Internal Hernia Through the Mesenteric Opening After Laparoscopy-Assisted Transverse Colectomy

Koichi Nagata, MD, Jun-ichi Tanaka, MD, PhD, Shungo Endo, MD, PhD, Kishiko Tatsukawa, MD, Eiji Hidaka, MD, PhD, and Shin-ei Kudo, MD, PhD

Abstract: We report a case of a rare complication in laparoscopic colectomy. A 55-year-old woman underwent a laparoscopy-assisted transverse colectomy for transverse colon cancer. On the 5th postoperative day, she developed bowel obstruction. Decompression by a long intestinal tube failed to resolve the bowel obstruction. She underwent operative intervention. Abdominal exploration showed jejunal loop caused by a strangulation forming on an internal hernia through the mesenteric opening at the anastomotic colonic stumps, which had not been sutured during the previous operation. Our experience might indicate the need for closure of small mesenteric opening after laparoscopic colectomy.

Key Words: internal hernia, intestinal obstruction, laparoscopic surgery, colon cancer, complication

(*Surg Laparosc Endosc Percutan Tech* 2005;15:177–179)

A 55-year-old woman was admitted to our hospital for surgical treatment of transverse colon cancer. Colonoscopic study demonstrated a 4.5 × 5.0-cm protruded lesion in the transverse colon. Biopsies were performed, and the results showed well-differentiated adenocarcinoma. The patient underwent a laparoscopy-assisted transverse colectomy for transverse colon cancer. Intracorporeal ligation of tumor-feeding vessels (middle colic vessels) at their origins was performed initially. After mobilization of hepatic and splenic flexures, the bowel loop was delivered under a wound protector through a 6-cm midline incision of the upper abdomen. The division of the marginal vessels and functional end-to-end anastomosis was performed extracorporeally with linear staplers (PROXIMATE Linear Cutter 75; Ethicon Endo-Surgery, Cincinnati, OH). The mesenteric opening region resulting from bowel resection was not closed for preference. After the closure of the minilaparotomy, the anastomosis and the small mesenteric opening region were checked laparoscopically and found to be normal without internal hernia. On postoperative day (POD) 5, she had nausea, vomiting, and abdominal distention. Plain abdominal x-ray film revealed air-fluid levels in the left upper quadrant (Fig. 1). This finding was thought to be compatible with an adhesive small bowel obstruction initially. Surgery was not chosen because her clinical symptoms and plain x-ray film improved after decompression by a long intestinal tube. Body temperature, pulse, and white blood cell count were within normal limits. There were no signs of clinical indication of strangulation suggesting a need for urgent surgery. She was treated conservatively with bowel rest and total parenteral nutrition. Despite this treatment, high-volume output from a long intestinal tube persisted (1600–3000 mL/day), and, on POD 15, an upper gastrointestinal radiologic contrast study demonstrated obstruction of the proximal jejunum (Fig. 2). She eventually underwent surgical intervention. At laparotomy, there was jejunal loop caused by a strangulation forming on an internal hernia through the mesenteric opening at the anastomotic colonic stumps, which had not been sutured during the previous surgical procedure. A part of jejunum, 80 cm in length corresponding to 5 to 85 cm of the jejunum from Treitz's ligament was strangulated (Fig. 3). After lysis of the adhesions and relieving of the strangulation, the jejunal loop was returned to the abdomen. The operation was concluded by sealing the mesenteric opening and closing the wall of the abdomen in layers. The postoperative course was uneventful. At 11 months' follow-up, there was no clinical or radiographic evidence of recurrence of the cancer or the internal hernia.

DISCUSSION

Laparoscopic adhesiolysis for small bowel obstruction was first reported in 1991.[1] Laparoscopic adhesiolysis has been shown to decrease the incidence, extent, and severity of intraabdominal adhesions when compared with open approach, thus potentially decreasing the recurrence rate for adhesive small bowel obstruction.[2,3] We expected this patient with a complete obstruction caused by adhesions preoperatively (Fig. 2) to not be a good candidate for laparoscopic adhesiolysis.[3]

Laparoscopy-assisted colectomy (LAC) becomes the procedure of choice for colonic disease at many facilities worldwide.[4–9] There are many recognized advantages of LAC, including decreased pain, improved cosmesis, decreased postoperative ileus, shortened hospital stay, and more rapid return to normal activities.

