseline characteristics of 37 lesions resected by endoscopy are

presented in Table 2.

Rectal carcinoid tumors had a smooth, round, mobile, submucosal, and nodular appearance. There were no tumors which showed central depression on colonoscopy (Fig. 1A).

EUS was performed before definitive treatment in 19 patients, including 6 patients who have the tumor size ≥1 cm (Fig. 1B). EUS findings typically showed a hypoechoic mass in the submucosal layer of the rectum. Through the work-up including abdominopelvic CT or EUS, no lymph node enlargement or distant metastasis were found in any patients. Therefore, endoscopic excision was tried for these patients. In regards to margin positivity after endoscopic resection, 29 patients (78.4%) had clear margins, and 8 patients (21.6%) had tumor involvement at the resected margins. In patient who showed tumor involvement at the resected margins, short-term follow-up (within 3 months) was done and revealed no residual tumors in all cases. Endoscopic examinations were performed periodically to monitor for local recurrence during the follow-up period. The median follow-up duration was 21.4±25.4 months (range 0-87 months). Six patients were followed up by more than 3 years, and no recurrences were identified.

2. Ki-67 expression

The average Ki-67 expression was 0.7±1.2% (Fig. 2). Subgroups of Ki-67 expression based on tumor size, patient age, gender, and margin positivity are presented in Table 3.

Table 2. Baseline Characteristics of Patients and Tumors

Parameter	Data
Age (yr)	56.3±10.7
Gender	
Male	29 (78.4)
Female	8 (21.6)
Tumor size (cm)	0.5±0.4
Mean follow-up (month)	21.4±25.4
Involvement of resection margin	
Yes	8 (21.6)
No	29 (78.4)
WHO classification	
G1	33 (89.2)
G2	4 (10.8)
G3	0 (0)
Ki-67 expression (%)	
<0.7	29 (78.4)
≥0.7	8 (21.6)

Values are presented as mean±SD or n (%).
WHO, World Health Organization; G, grade.

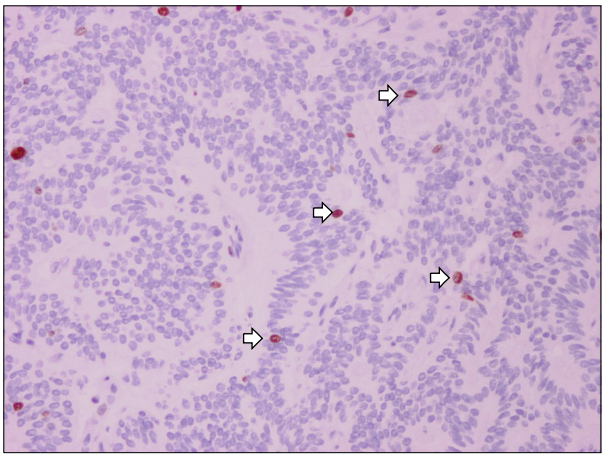

Fig. 2. Ki-67 staining in tumors resected by endoscopic mucosal resection (×200). On light microscopic examination, moderately uniform, small, round tumor cells with minimal cellular atypia were seen. The arrows indicate Ki-67 labeled nuclei, and the Ki-67 labeling index was 4% in this sample.

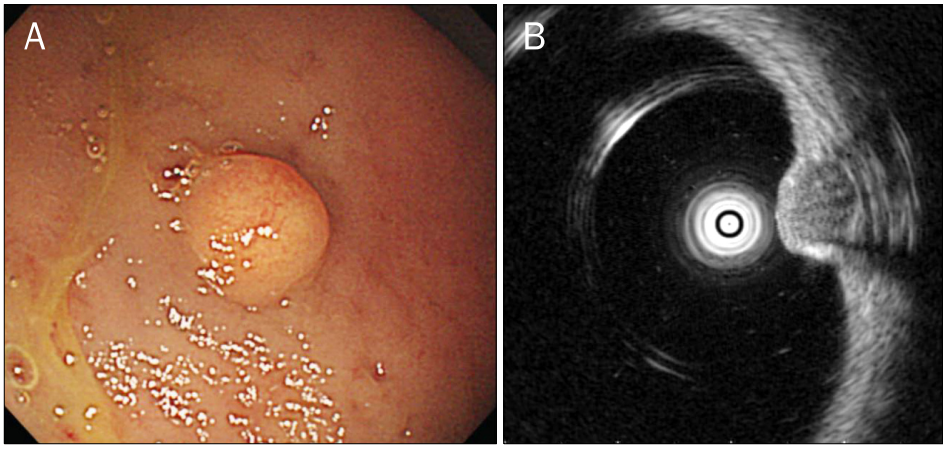

Fig. 1. (A) Endoscopic findings. Colonoscopy showed a 0.6 cm sized mild yellowish elevated lesion with intact mucosa at anal verge 7.0 cm site. (B) EUS findings. EUS showed a 0.7×0.6 cm sized hypoechoic lesion in the submucosal layer of the rectum.

Table 3. Comparative Results of Ki-67 Expression with Size, Age, Gender and Involvement of Resection Margin

Variable	Mean Ki-67 (%)	p-value
Tumor size (cm)		
<1	0.7	
≥1.0 and ≤1.5	0.8	0.785
Age (yr)		
≤55	0.7	0.880
>55	0.7	
Gender		
Male	0.7	
Female	0.8	0.771
Involvement of resection margin		
Positive	0.4	
Negative	0.8	0.498

$p<0.05$ accepted as statistically significant.

