Diseases of the Colon & Rectum

Early Results of Intersphincteric Resection for Patients with Very Low Rectal Cancer: An Active Approach to Avoid a Permanent Colostomy

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PURPOSE: Abdominoperineal resection has been the standard surgery for very low rectal cancer located within 5 cm of the anal verge. However, permanent colostomy exerts serious limitations on quality of life. The present study aimed to investigate curability and functional results of intersphincteric resection and additional partial external sphincteric resection for carcinoma of the anorectal junction. METHODS: Thirty-five patients were prospectively studied from November 1999 to September 2002. All patients displayed adenocarcinoma (T3: n = 26; T2: n = 7; T1: n = 2) located between 0 and 2 cm above the dentate line. Abdominotransanal rectal resection with total mesorectal excision was performed in all patients (total intersphincteric resection: n = 14; subtotal intersphincteric resection: n = 5; additional partial external sphincteric resection: n = 6). All patients underwent diverting colostomy, which was closed at a median of six months postoperatively. Twenty patients received preoperative radiochemotherapy. RESULTS: All patients had curative intent with microscopic safety margins (mean surgical cut end: 4 mm; mean distal cut end: 10 mm). No postoperative mortality was encountered. Morbidity was identified in 13 patients (perianastomotic abscess: n = 4; anastomotic leakage and fistula: n = 4; postoperative bleeding: n = 2; wound infection: n = 1; anastomotic stenosis: n = 1; anovaginal fistula: n = 1). One of these patients received a permanent colostomy. Five patients developed recurrence (liver: n = 1; lung: n = 2; local and lung: n = 1; abdominal wall: n = 1) during the median observation period (23 months). Two of these patients underwent curative resection of liver or lung metastases. Twenty-one patients have received stoma closure, and although continence was satisfactory in all, 5 displayed occasional minor soiling 12 months after stoma closure. Anal canal manometry demonstrated significant reduction in maximum resting pressure (median: 50 cmH_2O at 12 months after stoma closure), but acceptable function results were obtained. CONCLUSION: Curability and anal function were achieved by means of intersphincteric resection without or with additional partial external sphincteric resection. These procedures can be recommended for low rectal cancer patients who are candidates for abdominoperineal resection. [Key words: Very low rectal cancer; Intersphincteric resection; Curability; Permanent colostomy; Anal function]

With advances in rectal anastomosis techniques, sphincter-preserving operations have become usual for upper and middle rectal cancers. The advent of mechanical low stapling and double stapling techniques, and sutured coloanal anastomosis have facilitated easier anastomosis at the distal rectum. These methods have increased the frequency of sphincter salvage. Nevertheless, permanent colostomy is still

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performed in about 20 percent of patients with rectal cancer. Abdominoperineal resection (APR) is the standard surgery for low rectal cancers located below 5 cm from the anal verge, or below 2 cm from the dentate line. Quality of life (QOL) after APR is unsatisfactory, because permanent colostomy results in serious psychologic and social limitations.[1,2]

In recent years, intersphincteric resection (ISR) with coloanal anastomosis has been proposed to avoid permanent colostomy for rectal cancer located at or near the anorectal junction. Several studies have reported that functional results and local recurrence rates after ISR are satisfactory in patients with low rectal cancer.[3–6] All patients in these studies underwent ISR, and none required permanent colostomy.

Prevention of local recurrence and preservation of anal function represent important goals in the treatment of low rectal cancer patients. The aim of this study was to investigate curability and functional results in ISR and additional partial external sphincteric resection (ISR plus PESR) for patients with low rectal cancer located at the anorectal junction.

METHODS

Patients

From November 1999 to September 2002, 52 patients underwent surgery for very low rectal cancer located at the anorectal junction in our colorectal surgery division. A total of 35 patients underwent the partial sphincter-preserving operation (ISR or ISR plus PESR) with coloanal anastomosis situated below the dentate line. APR was done in four patients (3 patients with locally advanced, poorly differentiated adenocarcinomas, one patient with poor anal function). Ordinary coloanal anastomosis (CAA, situated just above the dentate line) was done in five patients, local excision was done in five patients with T1 tumors, and total pelvic excenteration was done in three patients with bulky tumors invading adjacent organs. These 35 patients with ISR or ISR plus PESR were investigated in this study. There were 29 males and 6 females, with a median age of 59 (range, 27–72) years. All 35 patients had T2 or T3 tumors (well or moderately differentiated adenocarcinomas) located between 1.5 and 5.0 cm from the anal verge. All tumors found infiltrating the rectal wall on digital examination, computed tomography (CT), magnetic resonance imaging (MRI), or endorectal ultrasonography were eliminated from consideration for local excision. Distribution of tumor localization was investigated using endoscopy. The distance between distal margin of tumor and anal verge was measured. In all patients, the objective during the operation was to achieve a distance of at least 1.0 cm between tumor and the distal plane of resection. Surgical technique was chosen according to tumor localization and TNM staging.[7] Staging evaluations included digital examination, colonoscopy with biopsy, endorectal ultrasonography, CT, and MRI. A lateral view of the rectum by means of double-contrast technique was also used to assess the level of the tumor. An exception to selection of ISR was made if malignant infiltration of the pelvic floor was suspected, if tumors displayed low differentiation on histopathology, or if preoperative anal function demonstrated marked insufficiency. Pathologic TNM classifications for the 35 patients comprised T1N0 (n = 2), T2N0 (n = 6), T2N1 (n = 1), T3N0 (n = 14), T3N1 (n = 5), T3N2 (n = 5), T3N1M1 (n = 1), and T3N2M1 (n = 1).

All specimens were examined to determine macroscopic and microscopic surgical margins (distal and radial (lateral)). Distances for distal margin clearance and radial clearance (surgical cut end) were measured microscopically.

Surgical Technique

Total or subtotal ISR was performed according to the methods previously described by Schiessel *et al.*[5] ISR was contraindicated if invasion of the striated pelvic floor muscles was observed in patients with T4 tumors. When the left colon and the rectum had been mobilized to the levator ani with total mesorectal excision,[8–10] the tumor was reevaluated through gentle synchronous palpation by the abdominal and perineal surgeons. The internal sphincter was exposed and circumferentially divided. Intersphincteric dissection was performed by an abdominal approach with scissors and occasionally with fingers.

Once the intersphincteric space was entered just on the intersphincteric groove by the perineal surgeons, dissection continued upward between the smooth and striated sphincters under permanent guidance by the abdominal surgeon (Fig. 1A). Total ISR involved complete excision of the internal sphincter for a tumor spreading beyond or to the dentate line. The distal cut end line was at the intersphincteric groove. Total excision of the internal sphincter was unnecessary in patients with tumor located above within 2 cm from the dentate line and with a long anal canal. Those patients underwent subtotal ISR (Fig. 1B). Sub-

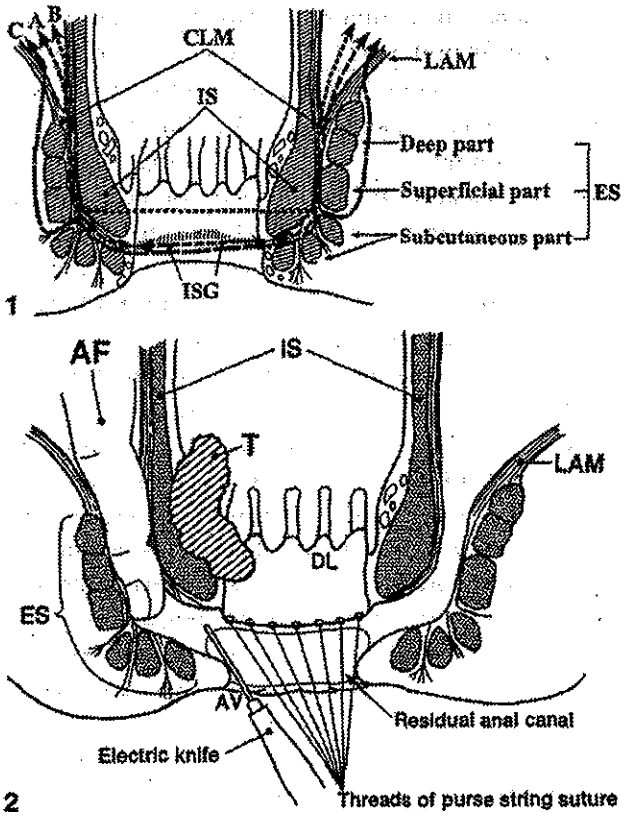

Figure 1. Methods of intersphincteric resection (ISR). A. Total ISR means complete resection of the internal anal sphincter. B. Subtotal ISR means partial preservation of the internal sphincter. C. ISR plus PESR means total ISR combined with partial resection of the external anal sphincter. CLM = conjoined longitudinal muscle; ES = external sphincter; IS = internal sphincter; ISG = intersphincteric groove, LAM = levator ani muscle.