The frequently reported complications after LAC are incisional infection, anastomotic fistula, abdominal abscess, intestinal obstruction, incisional hernia, perineal hernia, and others.[5–13] Intestinal obstruction is one of the common complications after LAC. The incidence is reported to be

Received for publication March 23, 2004; accepted March 8, 2005.
From the Digestive Disease Center, Showa University Northern Yokohama Hospital, Yokohama, Japan.
Reprints: Koichi Nagata, MD, Digestive Disease Center, Showa University Northern Yokohama Hospital, 35-1 Chigasaki-chuo, Tsuzuki-ku, Yokohama 224-8503, Japan (e-mail: Nagata7@aol.com).

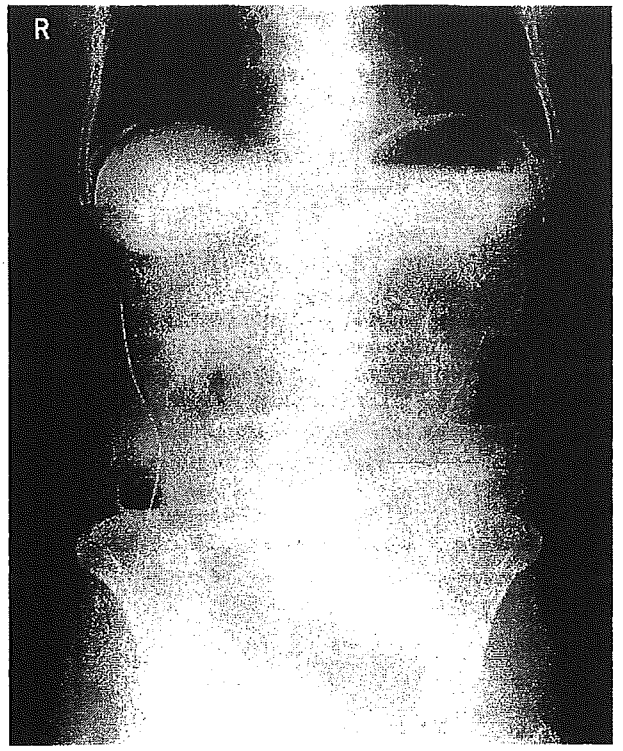

FIGURE 1. Plain abdominal x-ray. Air-fluid levels in the left upper quadrant.

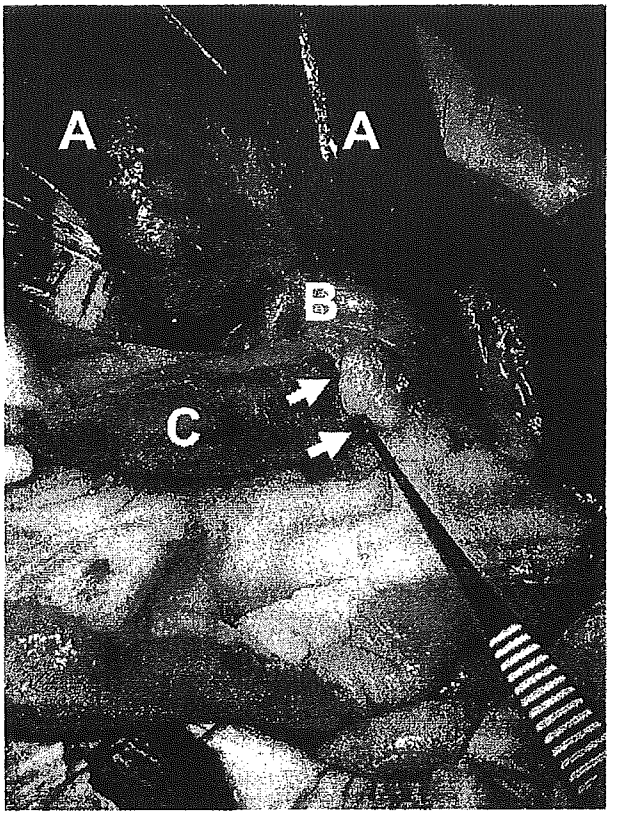

FIGURE 3. Intraoperative photograph. The jejunum (A) has herniated through the mesenteric opening (arrows) in the anastomotic colonic stumps (B). C, Distal side of the jejunum.