Table 4. Correlation of WHO Classification Grade with Size, Age, Gender and Involvement of Resection Margin

Variable	G1 (n=33)	G2 (n=4)	p-value
Tumor size (cm)	0.5±0.4	0.6±0.4	0.776
Age (yr)	56.6±10.5	53.5±13.2	0.589
Gender			
Male	26/33 (78.8)	3/4 (75.0)	0.867
Resection margin			
Positive	8/33 (24.2)	0/4 (0.0)	0.279

Values are presented as mean±SD or n (%).
$p<0.05$ accepted as statistically significant.
WHO, World Health Organization; G, grade.

In regards to tumor grading by WHO criteria, 33 patients had grade 1 tumors, and four patients had grade 2 tumors. In aspect of mitosis, no mitosis was seen in all cases. Tumor size, patient age, gender, and margin positivity were not related to tumor grade (Table 4).

When rectal carcinoid tumor specimens were divided into two groups based on mean value of Ki-67 expression, that was <0.7% or ≥0.7%, there was no correlation between Ki-67 expression and tumor size, patient age, gender, or margin positivity (Table 5).

DISCUSSION

GI carcinoid tumors arise from subepithelial neuroendocrine cells, penetrate the muscularis mucosa, and invade the submucosal layer at an early stage. Therefore, they appear as submucosal lesions on endoscopic finding. Since the widespread implementation of colorectal cancer screening,

Table 5. Correlation of Ki-67 Expression with Size, Age, Gender and Involvement of Resection Margin

	Ki-67 <0.7% (n=29)	Ki-67 ≥0.7% (n=8)	p-value
Tumor size (cm)	0.5±0.4	0.4±0.3	0.460
Age (yr)	56.1±10.5	56.8±12.0	0.888
Gender			
Male	22/29 (75.9)	7/8 (87.5)	0.493
Resection margin			
Positive	6/29 (20.7)	2/8 (25.0)	0.800

Values are presented as mean±SD or n (%).
$p<0.05$ accepted as statistically significant.

GI carcinoid tumors have begun to be discovered at an early stage.[12] Well-differentiated, grade 1 carcinoid tumors less than 10 mm in diameter that are confined to the submucosal layer can be treated with endoscopic resection. A variety of endoscopic techniques are used, including conventional polypectomy, band-snare resection, cap-assisted EMR and ESD.[13-15] Previous reports have shown that ESD results in a higher proportion of histologically complete resections with a similar complication rate to EMR.[16,17] In our study, most patients received EMR as treatment for their rectal carcinoid tumor; only two patients underwent ESD.

EUS has a high sensitivity (87%) in the diagnosis of rectal carcinoid tumors, which are usually well-demarcated isoechoic or hypoechoic masses.[13] The main applications of EUS in rectal carcinoid tumors are for the evaluation of tumor size, depth of invasion, and perirectal lymphadenopathy.[18] In our study, EUS was performed in 19 patients, and all tumors were confined to the submucosa without invasion of the muscularis propria. Especially, in 6 patients who had tumor sized ≥1 cm, EUS revealed that tumor was confined to the submucosa.

In this study, we evaluated the role of Ki-67 expression as a prognostic factor in low grade rectal carcinoid tumors. Ki-67 expression is widely used as a marker of proliferation in various types of tumors.[19-21] However, the role of Ki-67 expression as a prognostic factor in rectal carcinoid tumors has not been fully established.[21-24] Kawahara et al.[24] investigated 41 patients with GI carcinoid tumors. Fifty percents were Ki-67 positive in metastatic samples, while 0% were Ki-67 postitive in non-metastatic samples. Tumor cells were considered positive for Ki-67 when ≥10% of the cells were immunoreactive in the study. Hotta et al.[23] investigated 43 pa-

tients with rectal carcinoid tumors and found that the mean Ki-67 expression in metastatic samples was 3.9%, while in non-metastatic samples it was 1.0% ($p < 0.01$). Based on these results they suggested that Ki-67 expression is a reliable microscopic predictor of metastatic potential in rectal carcinoid tumors.[23,24] However, there are no reports on Ki-67 expression in low grade rectal carcinoid tumors.

In our study the mean Ki-67 expression was 0.7±1.2%, suggesting low malignant potential among our 37 patients with small, low grade rectal carcinoid tumors. Other prognostic factors such as tumor size, depth of invasion, and lymphovascular invasion were reviewed. There was no correlation between patient age, gender, tumor size, marginal involvement and Ki-67 expression.

The mean tumor size in our study was 0.52 cm, and most tumors had grade 1 histology. Therefore, low grade rectal carcinoid tumors and low Ki-67 expression suggested a good prognosis. We conclude that endoscopic resection is an effective therapeutic strategy for low grade rectal carcinoid tumors. EUS might be helpful to be performed prior to resection. If the tumor is less than 10 mm, without a central depression, with invasion restricted to the mid-submucosa, and no lymph node involvement or distant metastasis, endoscopic resection is indicated.

Our study has several limitations. The number of sample size was small, and all data were retrospectively reviewed. There were no comparable control groups, and all enrolled patients had non-metastatic, non-invasive tumors. These compositions of our study population may have contributed to the overall low levels of Ki-67 expression. The follow-up period of 10 years (with a median follow-up duration of 21.4±25.4 months) was relatively short.

Our study showed that Ki-67 expression was low in low grade rectal carcinoid tumors which are lack of central depression on colonoscopy. Therefore, we suggest that endoscopic resection seemed to be a safe and effective treatment for these tumors.

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