Figure 2. Peranal approach in ISR. Assistant's finger-tips (AF) means guidance by the abdominal surgeon. The anal orifice is closed by purse string suture to avoid spread of tumor cells during peranal operation. AV = anal verge; DL = dentate line; ES = external sphincter, IS = internal sphincter; LAM = levator ani muscle; T = tumor.

total ISR required partial preservation of the internal sphincter for tumors spreading within 2 cm of the dentate line. The distal cut end line was between the dentate line and the intersphincteric groove in this procedure. The dentate line was included in the resected specimen. A resection margin of at least 1 cm was always attempted. When patients had tumor invading the external sphincter, ISR plus PESR was undertaken (Fig. 1C). Only the subcutaneous part of the external sphincter was preserved in these patients. Between the subcutaneous and superficial part of the external sphincter, a visible margin was not obtained. However, a loose attachment was present between these muscle bundles, so they could be easily separated circumferentially with fingertips and scissors. The anal orifice was closed by purse string suture after the anoderm and subcutaneous tissue had been cut (Fig. 2). This procedure was used to avoid spread of tumor cells during the perianal phase of the intersphincteric dissection. After specimen removal and generous irrigation of the pelvic cavity, the sigmoid or descending colon was pulled down and coloanal anastomosis was performed according to the method described by Parks.[11] The coloanal anastomosis was situated between 5 and 10 mm below the dentate line in patients with subtotal resection of the internal sphincter, and 5 mm from the perineal skin in patients with total resection of the internal sphincter. Anastomoses were performed using transanal manual sutures in all patients. End-to-end anastomosis was performed in 28 patients, colonic J-pouch in 2 patients, and coloplasty pouch in 5 patients. A diverting transverse colostomy was established in all patients. Although colostomies in patients with ordinary CAA and very low anterior resection were closed three months after radical surgery, colostomies in patients with ISR were planned such that closure was done three months later postoperatively because of estimated insufficient anal function and postoperative complications.

Adjuvant Therapy

Preoperative radiochemotherapy was performed in patients with T3 tumors who agreed to preoperative adjuvant therapy (n = 20). These patients had 45 Gy delivered over a five-week period, followed by resection two weeks later. In addition, 5-fluorouracil (250 mg/m^2/day) in continuous infusion was administered to these patients during radiotherapy to increase radiotherapeutic efficacy.

Follow-Up and Functional Assessment

Follow-up examinations were performed every three months for two years postoperatively, and every six months after that. Patients underwent clinical examination, determination of continence status and frequency of daily defecation episodes, laboratory tests including tumor markers (CEA, CA19-9), and radiologic examination (liver and pelvic CT, and chest radiography).

Median follow-up was 23 (range, 6–35) months. No patient was lost in follow-up, and 56 percent of patients were observed for at least 24 months.

Table 1.
Distribution of Tumor Localization With Respect to Operative Procedure

Distance to Anal Verge	No. Patients	Procedure		
		Total ISR	Subtotal ISR	Total ISR + PESR
≤2 cm	11	6	1	4
2 to ≤3 cm	5	2	3	0
3 to ≤4 cm	12	4	6	2
4 to ≤5 cm	7	2	5	0
Total	35	14	15	6

ISR = intersphincteric resection; ISR + PESR = intersphincteric resection and additional partial external sphincteric resection.

Sphincter function was evaluated clinically and manometrically 3, 6, and 12 months after stoma closure. Patients were questioned about frequency of bowel movements during 24 hours, ability to defer defecation for 15 minutes (urgency), discrimination of feces from flatus, and continence. Continence status was determined according to the classification of Williams *et al.*,[12] which had been used for the last ten years at our institute. Satisfaction with results was also investigated by use of a questionnaire on the degree of satisfaction with bowel function based on continence, frequency, and evacuation. The degree of satisfaction was classified into four grades (excellent: pleased; good: satisfied; fair: somewhat dissatisfied; poor: very dissatisfied). Physiologic assessment included anal manometry with water-filled balloons. Maximum resting and squeeze pressures and anal canal length were evaluated 3, 6, and 12 months after stoma closure.

RESULTS

Radical resection of the tumor was achieved in all 35 patients. Surgery was judged curative in all cases. Mean distal margin clearance was 13 (range, 8–32) mm and the mean radial clearance was 4.2 (range, 1.3–11) mm. Two patients with liver metastasis underwent partial hepatic resection at the same time as rectal cancer surgery. Endoscopic distribution of tumors (distance between tumor distal margin and anal verge) is shown in Table 1. Surgical procedure was decided according to tumor localization. Total ISR with or without PESR was performed in 20 patients, subtotal ISR was done in 15 patients. In 11 patients with tumors located within 2 cm of the anal verge, 10 patients underwent total ISR with or without PESR. One patient with T1 tumor underwent subtotal ISR. In 19 patients with tumors located between 3 and 5 cm from the anal verge, 11 patients underwent subtotal ISR. All patients underwent coloanal anastomosis by manual suturing.

No postoperative mortality was encountered. Early complications in 11 patients (31 percent) included perianastomotic abscess and infection (n = 4), anastomotic leakage and fistula (n = 4), postoperative bleeding (n = 2), and wound infection (n = 1). There were eight patients (40 percent) in the preoperative radiochemotherapy group (n = 20) and three patients (20 percent) in the surgery-alone group. Six patients (30 percent) in the preoperative radiochemotherapy group had complications because of infection or anastomotic leakage. Of these 11 patients, one in the surgery-alone group experienced serious complications of postoperative massive bleeding and necrosis of the oral side colon in the anastomotic site. This patient required coloanal anastomosis removal to achieve homeostasis and to remove necrotic tissue, and APR was subsequently performed. Late complications in two patients included anastomotic stricture (n = 1) and anovaginal fistula (n = 1). These patients needed surgical treatment.

During the median observation time of 23 months, five patients developed recurrence. These comprised multiple lung metastases with local recurrence (n = 1), multiple lung metastases with regional lymph node metastasis (n = 1), solitary liver metastasis (n = 1), solitary lung metastasis (n = 1), and abdominal wall metastasis (n = 1). Patients with liver or lung metastasis alone received curative partial hepatic or lung resection. The other patients with multiple lung metastases were treated by radiochemotherapy.

Of the 34 patients (after excluding one who underwent APR), 21 received colostomy closure at a median of six (range, 3–15) months postoperatively. Stoma closure was postponed in four patients because of postoperative complications (n = 2) or insufficient anal function (n = 2). Five patients without problems are scheduled for closure, and the remaining four patients have had too short a postoperative period for stoma closure. Sphincter function was investigated in the 21 patients with stoma closure 3, 6, and 12 months after colostomy closure (Table 2). Fifteen of the 21 (71 percent) patients experienced six or more bowel movements per day, 16 (76 percent) reported urgency, and 11 (52 percent) were able to completely discriminate feces from flatus three months after stoma closure. Twelve months after stoma closure, 9

Table 2.
Functional Outcome (After Stoma Closure)[a]

	3 Months (n = 21) No. Patients (%)	6 Months (n = 12) No. Patients (%)	12 Months (n = 11) No. Patients (%)
Stool frequency (per day)			
≤3	—	3 (25)	3 (27.3)
4–5	6 (28.5)	2 (16.7)	6 (54.5)
6–8	9 (43)	6 (50)	2 (18.2)
≥9	6 (28.5)	1 (8.3)	—
Urgency (+)	16 (76.2)	4 (33.3)	4 (36.4)
Discrimination of feces from flatus	11 (52.4)	9 (75)	9 (81.8)

[a]n = 21, October 2002.

of 11 patients (82 percent) experienced five or fewer bowel movements per day and had complete discrimination, and 4 (36 percent) reported urgency. Continence status according to Williams *et al.*[12] is shown in Table 3. Three months after stoma closure, occasional minor soiling was present in 19 patients (91 percent) and major soiling occurred in 2 patients (10 percent). The two patients with major soiling had undergone ISR plus PESR. Although their anal function was improved 6 months after stoma closure, remarkable improvement of anal function was not recognized 12 months after stoma clause. There were no patients with perfect function 12 months after stoma closure in the ISR plus PESR group (n = 6). No patients experienced major soiling and incontinence, but occasional minor soiling occurred in 5 of 11 patients (45 percent) 12 months after stoma closure. There were no differences in anal function between the preoperative radiochemotherapy group and surgery-alone group.

Functional assessment by patients, who compared status before and after stoma closure, is shown in Table 4. Satisfaction was excellent for one patient (5 percent), good for eight (38 percent), fair for eight (38 percent), and poor for four (19 percent) patients 3 months after stoma closure. Satisfaction was improved 12 months after stoma closure, with excellent reported by three patients (27 percent), good by six (55 percent), and fair by two (18 percent). No patients reported poor satisfaction at this stage. All patients preferred the status after stoma closure to status with temporary colostomy.

Physiologic results by manometry 3, 6, and 12 months after stoma closure are shown in Table 5. Manometric studies demonstrated a significant decrease in resting pressure after ISR. Median resting pressure was 42 (range, 8–87) cmH_2O 3 months after stoma closure, 44 (range, 8–96) cmH_2O at 6 months, and 50 (range, 26–100) cmH_2O at 12 months. Maximum squeeze pressure also decreased at all time points. Squeeze pressure, however, tended to recover. Median squeeze pressure was 175 (range, 90–275) cmH_2O 12 months after stoma closure.