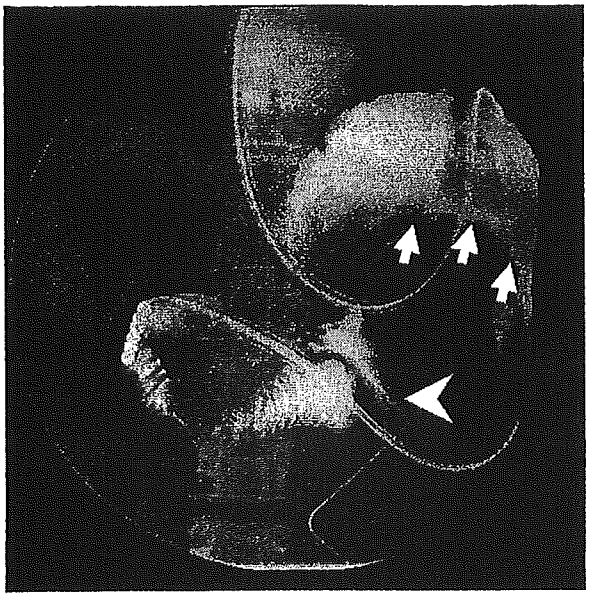

FIGURE 2. Upper gastrointestinal radiologic contrast study. The arrowhead is pointing obstruction of the proximal jejunum. The stomach is excluded upper side (arrows).

0.7%–2.7%.[5–14] The most frequent cause of intestinal obstruction is Richter's hernia at the trocar site. Internal hernia, herniation of the internal organs through defects in the intraabdominal cavity, is not a common cause of intestinal obstruction. Intestinal obstruction secondary to internal hernia after laparoscopic surgery is a rare event. Such conditions have been reported after laparoscopic Roux-en-Y gastric bypass,[15,16] laparoscopic donor nephrectomy,[17] and laparoscopic Nissen fundoplication.[18] However, few data exist regarding the complications of postoperative internal hernia after LAC.

A total of 269 patients underwent LAC without closing the mesenteric opening between April 2001 and October 2004 at our institution. This is the first case (one of the 269 patients; 0.37%) of internal hernia through the mesenteric opening after LAC. The data on the incidence of small bowel obstruction from internal hernia after LAC are scarce. In 1996, Kok et al[11] reported a case of small bowel hernia through a mesenteric defect after laparoscopy-assisted sigmoidectomy. In 1998, Elio et al[19] reported the case of ileal volvulus on internal hernia after laparoscopy-assisted left hemicolectomy. In 1999, Kawamura et al[20] reported the case of transmesenteric hernia after laparoscopy-assisted sigmoidectomy. To the best of our knowledge, only the above-mentioned cases were reported in the literature.

The omission of closing the mesenteric opening can expose the patient to the risk of small bowel obstruction from internal hernia. Although this complication is a rare event, it involves significant morbidity in an otherwise healthy patient who has undergone LAC.

Duepree et al[14] reported that the incidence of both ventral hernia and postoperative small bowel obstruction after laparoscopic resection is lower than that of after open surgery. There have been reported only 3 patients with small bowel obstruction from internal hernia after LAC, and the incidence is very low.[11,19,20] It would seem safe to assume that there is no need to close the mesenteric opening in all cases of laparoscopic colectomy. But if the mesenteric opening is small, then it is likely to cause internal hernia, and our experience might indicate the need to close the mesenteric opening by suturing or clipping after bowel resection. However, more cases are required to explain the indication for closure of mesenteric opening to prevent this complication.

REFERENCES

1. Bastug DF, Trammell SW, Boland JP, et al. Laparoscopic adhesiolysis for small bowel obstruction. *Surg Laparosc Endosc Percutan Tech.* 1991;1: 259–262.
2. Tittel A, Treutner KH, Titkova S, et al. Comparison of adhesion reformation after laparoscopic and conventional adhesiolysis in an animal model. *Langenbecks Arch Surg.* 2001;386:141–145.
3. Nagle A, Ujiki M, Denham W, et al. Laparoscopic adhesiolysis for small bowel obstruction. *Am J Surg.* 2004;187:464–470.
4. Huscher C, Silecchia G, Croce E, et al. Laparoscopic colorectal resection. A multicenter Italian study. *Surg Endosc.* 1996;10:875–879.
5. Wexner SD, Reissman P, Pfeifer J, et al. Laparoscopic colorectal surgery. Analysis of 140 cases. *Surg Endosc.* 1996;10:133–136.
6. Braga M, Vignali A, Gianotti L, et al. Laparoscopic versus open colorectal surgery. *Ann Surg.* 2002;236:759–767.
7. Clinical Outcomes of Surgical Therapy Study Group. A comparison of laparoscopically assisted and open colectomy for colon cancer. *N Engl J Med.* 2004;350:2050–2059.
8. Lacy AM, Garcia-Valdecasas JC, Delgado S, et al. Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial. *Lancet.* 2002;359:2224–2229.
9. Leung KL, Kwok SP, Lam SC, et al. Laparoscopic resection of rectosigmoid carcinoma: prospective randomised trial. *Lancet.* 2004;363:1187–1192.
10. Fielding FA, Lumley J, Nathanson L, et al. Laparoscopic colectomy. *Surg Endosc.* 1997;11:745–749.
11. Kok KY, Ngoi SS, Kum CK, et al. Laparoscopic-assisted large bowel resection. *Ann Acad Med.* 1996;25:650–652.
12. Campos FG. Complications and conversion in laparoscopic colorectal surgery. Results of a multicenter Brazilian trial. *Surg Laparosc Endosc.* 2003;3:173–179.
13. Lacy AM, Garcia-Valdecasas JC, Delgado S, et al. Postoperative complications of laparoscopic-assisted colectomy. *Surg Endosc.* 1997;11: 119–122.
14. Duepree HJ, Senagore AJ, Delaney CP, et al. Does means of access affect the incidence of small bowel obstruction and ventral hernia after bowel resection? Laparoscopy versus laparotomy. *J Am Coll Surg.* 2003;197: 177–181.
15. Filip JE, Mattar SG, Bowers SP, et al. Internal hernia formation after laparoscopic Roux-en-Y gastric bypass for morbid obesity. *Am Surg.* 2002;68:640–643.
16. Higa KD, Ho T, Boone KB. Internal hernias after laparoscopic Roux-en-Y gastric bypass: incidence, treatment and prevention. *Obes Surg.* 2003;13: 350–354.
17. Regan JP, Cho ES, Flowers JL. Small bowel obstruction after laparoscopic donor nephrectomy. *Surg Endosc.* 2003;17:108–110.
18. Malas MB, Katkhouda N. Internal hernia as a complication of laparoscopic Nissen fundoplication. *Surg Laparosc Endosc.* 2002;12: 115–116.
19. Elio A, Veronese E, Frigo F, et al. Ileal volvulus on internal hernia following left laparoscopic-assisted hemicolectomy. *Surg Laparosc Endosc.* 1998;8:477–478.
20. Kawamura YJ, Sunami E, Masaki T, et al. Transmesenteric hernia after laparoscopic-assisted sigmoid colectomy. *JSLS.* 1999;3:79–81.

Flat and Depressed Lesions of the Colorectum

SHIN-EI KUDO and HIROSHI KASHIDA
Digestive Disease Center, Showa University Northern Yokohama Hospital, Chigasaki-chuo, Tsuzuki-ku, Yokohama, Japan

Although flat and depressed-type lesions are found by regular endoscopic view, magnification and pit pattern observation are vital parts of a precise diagnosis of the lesion. The depressed-type lesions have a prominent tendency to show malignant character, and the recognition and timely treatment of this lesion is inevitable in improving the morbidity and mortality from colorectal cancer. A magnifying colonoscope has the capability of a regular colonoscope with an additional feature: magnification. With chromoscopic techniques, the surface pattern of the mucosal pits can be observed. The pit-pattern classification correlates well with actual histologic findings and provides important additional information before endoscopic treatment of the lesion.

The depressed-type colon cancer first was described in 1977.[1,2] The occurrence of its lesion is accepted widely and now is reported throughout the world.[3] The advent of commercially available magnifying video-colonoscopes with high-power resolution in 1993 accelerated the study of the microstructure of colonic lesions. The combination of chromoscopy and magnifying colonoscopy is useful for detecting small localized lesions for the differential diagnosis and for determining not only the lateral margins but also the depth of a lesion.[4] We use colonoscopes with magnification routinely for colonoscopic examinations. Therefore, we can perform detailed examination with magnification immediately at the time a lesion is found in the colon. The openings of the colonic crypts are referred to as *pits*, and the specific arrangement of the openings of the glands in various kinds of lesions is called the *pit pattern*.[4,5] The aim of this study was to clarify the characteristics and importance of flat and depressed lesions of the colorectum.