DISCUSSION

It is generally agreed that most middle-rectum carcinomas can be treated using low anterior resection. In recent years, however, the need for a 5-cm distal margin has been challenged, and a distal margin of 1 to 2 cm is now considered sufficient in most instances. This change in philosophy and the introduction of circular stapling devices that facilitate the performance of low anastomosis have meant that low anterior resection can be performed for most rectal carcinomas, even those located in the distal third of the rectum. Sphincter-saving operations such as ultra-low and coloanal anastomosis, for carcinoma of the lower third of the rectum, have also been reported by specialized teams, with local recurrence ranging from 4 to 13 percent.[13–18] Although ultralow and coloanal anastomosis have been associated with some controversial functional results, it is widely accepted that patients without a permanent stoma experience significantly better QOL.[19,20] However, most tumors in these studies have been situated at or above 5 cm from the anal verge. The few studies that have reported oncologic and functional results after ISR concentrated on low rectal cancers located below 5 cm from the anal verge, such as carcinoma of the anorectal junction.[3–6] Some fears of incontinence or poor anal function exist for ISR, because the distal cut end line exists below the dentate line and the internal sphincter is removed. The present study was designed to investigate curability and functional outcome of ISR and ISR plus PESR in patients with tumors of the anorectal junction who would generally undergo APR. The possibility of avoiding APR was also evaluated.

Table 3.
Functional Outcome (After Stoma Closure)[a]

Continence	3 Months (n = 21) No. Patients (%)	6 Months (n = 12) No. Patients (%)	12 Months (n = 11) No. Patients (%)
Perfect			
Continence to solids, liquids, and flatus			3 (27.3)
Incontinent of flatus			
Continence to solids and liquids but not to flatus		2 (16.7)	3 (27.3)
Occasional minor soiling			
Continence to solids, occasional incontinence to liquids	19 (90.5)	10 (83.3)	5 (45.4)
Major soiling			
Frequent episodes of incontinence to liquids	2 (9.5)		
Incontinence			
Frequent episodes of incontinence to solids and liquids			

[a]n = 21.

Table 4.
Functional Assessment by Patients (After Stoma Closure)

Satisfaction with Results	3 Months (n = 21) No. Patients (%)	6 Months (n = 12) No. Patients (%)	12 Months (n = 11) No. Patients (%)
Excellent	1 (4.8)	1 (8.3)	3 (27.3)
Good	8 (38.1)	6 (50)	6 (54.5)
Fair	8 (38.1)	5 (41.7)	2 (18.2)
Poor	4 (19)	0	0
Prefer the present time to the past with stoma		Yes 12 (100) No 0 (0)	Yes 11 (100) No (0)

Table 5.
Physiologic Results (After Stoma Closure)

	3 Months Median (Range)	6 Months Median (Range)	12 Months Median (Range)
Maximum resting pressure (70–120 cm H_2O)	42 (8–87)	44 (8–96)	50 (26–100)
Maximum squeeze pressure (200–350 cm H_2O)	120 (70–215)	148 (88–220)	175 (90–275)
Anal canal length (cm)	3.6 (1.8–4.6)	3.6 (1.8–4.6)	3.3 (2.5–4.2)

In this study, 35 patients with low rectal cancers located below 5 cm from the anus underwent ISR or ISR plus PESR. Patients with low-differentiated carcinomas on histopathology were excluded from the study, as survival rate is poor in patients with low-differentiated rectal carcinoma. Braun *et al.* reported five-year survival rates following coloanal anastomosis and APR with respect to histopathologic grading as 83 percent and 68 percent for high-grade differentiation, 64 percent and 48 percent for medium-grade, and 33 percent and 44 percent for low-grade, respectively.[6] Initially, some doubt existed as to whether safety margins were obtained using these new surgical procedures. One of the important criteria for assessing therapeutic results in rectal cancer is local control. Cancer-free margins were obtained, however, in all 35 patients in the present investigation. Mean distal margin of clearance was 13 mm and mean radical clearance was 4 mm. These results show that radical tumor resection was achieved in all patients by ISR or ISR plus PESR. Curability with these new surgical procedures was verified histologically. Although median duration of follow-up was very short (23 months), only 1 of the 35 patients developed local recurrence. Schiessel *et al.* reported that 4 of 38 patients developed local recurrence during a median observation of three years.[3] Rullier *et al.* reported a similar result.[4] Local control in our data does not differ from that in these reports, and acceptable local control can be obtained by ISR or ISR plus PESR in patients with low rectal cancer.

Although Schiessel *et al.* reported a low morbidity

rate (7 of 38 patients),[3] morbidity in our study was high, with 11 of 35 patients having early complications. One patient required removal of the coloanal anastomosis and needed permanent colostomy. But complications in other patients were not serious, and postoperative mortality was not encountered. There was a tendency to have complications caused mainly by infection in patients with preoperative radiochemotherapy. Careful treatment is needed for these patients. The present results confirm, however, that ISR is a relatively safe procedure.

The diverting stoma was closed at a median of 6 months postoperatively in 21 patients in the present study, excluding the patient with APR. Stoma closure was postponed in four patients with late complications caused by anastomotic stricture (n = 1), anovaginal fistula (n = 1), and marked decrease of anal canal tonus (n = 2). Stoma closure is planned for these patients after resolution of problems. One of them underwent an additional flap operation, one received bogie treatment, and the other two patients received biofeedback therapy. Their anal status is improved at the present time. Five patients without problems are also awaiting stoma closure. These nine patients will be without stoma soon. The remaining four patients have had too short a postoperative period to undergo stoma closure.

Approximately 30 to 60 percent of low colorectal or coloanal anastomoses induce functional disturbances collectively termed *anterior resection syndrome*.[21–26] Some patients display a higher frequency of bowel movement and urgency and continence disorders. Most authors believe preservation of the whole anal sphincter and mucosa of the anal cancer mucosa is necessary for maintenance of good continence. Functional results after coloanal anastomosis disprove the hypothesis that total loss of the rectal cuff must lead to anal incontinence. Total loss of the rectum by ISR sacrifices reservoir function and the ability for repulsion, as expressed by increased frequency of bowel movements and decreased warning period.[6] APR, therefore, represents a standard surgical procedure when the distance between the lower edge of the tumor and the anal ring is <2 cm.[27] However, in a study by Williams and Johnston, patients with stomas displayed higher scores on depression (32 percent in APR *vs.* 10 percent in low anterior resection (LAR), reported altered body image (66 percent in APR *vs.* 5 percent in LAR), and returned to employment less frequently (40 percent in APR *vs.* 83 percent in LAR). A recent study on QOL by Sprangers *et al.*[2] demonstrated that although both patients with stoma and those with ultra-LAR reported restricted social function, such problems were more prevalent among patients with permanent stomas. Conversely, Grumann *et al.*[28] reported that patients with APR did not experience poorer QOL than patients undergoing anterior resection, and patients with LAR demonstrated lower QOL than those with APR. Functional results actually influence QOL.[21] In the present study, manometric results demonstrated a significant decrease in resting pressure, probably representing one of the reasons for minor soiling and occasional incontinence to liquids. Although all patients had continence for solid stool, five patients (45 percent) had occasional minor soiling at 12 months after stoma closure. Three of these patients underwent ISR plus PESR. There was a tendency to improve anal function slowly in patients with ISR plus PESR. However, no patients experienced major soiling with incontinence. Although a few patients in the present study had perfect continence, all patients with stoma closure preferred stomaless status to status with diverting stoma. In other series,[3–6] perfect continence in almost 80 percent of patients is probably the result of the undisturbed external sphincter muscle, as indicated by normal squeeze pressure.

The oncological and functional results obtained in this study seem acceptable, although the short follow-up (median, 23 months) must be considered. Even in continent patients, however, high stool frequency associated with urgency can be troublesome. Additional procedures such as colonic pouch, coloplasty,[29] or creation of a smooch muscle cuff[30] may facilitate functional improvement. We conclude that, excluding patients with low-differentiated tumors or with tumors fixed to the pelvic wall, ISR represents a safe and oncologically radical procedure allowing preservation of anal function in patients with very low rectal cancer.

CONCLUSION

Curability and acceptable anal function were obtained by ISR or ISR plus PESR in patients with very low rectal cancers. These procedures can be recommended for APR candidate patients with tumors located on or near the anorectal junction. Permanent colostomy can be avoided in almost all rectal cancer patients.

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Original Paper

The Risk of Lymph Node Metastasis in T1 Colorectal Carcinoma

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KEY WORDS:
Colorectal T1 carcinoma; Lymph node metastasis

ABSTRACT

Background/Aims: The purpose of this study was to evaluate the risk of lymph node metastasis in patients with T1 colorectal carcinoma based on a uniform histopathology system, and to accomplish guidelines for additional surgery for endoscopically or locally removed T1 colorectal carcinoma.

Methodology: A review was performed of 301 patients who underwent curative resection for T1 colorectal carcinoma between January 1970 and March 2001. The following clinicopathologic variables were evaluated using univariate and multivariate analysis: sex, age, location, size of tumor, macroscopic appearance, depth of submucosal invasion, lymphovascular invasion, and histologic grade. Lesions were subdivided according to the depth of submucosal invasion: sm1, submucosal invasion up to 500μm from the muscularis mucosa; sm2, submucosal invasion between 500 and 1000μm; sm3, submucosal invasion beyond 1000μm.