Materials and Methods

Our database consists of prospectively collected data of consecutive patients who underwent colonoscopic examinations for any indication over a period of 20 years: from 1985 to 2004. The colonoscopic examinations were performed with magnifying colonoscopes (CF 240ZI, 260AZI, or 200ZI; Olympus, Tokyo, Japan). When a lesion was suspected or identified, chromoscopy was performed with .2% indigo carmine dye and, in addition, .05% crystal violet dye as indicated. All resected lesions were documented with pit-pattern findings, diameter, and final pathologic findings. The number of endoscopically or surgically treated neoplastic lesions was 21,262. The lesions were divided into 3 groups: protruded, flat-elevated, and depressed. The pit patterns, or microstructures of the surface of the lesions, were classified as type I, II, IIIs (small), IIIL (large), IV, VI (irregular), or VN (nonstructural), as previously reported (Figure 1).[6] Indigo carmine dye accumulates in the glandular orifices, which usually clarifies the pit pattern. However, in some lesions, especially those with type IIIs and type V pit patterns, the openings are too narrow, too distorted, or too sparse for enough dye to be retained. In such cases, crystal violet dye is used, which stains the absorbent epithelium of the colon and makes the pit pattern conspicuous.

Results

The rates of invasive cancer in early colorectal neoplasms that appear as protruded and flat elevated lesions between 6 and 10 mm in diameter were 1.3% and .18%, respectively (Table 1). The invasive rate with depressed lesions for the same size group was 43.2%. On magnifying colonoscopy, most (95.7% and 95.7%) protruded and flat neoplasms showed type IIIL or IV pit pattern. Eighty-six percent of the depressed lesions were characterized by type IIIs, VI, or VN pit pattern. The pit patterns correlated well with the final histologic diagnosis (Table 2). Type IIIs, IIIL, and IV pit patterns were typical of adenomas, and were seen only rarely in invasive cancers (0%, 3.3%, 2.3%, respectively). On the other hand, 88.9% of type VN lesions were invasive.

Discussion

The gross appearances of early colorectal neoplasms are divided into 3 categories: protruded, flat elevated, and depressed.[7] Recognition of depression is very important because depressed lesions often are associated with invasive cancer when they are very small. Detecting a tiny area with a slight color change is important; some lesions look slightly reddish and some look pale or discolored. Bleeding spots, interruption of the capillary network pattern, or

1542-3565/05/$30.00
PII: 10.1053/S1542-3565(05)00283-1

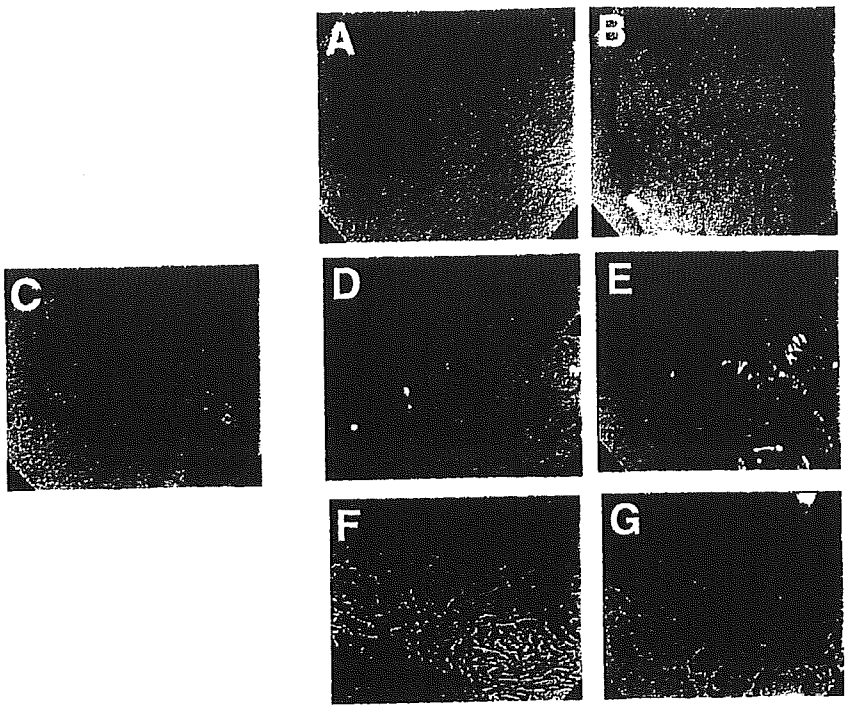

Figure 1. Pit pattern classification. (*A*) Type I: normal roundish pits; (*B*) type II: large star-shaped pits; (*C*) type IIIs: small roundish pits; (*D*) type IIIL: long tubular pits; (*E*) type IV: branched pits; (*F*) type VI: irregular pits; (*G*) type VN: nonstructural pit pattern. Types I and II are non-neoplastic pit patterns. Types IIIs, IIIL, and IV are adenomatous pit patterns. Types VI and VN are cancerous pit patterns.