Results: The overall lymph node metastasis rate was 6.3 per cent (19 of 301). Depth of submucosal invasion (sm3) and presence of lymphovascular invasion were significant risk factors for lymph node metastasis both univariately and multivariately.

Conclusions: The findings of the current study demonstrated that significant risk factors for lymph node metastasis were level of submucosal invasion (sm3) and the presence of lymphovascular invasion. Surgery is indicated for patients with adverse factors.

INTRODUCTION

With advances in endoscopic skills and instruments, increasing numbers of T1 colorectal carcinomas are being detected. It is widely accepted that once the cancer cells have invaded into the submucosal layer, they can metastasize to the regional lymph nodes or even distally to the liver or other organs. Despite recent advances in the knowledge of various clinical, biologic, and pathologic features that relate to the prognosis of colorectal carcinoma, the risk of lymph node metastasis in patients with colorectal carcinoma invading the submucosal layer is still undetermined because of the lack of a reliable data for analysis based on a uniform histopathology system (1). Although it is widely accepted that inadequate excision (i.e. incomplete polypectomy or a margin of resection which is not clearly cancer free) is one of the high risk factors for adverse outcome, controversy exists regarding additional surgery for endoscopically removed colorectal carcinoma invading the submucosal layer (1-7).

The present study was undertaken in an attempt to determine the risk of lymph node metastasis in patients with colorectal carcinoma invading the submucosal layer and to develop clinical guidelines for treatment of colorectal carcinoma invading the submucosal layer, using a histopathologic staging system obtained from resected T1 lesions.

METHODOLOGY

The records of 301 patients with T1 colorectal carcinoma who underwent bowel resection, or local excision or endoscopic resection followed by bowel resection at the Keio University Hospital between January 1970 and March 2001 were retrospectively reviewed. Patients who underwent local excision or endoscopic resection only, and those with ulcerative colitis or familial polyposis were excluded from this study. Thus, all patients in the study group had undergone exploration to determine lymph node status.

All the lesions were divided into pedunculated or sessile forms according to their macroscopic appearances. All patients underwent histopathologic evaluation of the level of submucosal invasion, lymphovascular invasion, and histologic grade of adenocarcinoma. Lesions were subdivided according to the depth of submucosal invasion described by Okabe (8) as follows: sm1, submucosal invasion up to 500μm from the muscularis mucosa; sm2, submucosal invasion between 500 and 1000μm from the muscularis mucosa; sm3, submucosal invasion beyond 1000μm from the muscularis mucosa. In the cases that the muscularis mucosa was destroyed by the infiltration of the tumor, the vertical distance from the surface of the tumor was measured.

Statistical analysis was performed using the Student's *t* test for continuous variables, Fisher's exact test and the chi-square test for univariate comparisons, and the logistic regression analysis for multivariate analysis to evaluate the variables as potential risk factors for lymph node metastasis. *P* values less than 0.05 in both univariate and multivariate analysis were

Hepato-Gastroenterology 2004; 51:998-1000

regarded as significant.

RESULTS

Nodal involvement was found in 19 (6.3 percent) patients with colorectal carcinoma invading the submucosal layer among 301 patients who underwent bowel resection. According to the TNM classification, 18 (94.7 percent) of the 19 patients were pN1 and only one (5.3 percent) patient was pN2. The site of lymph node metastasis was limited to the paracolic or pararectal lymph nodes, except one patient who had intermediate lymph node metastasis.

Univariate analysis revealed that the level of submucosal invasion (sm3; P=0.0004) and lymphovascular invasion (present; P=0.0003) were significant predictors for lymph node metastasis of colorectal carcinoma invading submucosal layer. Other clinicopathologic factors, sex, age, location of tumor, size of tumor, macroscopic appearance, and histologic grade did not reveal any statistical difference in lymph node metastasis (**Table 1**).

According to logistic regression analysis, depth of submucosal invasion was most independently associated with lymph node metastasis, followed by lymphovascular invasion (**Table 2**).

The incidence of lymph node metastasis according to the level of submucosal invasion and lymphovascular invasion is presented in **Table 3**. The rate of nodal involvement for patients with sm3 or presence of lymphovascular invasion was 10.6 percent (19 of 180), whereas that for patients with sm1+sm2 and absence of lymphovascular invasion was 0 percent (0 of 121).

TABLE 1. Univariate Analysis of Clinicopathological Factors and Metastasis of Colorectal Carcinomas Invading the Submucosal Layer

	Lymph node metastasis		
Clinicopathologic factors	**Present**	**Absent**	***P***
Sex			
Male	10	208	0.0596
Female	9	74	
Age (mean±SD)	57.8±8.9	62.4±11.3	0.0789
Location of tumor			
Colon	10	206	0.0669
Rectum	9	76	
Size of tumor (mm; mean±SD)	25.4±25.0	20.3±12.4	0.1146
Macroscopic appearance			
Pedunculated	7	161	0.0983
Sessile	12	121	
Level of submucosal invasion			
sm1+sm2	3	166	0.0004
sm3	16	116	
Lymphovascular invasion			
Present	13	76	0.0003
Absent	6	206	
Histologic grade of adenocarcinoma			
Well differentiated	12	218	0.1637
Moderately differentiated	6	61	
Poorly differentiated	1	3	

TABLE 2. Multivariate Analysis with Respect to Lymph Node Metastasis of Colorectal Carcinomas Invading the Submucosal Layer

	Multivariate analysis			
Clinicopathologic factors	χ^2	***P* value**	**Risk ratio**	**95% CI**
Sex				
Male	1.896	0.169	2.066	0.736-5.801
Female				
Age	2.573	0.109	0.961	0.915-1.009
Location of tumor				
Colon	0.015	0.903	1.072	0.349-3.290
Rectum				
Size of tumor (mm)	0.995	0.319	1.015	0.986-1.044
Macroscopic appearance				
Pedunculated	1.941	0.164	2.203	0.725-6.690
Sessile				
Level of submucosal invasion				
sm1+sm2	8.922	0.003	8.592	2.095-35.241
sm3				
Lymphovascular invasion				
Present	6.491	0.011	4.045	1.380-11.853
Absent				
Histologic grade				
Well differentiated	0.130	0.903	1.235	0.391-3.905
Moderately or poorly differentiated				

DISCUSSION

It is generally accepted that colorectal carcinoma *in situ* does not metastasize and patients with these lesions can be treated adequately by endoscopic resection or local excision alone. However, once the cancer cells have invaded into the submucosal layer, they can metastasize to the regional lymph nodes or even distally to the liver or other organs. Lymph node metastasis is still the most important indicator of unfavorable clinical outcome, and determination of risk factors associated with lymph node metastasis in patients with submucosal colorectal carcinoma is essential for the selection of appropriate treatment. There are several clinicopathologic factors which have been reported to predict lymph node metastasis of submucosal carcinoma, and the findings of this study, in combination with previous published results, may help surgeons to select the patients for additional surgery after endoscopic resection or local excision for colorectal carcinoma invading the submucosal layer.

Several studies have demonstrated that deep submucosal invasion (1,5,9,10), the presence of lymphovascular invasion (2-4,6,8,11), low grade differentiation (2-4,6,8,11), a sessile or flat macroscopic appearance of the tumor (5), and a tumor at or near the resection margin after endoscopic resection or local excision (2-4,6,7,11) were adverse risk factors for lymph node metastasis with submucosal invasion. Needless to say, patients with T1 tumors at or near the resection margin after endoscopic resection or local excision require further treatment and we excluded this factor from our analysis. In the present study, the risk of lymph node metastasis was significantly associated with deep submucosal invasion and the presence of lymphovascular invasion. The overall incidence of lymph node metastasis was 6.3 percent (19 of 301), and all 19 patients belonged to the group with high risk submucosal lesions for lymph node metastasis, which were defined as having sm3 invasion and/or presence of lymphovas-

TABLE 3 Incidence of Lymph Node Metastasis according to the Level of Submucosal Invasion and Lymphovascular Invasion

Level of submucosal invasion	Lymphovascular invasion	
	Present	Absent
sm1	1 of 9 (11.1)	0 of 66 (0)
sm2	2 of 39 (5.1)	0 of 55 (0)
sm3	10 of 41 (24.4)	6 of 91 (6.6)

Values in parentheses are percentages.

cular invasion.

Several criteria for classifying submucosal colorectal carcinoma have been postulated. Haggitt *et al.* (9) proposed a classification for pedunculated and sessile polyps separately according to the level of submucosal invasion. Kudo *et al.* (10) divided the submucosal layer into three equally, vertically with a subdivision horizontally. On the other hand, slight submucosal extent (i.e. sm1) is defined as invasion from the muscularis mucosa to a depth of 200 to 300μm by the Japanese Society for Cancer of the Colon and Rectum, although criteria for sm2 and sm3 are not stated (12). However, Kikuchi *et al.* (5) pointed out a problem on Haggitt's system, especially with sessile polyps. According to Haggitt's system, all sessile polyps are classed as level 4 (sm3). Kikuchi *et al.* reported that sessile polyps could be classified as sm1, sm2, or sm3, and there was neither lymph node metastasis nor local recurrence in their sm1 patients. On the other hand, it is often difficult to classify endoscopically removed specimen according to Kudo's method, because most of the specimens do not contain muscularis propria. Recently, classification by absolute value of the depth of submucosal invasion has been reported, and we assessed all the specimens using the criteria described by Okabe (8) in this study.