slight deformation of the colonic wall may suggest the existence of a depressed lesion. The depressed-type colorectal cancers either can be absolutely depressed or accompanied by a slightly elevated margin. Some lesions with depression are elevated as a result of submucosal invasion and proliferation of the tumor cells.[8] Such lesions must not be mistaken for ordinary elevated neoplasms because they are quite different from each other in biological behavior. Chromoscopy is useful for confirming small colorectal lesions, for determining their lateral extent, and for clarifying their gross configuration; this is useful especially in determining the presence or absence of depression within the lesions.

There is some confusion about the depressed and flat lesions.[9] Lesions that are called *flat* adenomas are not absolutely flat, but often are elevated slightly. It is true that some adenomas appear to have a depression and resemble depressed-type early cancers; the depression in a depressed lesion is rather extensive and clearly demarcated. In contrast, the depression in flat elevated adenomas actually is an ill-defined pseudodepression with only a thorny or groove-like appearance. Depressed lesions are not part of flat adenomas but should be regarded as a different entity, because the latter almost invariably are benign. Invasive rates in flat elevated adenomas are slightly lower than but not remarkably different from those in protruded polyps. Flat lesions usually are benign or only focally malignant and grow very slowly, not becoming invasive until they are rather large. In contrast, depressed lesions apparently grow rather rapidly, advancing at an early stage. The depressed-type lesions are reported not to have K-*ras* point mutation, although their genetic alterations are not clear.[10] It is certain that they arise without adenoma-carcinoma sequence.[8]

Table 1. Rate of Submucosal (T1) Cancer in Early Colorectal Neoplasms

	Size (*mm*)					
	0–5	6–10	11–15	16–20	21+	Total
Depressed	20/249	64/148	49/70	19/22	14/16	166/505
	8.0%	43.2%	70.0%	86.4%	87.5%	32.9%
Flat-elevated	2/6573	2/1120	13/533	19/182	61/276	97/8684
	.03%	.18%	2.4%	10.4%	22.1%	1.1%
Protruded	0/5909	57/4464	85/1095	64/387	65/218	271/12,073
	0%	1.3%	7.8%	16.5%	29.8%	2.2%
Total	22/12,731	123/5732	147/1698	102/591	140/510	534/21,262
	.17%	2.1%	8.7%	17.3%	27.5%	2.5%

NOTE. Data from April 1985 to August 2004.

Table 2. Pit Pattern and Histology of the Lesion

Pit pattern	Adenoma		Cancer (T1 stage)	Total
	Low grade	High grade		
IIIL	1984	464	0	2448
	81.0%	19.0%		
IV	266	261	18	545
	48.8%	47.9%		
IIIs	21	21	1	43
	48.8%	48.8%		
VI	32	140	59	231
		60.6%	25.5%	
VN	0	8	64	72
			88.9%	
Total	2303	894	142	3339

NOTE. Data from April 2001 to August 2004.

Dye spraying can be used during routine examination with an ordinary colonoscope, but it is especially useful when combined with magnifying colonoscopy. The magnified view can be obtained instantaneously simply by slightly rotating the magnification knob of the scope or stepping on the foot controller. Zoom colonoscopes have all the basic functions of conventional colonoscopes; therefore, they can be used during routine examinations with an ordinary view. The combination of chromoscopy and magnifying colonoscopy is useful for the differential diagnosis of a colorectal lesion and for predicting the depth of a cancer because it enables one to observe the detailed structure of the lesion. There is definite correlation between the gross appearance and the pit pattern of a colorectal lesion.[4,11] Depressed lesions present with type IIIs or V pit pattern; the latter implies that the lesion is cancerous. Almost all flat and protruded neoplasms have type IIIL or IV pits.