Many recent studies have demonstrated that laparoscopic surgery is technically feasible and oncologically appropriate with favorable short- and long-term outcomes in patients with T1 colorectal cancer (13,14). However, the treatment of T1 carcinoma of the lower rectum is controversial. In our institution, radical surgery with laparoscopic low anterior resection or abdominoperineal resection is often indicated for most patients with T1 lesions with adverse risk factors, if there is no contraindication. Several studies have suggested that a combination of local excision and adjuvant chemoradiotherapy may be a potential option for the treatment of T1 carcinoma of the lower rectum instead of "invasive" radical resection (15-17). Although most patients with lesions of T1 carcinoma in the lower rectum in whom local recurrence develops after local excision can be salvaged by radical resection, the long-term outcome remains unknown (18,19).

In conclusion, this study demonstrates that the level of submucosal invasion and lymphovascular invasion are significant risk factors for lymph node metastasis. In addition to patients with tumors at or near the resection margin after endoscopic resection or local excision, further treatment is indicated for patients with T1 lesion with adverse factors, sm3 invasion and/or presence of lymphovascular invasion.

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ORIGINAL ARTICLE

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Gene expression of 5-fluorouracil metabolic enzymes in primary colorectal cancer and corresponding liver metastasis

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Abstract *Purpose*: Expression of thymidylate synthase (TS) and the 5-fluorouracil (5-FU) metabolic enzymes, including dihydropyrimidine dehydrogenase (DPD), orotate phosphoribosyl transferase (OPRT), thymidine phosphorylase (TP), and uridine phosphorylase (UP), has been reported to be associated with the sensitivity to 5-FU-based chemotherapy in colorectal cancer. We evaluated the correlation of the expression of these genes between primary tumors and corresponding liver metastases. *Method*: The mRNA levels of TS, DPD, OPRT, TP, and UP were measured by real-time quantitative RT-PCR in samples from 23 consecutive patients with both primary colorectal adenocarcinoma and liver metastasis. *Results*: The DPD, OPRT, TP, and UP mRNA levels were significantly higher in liver metastases than in primary tumor (expression in relation to that of β-actin mRNA: 0.42 vs 0.16, $P=0.00053$; 1.4 vs 0.92, $P=0.016$; 23 vs 11, $P=0.00014$; 0.36 vs 0.25, $P=0.0026$; respectively). However, the TS mRNA level did not differ significantly between liver metastases than primary tumor (0.20 vs 0.16, $P=0.28$). No correlation was observed for any gene between primary tumor and liver metastases. In both primary tumor and liver metastasis, the TS mRNA levels correlated significantly with the OPRT mRNA level (primary $r_S=0.83$, $P=0.00000081$; liver metastasis $r_S=0.49$, $P=0.017$), while the DPD mRNA level correlated significantly with the TP mRNA level $r_S=0.81$, $P=0.0000024$; $r_S=0.63$, $P=0.0014$; respectively). *Conclusions*: The differential gene expression of 5-FU metabolic enzymes between primary colorectal cancer and corresponding liver metastases should be taken into consideration when estimating the sensitivity to 5-FU-based chemotherapy in colorectal cancer. The gene expression of TS and OPRT, which are involved in de novo pyrimidine synthesis, and that of DPD and TP, may be coregulated.

Keywords Thymidylate synthase · Dihydropyrimidine dehydrogenase · Orotate phosphoribosyl transferase · Thymidine phosphorylase · Uridine phosphorylase

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Introduction

Thymidylate synthase (TS) protein and gene expression in human colorectal cancers has been investigated as a predictor of response to chemotherapies based on 5-fluorouracil (5-FU), and as a prognostic marker [1, 2, 5, 9, 10, 20, 24, 29]. Previous studies have suggested that high TS expression in advanced colorectal cancers, determined by several methods (immunohistochemical staining, enzyme activity, and reverse transcription PCR), is followed by non-response to 5-FU and poor prognosis.

The other 5-FU metabolic enzymes have been also examined as predictors of sensitivity to 5-FU. Dihydropyrimidine dehydrogenase (DPD) is the first and rate-limiting enzyme for the catabolism of 5-FU, and its activity or mRNA level is high in various human cancers and cell lines with low sensitivity to 5-FU [3, 11, 16, 19, 33]. Ichikawa et al. [17] and Salonga et al. [33] have reported that patients with both low DPD and low TS mRNA expression in primary colorectal cancers respond to 5-FU-based chemotherapy, and their prognosis is better than patients with both high DPD and high TS expression. Other studies have indicated that the expression levels of the first metabolic enzymes of 5-FU, namely orotate phosphoribosyl transferase (OPRT) [6, 18, 30, 31], thymidine phosphorylase (TP) [6, 12, 25, 27, 28, 33, 34], and uridine phosphorylase (UP) [6, 8, 18, 25, 35], might also correlate with sensitivity to 5-FU. High TP gene expression has been shown to be followed by low

Table 1 Primers and probes

	Forward primer	Reverse primer	Probe
TS	TS-1	TS-r3	TS-P2
	GAATCACATCGAGCCACTGAAA	CAGCCCAACCCCTAAAGACTGA	TTCAGCTTCAGCGAGAACCCAGA
DPD	DPD-F11	DPD-R11	DPD-P11
	AATGATTCGAAGAGCTTTTGAAGC	GTTCCCCGGATGATTCTGG	TGCCCTCACCAAAACTTTCTCTCTTGATAAGGA
OPRT	OPRT-1107F	OPRT-1282R	OPRT-1200PF
	TCCTGGGCAGATCTAGTAAATGC	TGCTCCTCAGCCATTCTAACC	CTCCTTATTGCGGAAATGAGCTCCACC
TP	TP-700F	TP-770R	TP-722P
	CCTGCGGACGGAATCCT	GCTGTGATGAGTGGCAGGCT	CAGCCAGAGATGTGACAGCCACCGT
UP	UP-586F	UP-792R	UP-743PF
	TGACTGCCCAGGTAGAGACTATCC	AGACCTATCCCACCAGAAGTGC	TGCTCCAACGTCACTATCATCCGCAT
β-ActinACTB	ACTB-517F	ACTB-811R	ACTB-547PF
	TCACCCACACTGTGCCCATCTACGA	CAGCGGAACCGCTCATTGCCAATGG	ATGCCCTCCCCCATGCCATCCTGCGT

chemosensitivity to 5-FU in colorectal carcinoma [33]. Chung et al. have reported that OPRT, TP, and UP gene expression is downregulated in gastric cell lines with 5-FU resistance [6]. These results indicate that multiple analysis of the expression of 5-FU metabolic genes may predict sensitivity to 5-FU more precisely.

However, the relationship between the expression of these genes in primary cancers and their expression at metastatic sites has not been adequately evaluated. We have previously shown that TS gene expression is lower, and DPD gene expression is higher, in liver metastases than in primary colorectal cancers [36, 39]. As high expression of TS, DPD and TP protein or mRNA has been reported to be associated with low sensitivity to 5-FU [2, 17, 20, 24, 33], the prediction of 5-FU chemosensitivity by analysis of expression in the primary tumor may be inaccurate in patients whose TS, DPD and TP gene expression is markedly higher or lower in their liver metastases than in the primary tumor.

In the present study, the expression of TS, DPD, OPRT, TP, and UP genes in primary colorectal cancer was compared with that in the corresponding liver metastases by real-time quantitative RT-PCR.

Materials and methods

Patients and samples

We analyzed pairs of primary colorectal adenocarcinomas and corresponding liver metastatic tumors from 23 patients (13 males and 10 females, average age 62.2 years) who had undergone surgical resection of primary colorectal cancer between October 1997 and October 2001 at the Department of Digestive Surgery, Tokyo Medical and Dental University, Tokyo, Japan. This study was approved by the Institutional Review Board of the Tokyo Medical and Dental University, and written consent was obtained from all patients. Of the liver metastasis samples, 12 were obtained by surgical resection, and 11 by intraoperative core-needle biopsy at the time of resection of the primary tumor. Seven were metachronous metastases and two of the patients had received 5'-deoxy-5-fluorouridine orally as adjuvant therapy after the primary resection. One of these two patients discontinued adjuvant therapy after only 4 weeks, while the other completed a 1-year course of adjuvant therapy. Resection of the liver metastases in this patient was performed at least 6 months after completion of the adjuvant therapy regimen.

Immediately following surgery, each tissue sample was frozen in liquid nitrogen and stored at −80°C until preparation of RNA extracts. A gastrointestinal pathologist evaluated the remaining specimens. No contamination of the normal colonic mucosa or liver tissue in the tumor samples was histologically identified.

Total RNA extraction and cDNA synthesis

Our procedure has previously been described in detail [36, 38, 39]. In brief, total RNA was extracted using an RNeasy Minikit (Qiagen, Chatsworth, Calif.). The amount of total RNA was estimated by measuring absorbance, the quality was determined by electrophoresis through agarose gel in the presence of formaldehyde, and the rRNA bands were visualized. Then up to

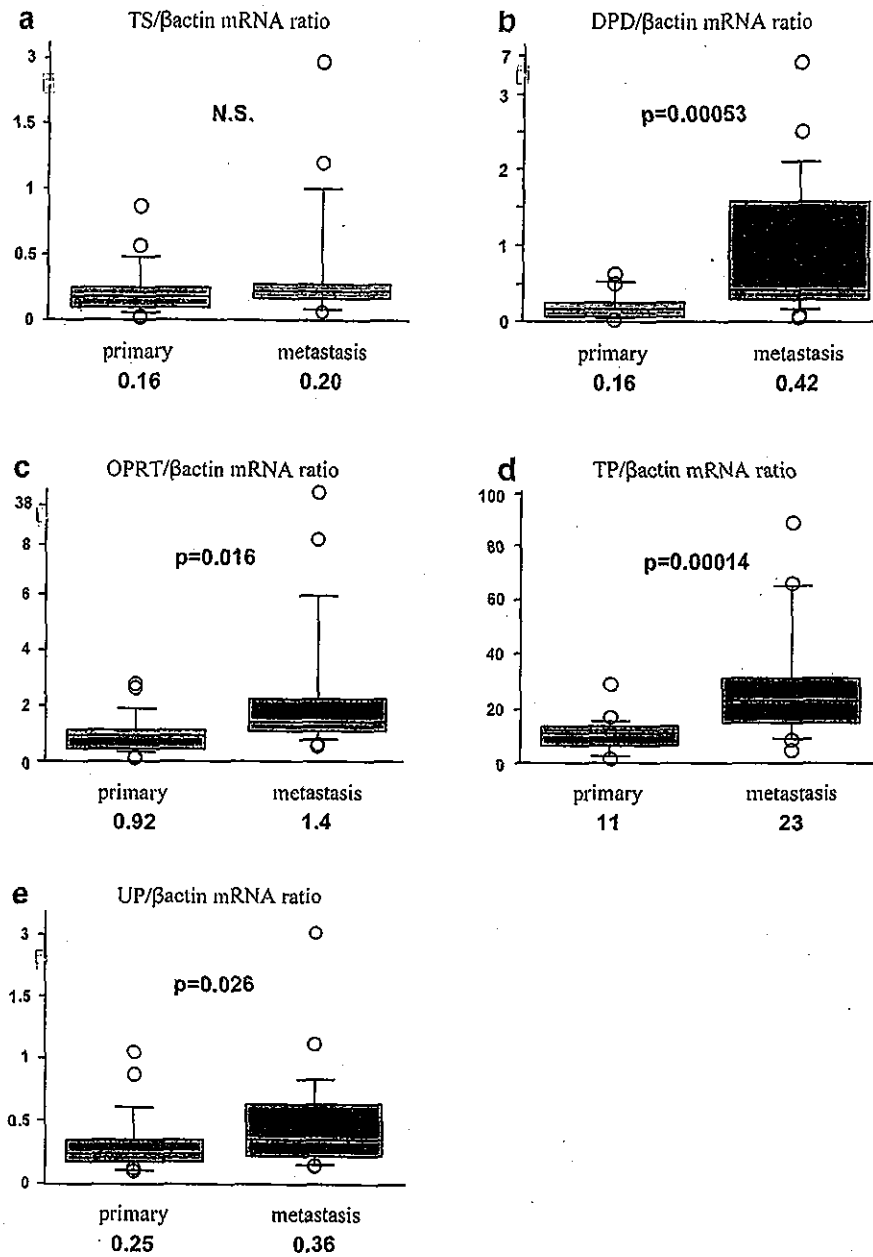

Fig. 1a–e Gene expression in primary tumors and liver metastases. The median values are shown

10 μg of the prepared RNA was reverse-transcribed to synthesize cDNA using the oligo(dT) primer, Superscript II (Life Technologies, Gaithersburg, Md.), as previously described.

Real-time quantitative RT-PCR assay

The mRNA levels of TS, DPD, OPRT, TP, and UP were evaluated by real-time quantitative RT-PCR [15, 29, 30] (TaqMan PCR) using an ABI Prism 7700 sequence detector (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The β-actin gene was used as the endogenous control gene. Primers and TaqMan probes for each gene were designed based on the nucleotide sequence of human TS, DPD, OPRT, TP, UP and β-actin (Table 1). The PCR mixture contained 10 μl of each appropriately diluted cDNA sample (standard curve points and patient samples), 200 n*M* forward primer, 200 n*M* reverse primer, 100 n*M* TaqMan probe, and 12.5 μl TaqMan Universal PCR Master Mix (Perkin-Elmer Applied Biosystems), in a final volume of 25 μl. The PCR profile consisted of one incubation at 50°C for 2 min, one incubation at 95°C for 10 min, and 45 cycles of amplification for 15 s at 95°C, and 1 min at 60°C.

The amount of PCR product was determined using a standard curve of cDNA synthesized from human tumor xenograft. Each PCR run included the seven points of the standard curve (fourfold serially diluted cDNA with 100 ng/μl) and negative controls. The range of the standards was 64 to 0.00391 ng/10 μl. All samples were run in duplicate PCR experiments. The mean was then used; a few samples with more than a twofold difference in the amount of PCR product were retested. Some samples out of the range of the respective points on the standard curve were also retested using altered cDNA concentrations.

The relative amount of each gene's mRNA was expressed as the ratio of each mRNA to that of β-actin.

Statistical analysis

The mRNA levels and clinicopathological factors were compared using the Mann-Whitney *U*-test. The mRNA levels of the primary colorectal cancers and those of the liver metastases were compared

using the Wilcoxon signed-ranks test. The relationship between each gene's mRNA level in the primary cancer and that in the liver metastases, and the relationships among the mRNA levels in primary cancers or liver metastases were assessed using Spearman's rank correlation. Statistical significance was established at the $P < 0.05$ level for each analysis.

Results

Messenger RNA levels of the 5-FU metabolic enzymes were assessed in 23 pairs of primary colorectal cancers and corresponding liver metastases. The difference in the quantities between duplicate PCR products was less than 10%. No significant differences in mRNA levels were observed for any clinicopathological features such as gender, age, location of primary tumor, number of liver metastases and method of obtaining samples from liver metastases. Synchronous and metachronous liver metastasis showed the same levels of gene expressions (median values: TS 0.20 vs 0.22, $P=0.69$; DPD 0.57 vs 0.42, $P=0.64$; OPRT 1.6 vs 1.4, $P=0.64$; TP 26 vs 21, $P=0.42$; UP 0.44 vs 0.32, $P=0.74$).

DPD, OPRT, TP and UP mRNA levels in the liver metastases were significantly higher than those in the corresponding primary tumors (DPD 0.42 vs 0.16, $P=0.00053$; OPRT 1.4 vs 0.92, $P=0.016$; TP 23 vs 11, $P=0.00014$; UP 0.36 vs 0.25, $P=0.026$; Fig. 1). The TS mRNA level did not significantly differ between the liver metastases and the primary tumors (0.20 vs 0.16, $P=0.28$). No significant correlation between the mRNA levels of the primary tumors and those of their corresponding liver metastases was noted for any of the genes (TS $P=0.48$, DPD $P=0.94$, OPRT $P=0.19$, TP $P=0.81$, UP $P=0.90$). There was a significant correlation between the OPRT and TS mRNA levels both in the primary tumors ($r_S=0.83$, $P=0.00000081$; Fig. 2a) and in the liver metastases ($r_S=0.49$, $P=0.017$; Fig. 2b). A similar relationship was noted between DPD and TP mRNA levels in both primary tumors ($r_S=0.81$, $P=0.0000024$; Fig. 3a) and liver metastases ($r_S=0.63$, $P=0.0014$; Fig. 3b). Other correlations among the genes were not found.

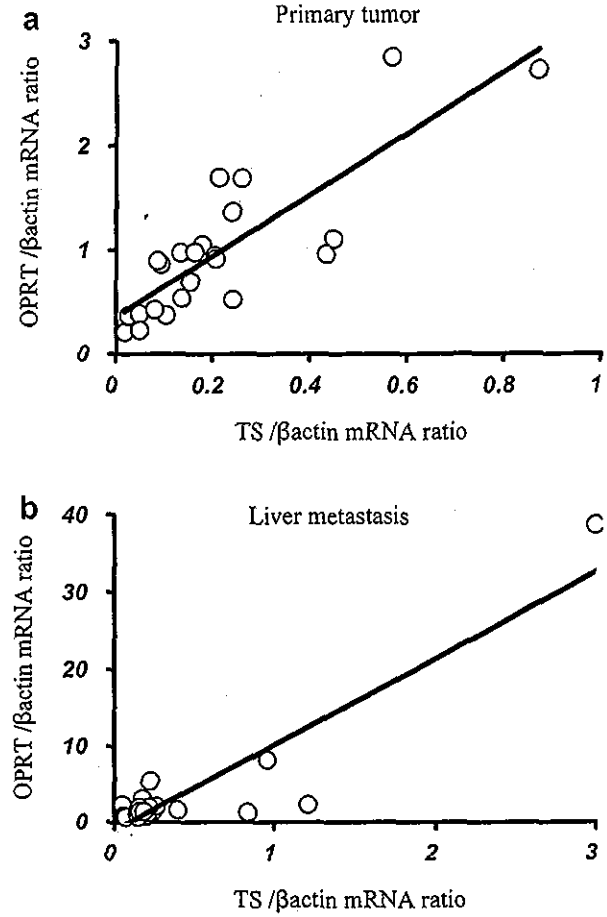

Fig. 2a, b TS and OPRT mRNA levels are significantly correlated in both primary colorectal cancers (a $r_S=0.83$, $P=0.00000081$) and liver metastases (b $r_S=0.49$, $P=0.0017$)