There also is correlation between the pit pattern and the histology of the lesion. The pit patterns are useful for distinguishing neoplastic lesions from nonneoplastic changes. In neoplastic lesions, pit-pattern analysis is useful for distinguishing between low-grade adenomas and invasive cancers. The majority of the lesions that present only type IIIs, IIIL, or IV pits are low-grade adenomas. Type V pit pattern is typical of cancers and can be subdivided into 2 subtypes. In deeply invasive or advanced cancers the surface of the lesion is rough and often ulcerated; therefore it almost is devoid of pits and looks nonstructural. Such a pit pattern is named type VN (nonstructural [N]). In severely dysplastic adenomas and minimally invasive carcinomas, the pit pattern is not completely nonstructural, but fairly irregular. Such an irregular pit pattern is named type VI (irregular [I]).

Treatment selection should be based on an accurate prediction of cancer invasion of the colorectal lesions.[12] We suggest the use of the highly accurate pit-pattern diagnosis for the determination of treatment. The lesions with type I or II pit pattern almost always are nonneoplastic; therefore, they need not be treated except for submucosal tumors such as carcinoids. Those with type IIIs, IIIL, or IV pit pattern usually are benign adenomas that can be treated endoscopically. The lesions with type VI encompass a variety of lesions from benign adenoma to invasive carcinoma. Therefore, lesions with type VI pit pattern first are treated endoscopically, and additional surgical colectomy and lymph node dissection is considered after the histologic analysis of the excised specimen. The lesions with type VN pit pattern usually are invasive cancers that should be referred for surgery.

Limitations

It cannot be denied that there are some limitations to the pit-pattern diagnosis because pit patterns are the changes of the surface of lesions and do not permit direct analysis of the deeper part. However, the changes in the deeper layers also are reflected on the surface to some extent; therefore, pit patterns generally are more useful in practice.[13]

Conclusions

Flat, and especially depressed, lesions are important in the colorectum. Chromoscopy is important for an accurate diagnosis of these lesions. The pit-pattern analysis helps predict the histology of the lesions and therefore is useful in determining the treatment selection.

References

1. Kariya J, Mizuno K, Mayama M. A case of early colonic cancer type IIc associated with familial polyposis coli (in Japanese). Stomach Intest 1977;12:1359–1364.
2. Kudo S, Muto T. Superficial depressed type (IIc) of colorectal carcinoma (in Japanese). Gastroenterol Endosc 1986;28: 2811–2813.
3. Lambert R, Lightdale CJ. The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon. Gastrointest Endosc 2003;58:s1–s50.
4. Kudo S, Rubio CA, Teixeira CR, et al. Pit pattern in colorectal neoplasia: endoscopic magnifying view. Endoscopy 2001;33: 367–373.
5. Kudo S, Hirota S, Nakajima T, et al. Colorectal tumours and pit pattern. J Clin Pathol 1994;47:880–885.
6. Kashida H, Kudo S. Magnifying colonoscopy, early colorectal cancer, and flat adenomas. In: Waye JD, Rex DX, Williams CB, eds. Colonoscopy: principles and practice. Oxford: Blackwell, 2003:478–486.
7. Kudo S, Kashida H, Nakajima T, et al. Endoscopic diagnosis and treatment of early colorectal cancer. World J Surg 1997;21: 694–701.
8. Kudo S, Kashida H, Tamura T, et al. Colonoscopic diagnosis and management of non-polypoid ECC (early colorectal cancer). World J Surg 1999;23:694–701.

9. Kudo S, Kashida H, Tamura S, et al. The problem of "flat" colonic adenoma. Gastrointest Endosc Clin N Am 1997;7:87–98.
10. Fujimori T, Satonaka K, Yamamura-Idei Y, et al. Non-involvement of ras mutation on flat colorectal adenomas and carcinomas. Int J Cancer 1994;57:51–55.
11. Kudo S. Early colorectal cancer: detection of depressed types of colorectal carcinoma. Tokyo: Igaku-Shoin, 1996.
12. Kudo S. Endoscopic mucosal resection of flat and depressed types of early colorectal cancer. Endoscopy 1993;25:455–461.
13. Huang Q, Fukami N, Kashida H, et al. Interobserver and intraobserver consistency in the endoscopic assessment of colonic pit patterns. Gastrointest Endosc 2004;60:520–526.

Address requests for reprints to: Shin-ei Kudo, MD, PhD, Digestive Disease Center, Showa University Northern Yokohama Hospital, 35-1 Chigasaki-chuo, Tsuzuki-ku, Yokohama 224-8503, Japan. e-mail: kudos@med.showa-u.ac.jp or kashi-md@xf6.so-net.ne.jp; fax: (81) 45-949-7535.