Discussion

We demonstrated that DPD, OPRT, TP, and UP mRNA levels in liver metastases were significantly higher than in their corresponding primary colorectal cancers, although no correlation was observed between primary tumors and liver metastases. Previously, we have shown that DPD gene expression in colorectal cancers is associated with tumor progression, and that higher DPD gene expression is present in liver metastases than in primary tumors [36]. Johnston et al. have suggested that suppression of translation of DPD mRNA is removed in tumor tissue, and proposed a general mechanism by which pyrimidine nucleotide biosynthesis and degradation are coregulated to maintain a growth advantage in the tumor [21]. Takebayashi et al. have reported that TP, which catalyses the reversible phosphorolysis of thymidine and its analogues to their respective bases 2-deoxyribose-1-phosphate, is associated with tumor progression [37]. Previous investigators have shown that the expression of the initial 5-FU-anabolizing enzymes (OPRT, UP, TP, etc.) is higher in various human cancers than in normal tissues [7, 22, 26, 32], and suggested that the increase in the expression of these enzymes may be an advantage via increased pyrimidine nucleotide biosynthesis for cell proliferation in cancer. The gene expression of thymidine kinase (TK), one of the enzymes involved in salvage DNA synthesis, correlates with malignant potential in ovarian tumors, as well as with TP gene expression [13].

In the present study, a linear relationship between TS and OPRT mRNA levels was observed in both primary colorectal cancers and liver metastases. Kasahara et al. have reported that TS gene expression correlates closely with E2F1 expression, and speculated that one mechanism by which tumor cells increase TS expression may be overexpression of E2F1, which induces S-phase-acting proteins such as TS [23]. Fujiwaki et al. have

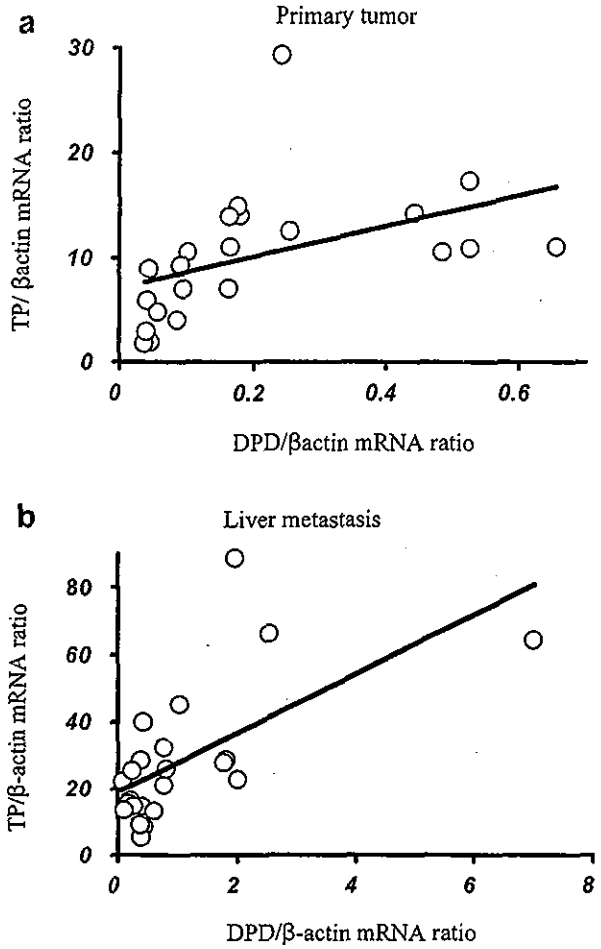

Fig. 3a, b DPD and TP mRNA levels are significantly correlated in both primary colorectal cancers (a $r_S = 0.81$, $P = 0.0000024$) and liver metastases (b $r_S = 0.63$, $P = 0.014$)

demonstrated a linear relationship in gene expression levels between TK1 and TS in ovarian cancer, suggesting that enzymes for DNA biosynthesis may be controlled by several similar mechanisms [13]. TS and OPRT, which are involved in de novo pyrimidine nucleotide biosynthesis, may be coregulated in cancer cell proliferation. DPD and TP gene expression were also positively correlated in both primary cancers and liver metastases. Collie-Duguid et al. suggested that the expression of DPD and TP protein is coregulated [7].

We measured gene expression using the TaqMan RT-PCR assay. This method is a more precise and reproducible semiquantitation of gene expression than conventional RT-PCR assays using agarose gel, because it is based on threshold values in the exponential phase of the PCR rather than end-point measurement of the amount of PCR product [4]. In addition, this PCR assay is suitable for smaller samples such as biopsy specimens and can measure a larger number of enzymes in a shorter time than other methods, including enzymatic activity or protein assays [24].

Increased OPRT gene expression in liver metastases may be associated with increased sensitivity to 5-FU, because OPRT is a 5-FU-anabolizing enzyme and is considered the main pathway of 5-FU initial phosphorylation in human cancers [14]. On the other hand, increased TS, DPD, and TP may be associated with decreased sensitivity to 5-FU. In patients with extremely low expression of OPRT mRNA or high levels of TS, DPD, or TP gene expression in liver metastasis, it may be difficult to predict 5-FU sensitivity of the metastasis via analysis of the gene expression of the primary site.

The present study showed that the expression of 5-FU metabolic genes in liver metastases does not correlate with that in the corresponding primary tumor. The difference in the expression of 5-FU metabolic genes between the primary site and liver metastases should be taken into consideration when predicting the sensitivity to 5-FU-based chemotherapy of colorectal cancer.

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LOWER DIGESTIVE TRACT STRICTURE

EXPERIENCES OF SELF-EXPANDABLE METALLIC STENT FOR COLORECTAL OBSTRUCTIONS: 70 CASES

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ABSTRACT

Clinical utilization of self-expandable metallic stent (EMS) endoprosthesis has come later for colorectal diseases than for other lesions. Recently, EMS has been used for palliative insertions for strictures caused by malignant diseases or as a 'bridge to surgery' for obstructive colorectal cancers, with good clinical results increasingly reported in many western countries. Its application for benign strictures has been reported, but we believe that the surgical indications require more careful analysis because of the absence of data concerning long-term prognosis. The advantage of this technique in the treatment of colorectal strictures is that it limits invasiveness, such as in palliative or temporary stoma creation, thereby improving patient quality-of-life. Therefore, we believe that EMS endoprosthesis will play a key role in this field. We are awaiting the introduction of the metallic stent for the colon and the associated kit, as well as the Japanese government's approval for reimbursement for this procedure.

Key words: self-expandable metallic stent, colorectal obstructions, obstructive colorectal cancer, endoprosthesis.

INTRODUCTION

Self-expandable metallic stent (EMS) endoprosthesis for colorectal diseases has been slowly utilized because of the difficulty resulting from colorectal characteristics such as flexuous structure and thin intestinal wall. In the late 1990s, however, this procedure started to be applied to clinical use, and an increasing number of cases have been reported. This study attempted to clarify the current status and challenges of metallic stent endoprosthesis for colorectal strictures, not only in our experience, but also from evaluation of other relevant clinical reports.

EMS ENDOPROSTHESIS FOR COLORECTAL MALIGNANT DISEASES

EMS endoprosthesis is mainly used in the treatment of malignant disease. Although many reports discuss its use in palliation of malignant disease, favorable results have been reported when it was applied for preoperative relief of stricture for obstructive colorectal cancer. In Japan, it has not been widely accepted as a standard procedure because the cost is not reimbursed by the public insurance system. However, its efficacy has been reported in many countries in the world. Knot *et al.*[1] reviewed clinical papers written in English in 2003: analysis of 598 cases revealed 92% successful stent insertion, 88% clinical efficacy, 90% efficacy for palliative purpose, 85% success in preoperative insertion (95% of these cases were performed one stage operation), three deaths (0.5%), 4% perforation, 10% stent migration, and 10% re-obstruction in palliative purpose insertion. The report concluded that EMS insertion is a safe and effective procedure; however, there was a higher risk of perforation when its insertion was combined with balloon dilation.

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Palliative EMS endoprosthesis for malignant colorectal obstruction

EMS insertion for colorectal obstruction in palliative therapy involves stent deployment for a relatively long-term, and mainly targets primary malignant diseases or recurrence disease for which curative surgeries are not indicated for reasons of distant metastasis or poor general condition and cases that are conventionally treated by bypass operation or stoma creation. We assayed EMS insertion for 10 colorectal cancer cases, which consisted of four rectal cancer cases (re-inserted cases are included), two sigmoid colon cancers, two descending colon cancers (Fig. 1), and two transverse colon cancers; we successfully inserted stents for all these cases. According to stent insertion reports for obstructive colorectal cancer in 2002 (discovered using the Japan Centra Revuo Medicina database) 51 cases were performed and reported in Japan, including our cases.[2] The data show that this procedure was performed mainly for the rectum and sigmoid colon. Approximately 40% of obstructions were caused by peritoneal disseminative metastasis and recurrence, and approximately 30% by local recurrence. All stent-inserted cases resulted in improvement of both the obstructing condition and the patient's quality-of-life. Nevertheless, complications were observed: one case (2%) of perforation during follow-up, one case (2%) of high bowel frequency after insertion, one case (2%) of pain at the stent site, and four cases (8%) of deviation of stents. Although re-obstruction was observed in four cases (8%), all cases were managed using either laser (1 case) or re-insertion of stent (2 cases). The observed stent

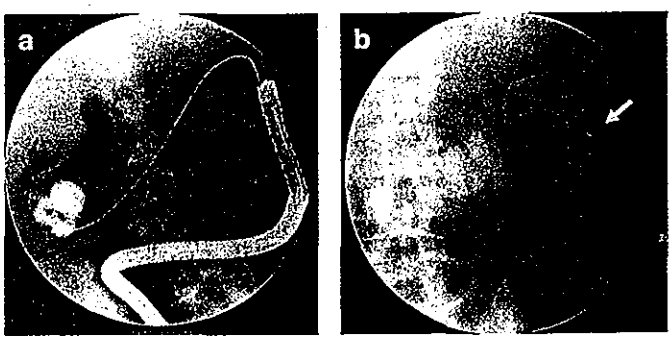

Fig. 1. Palliative EMS insertion for descending colon cancer with multiple liver metastasis.(Case: 38-year-old female). (a) Fluorography with water soluble contrast agent of proximal side of the stricture through the sheath. (b) Plain XP after EMS (Ultraflex esophageal stent) insertion. Allow shows metallic clip for marking of the distal side of the stricture.

patency period was 10–406 days (mean: 114 ± 104 days) except for one case of death by myocarditis and one case of stent extraction due to insertion site pain. These data prove the long-term patency of the stents. After stent insertion, it is essential to follow the patient by regular examination, abdominal X-ray, and barium enema in order to detect early stage complications, and to take immediate appropriate countermeasures.

Preoperative 'bridge to surgery' EMS insertion for obstructive colorectal cancer

With improved diagnostic technologies, a number of advanced colorectal cancers have been detected by the occurrence of ileus (reported incidence 3.1%~22.7%).[3,4] In ileus, the risk of surgical contamination increases the risk of postoperative complications. It is better to avoid emergency operations to achieve maximum ileus release by conservative therapy. Elective surgeries can then be performed after the patient's general condition improves. Ileus on the left colon, however, is treated by emergency surgery such as Hartmann's operation, and anastomosis was performed on the second operation, the so-called two-stage operation, because release of ileus is difficult using the nasogastric intestine decompression method with an ileus tube.[5] This two-stage operation significantly impacted our patients' condition. So, transanal decompression method has been recently discussed. The introduction of this method has had a dramatic change on clinical decision-making in the treatment of ileus caused by colorectal cancer (Fig. 2), by enabling the same process as elective surgeries for colorectal cancers: preoperative inspection and mechanical cleansing of the proximal side of the colon after colorectal cancer ileus was decompressed transanally. EMS insertion for obstructive colorectal cancer is the procedure that is performed transanally with the aim of improving the obstructive conditions. A few days after the release of ileus is confirmed, a barium enema is performed to gather information about the proximal bowel. Then oral intake should be started. About one week after the insertion, mechanical preoperative preparation (which is identical to the conventional method) using polyethylene glycol 21, is performed and the patients are prepared for surgery. We developed this procedure, Stent

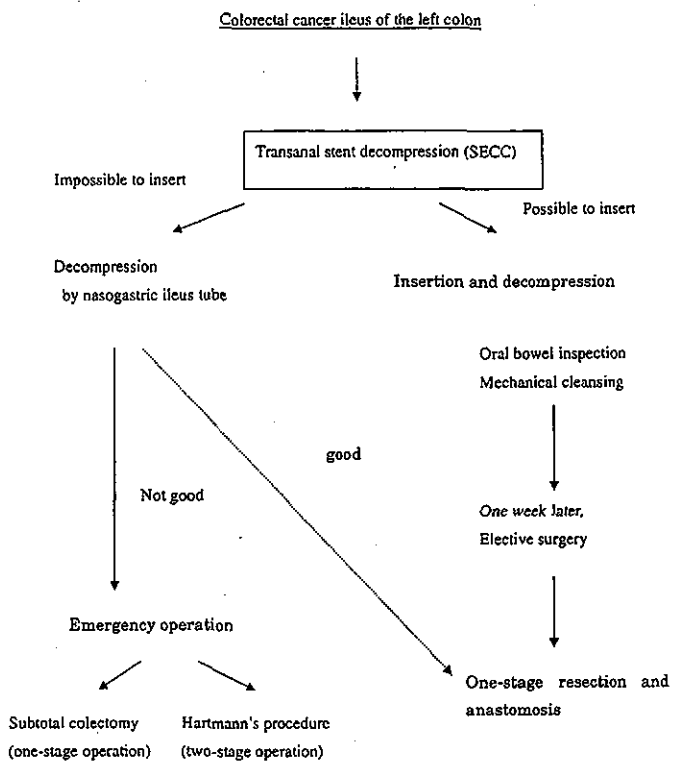

Fig. 2. Flow chart for treatment of left colon cancer ileus.

Endoprosthesis for Colorectal Cancer (SECC) in November 1993 and published a clinical paper in 1996.[6] In recent years, reports of the same procedure have published in the Western world.[7,8]

As of November 2003, we have performed 60 cases of SECC and 52 cases (87%) were successfully inserted. Within failed insertion cases, the tumor lesions were located around sigmoid-descend junction. In almost rectum cases, SECC was successfully inserted. The period from stent insertion to surgery was 16 days at most, with a mean of 6.3 days. No complication was observed in complete insertion cases, all of which underwent mechanical preparation using polyethylene glycol. Preoperative bowel preparation was satisfactory in almost all cases. Among the failed insertion cases, three perforations (5%) and two migrations at insertion (3%) were observed as complications caused by SECC. Among these cases, emergency operations were required in four cases, which does not indicate worse prognosis due to SECC introduction, because these were ileus cases that needed emergency operations. A small amount of melena was observed in all case just after stent insertion, but it was transient and had no impact on the patients' general condition. Slight pain and an unpleasant feeling at the stent site were noted by approximately half of patients, but no one required analgesic and these complaints gradually decreased.

For obstructive colorectal cancers, the elective surgeries following SECC therapy improved short-term operative results to a far greater extent than in the emergent operation group.[9,10] In particular, the incidence of infection was considerably lower. The insertion of stents at the cancer site is controversial with respect to long-term results because it violates a principle of cancer surgery, the so-called 'non-touch

Table 1. Comparison between preoperative stent endoprosthesis (SECC) and transanal ileus tube placement for obstructive colorectal cancer

	SECC	Transanal ileus tube
Rate of successful insertion and decompression	good	good
Ease of insertion	same	same
Speed of decompression	rapid	slow
Management after insertion	easy	slightly complicated
Oral intake after insertion	possible	difficult
Quality-of-life at insertion	good	slightly compromised
Impact on tumors	relatively invasive	protective
Long-term placement	possible	unknown
Available in market	under consideration	available from 3 companies

isolation'. However, there has been no significant difference in long-term prognosis between the SECC group and the emergent operation group.[10]

Regarding the transanal decompression method, many of the reported cases in Japan used transanal ileus tubes.[11] Compared with this procedure, SECC requires no tube placement, produces no uncomfortable feeling in the nasal cavity and anus, and results in better patient quality-of-life (Table 1). Moreover, there is no restriction of physical activities and patients can manage fecal evacuation. In addition, there is no problem with foul odors from patients due to tube placement, so patient anxiety is negligible. The stent lumen is wider than the transanal decompression tube and releasing ileus is much faster with stents than tubes.

STENT ENDOPROSTHESIS FOR BENIGN DISEASES

Stent endoprosthesis has been applied mainly to malignant diseases, but recently it has been attempted in the treatment of benign diseases. It has been utilized for stenosis at the anastomotic site,[12] torsion,[13] stricture caused by diverticulitis,[12,14] stricture caused by Behcet's disease,[15] and stricture caused by ulcerative colitis.[16] Stent endoprosthesis for the lower intestine has proven effective for these benign strictures, with swift improvement of patient quality-of-life. However, Concern regarding long-term stent placement suggests that more careful examination of the indications is indispensable. According to the guidelines established by the Japan Gastroenterological Endoscopy Society[17] benign stricture is a contraindication of stent endoprosthesis; palliation for malignant diseases remains the basic indication. In fact, there are reports of fistula and perforation after stent insertion for diverticulitis, which necessitated stent removal due to severe pain caused by stent insertion for anastomotic stenosis.[12] Due to the reasons mentioned above, stent endoprosthesis for benign diseases should be viewed as a temporary therapy for conditions for which no other therapy is indicated or in situations where resistance was observed to more standard therapies (e.g. balloon dilation).[12,13]

CONCLUSIONS

In the treatment of colonic stricture patients, stent therapy plays an important role, as it limits invasiveness and improves patient quality-of-life. We desire the arrival of the colonic stent in the Japanese market and hope that the procedure will soon be reimbursed by the public insurance system of the Japanese Ministry of Health, Labour and Welfare.

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