大腸癌診断における3D-CT検査の役割

昭和大学横浜市北部病院消化器センター
永田 浩一，遠藤 俊吾，工藤 進英
昭和大学横浜市北部病院放射線部
太齋 圭輔，守屋 克之

KEY WORDS
●CT colonography
●virtual colonoscopy
●CT air-contrast enema
●大腸癌

CT colonography for diagnosis of colorectal cancer.

Koichi Nagata
Shungo Endo(助教授)
Shin-ei Kudo(教授)
Keisuke Dasai
Katsuyuki Moriya

はじめに

1994年にViningらが大腸の三次元CT画像(3D-CT)を発表してから10年以上が経過した[1]。この発表以降，multidetector-row CT (MDCT)およびワークステーションなどの飛躍的な発達に伴い，日常臨床に十分応用可能な3D-CT画像が得られるようになり，大腸癌診断における3D-CT検査が広まりつつある。しかし，日常臨床で3D-CT検査を行うにあたり，各施設で検査目的，撮影方法を模索・検討しているのが現状である。

そこで，本稿では大腸癌診断における3D-CT検査について①現状，②役割と有用性，③腸管前処置と画像構築に分けて，筆者らが行っている方法を紹介しながら概説する。

Ⅰ．大腸癌診断における3D-CT検査の現状

大腸癌における3D-CT診断の役割は，大腸癌検診(スクリーニング)と精密検査(術前検査)とに大別される。その利用方法は米国と日本とでは大きく異なっている。

1．米国の現状

米国では，下部内視鏡の検査費用が高額であること，熟練した内視鏡検査医の不足などの理由から，民間のイメージング・センターを中心に3D-CT検査が大腸癌検診(一次・二次スクリーニング)の1つとして臨床応用されている[2]-[4]。しかし，2003年から2005年にかけて米国で行われた複数の大規模な多施設共同臨床試験では，3D-CTによる病変別の診断能が試験間で大きく異なる結果となった。10mm以上の

ポリープ別のsensitivityをPickhardtらは3D-CTで92.2%，大腸内視鏡検査で88.2%と報告したが[5]，Rockeyらは3D-CTで53%，大腸内視鏡検査で99%と報告しており，2つの試験結果が大きく食い違う結果となっている[6]。このため，米国では3D-CT検査による大腸癌検診の臨床応用が始まっているものの，学会などから有効な大腸癌検診法とはいまだに認められず，保険も適応されていないのが現状である。

2．日本の現状

本邦では，大腸癌検診に3D-CT検査はほとんど活用されていない。その理由の第1として，内視鏡検査が世界をリードし，発達・普及していることがあげられ，二次スクリーニングでは大腸内視鏡検査が行われることが多いからである。

第2に，3D-CT検査では表面型大腸癌の診断能が低いことが指摘されている[7)-9)]。病変の色調(表面の色調，模様，毛細血管や出血など)，質感(緊満感など)，変形(鉗子や送気などによる変形)といった大腸内視鏡検査で得られる情報が3D-CT検査では捉えられない。3D-CT検査では主に周囲粘膜との高低差(凹凸)や変形で病変を認識するため，表面型大腸癌を検出することは難しい。

第3に，検診で3D-CT検査を行う場合は，医療被曝の問題がある。Gonzalezらは，日本を含む15ヵ国を対象に診断X線による発癌リスクに関する論文を発表し，そのなかで日本は欧米諸国に比較して医療被曝が突出して高いことを指摘している[10]。放射線診断による早期病変の発見などの利益も多いため，一概に悪いこととして片付けられないが，マスコミに取り上げられたこともあり，社会的反響が大きかったことも事実である。したがって，3D-CT検査を大腸癌検診に導入する場合には，撮影方法(被曝量，撮影回数)，目的(大腸癌検診かwhole body検診か)，撮影頻度・間隔などの点から，その妥当性を十分に検討する必要がある。

このような背景から，本邦では，3D-CT検査は術前の精密検査として利用されることが多いのが現状である。

Ⅱ．大腸癌診断における3D-CT検査の役割と有用性

昭和大学横浜市北部病院消化器センターでは2001年4月より大腸癌術前検査での注腸検査を省略し，3D-CT検査を行っている。検査目的は，病変の部位診断と深達度診断の補助，さらに遠隔転移やリンパ節転移診断などの病期診断を一度に行うことである。

2005年8月までに500症例，569病変に対して3D-CT検査を行い，病変の描出率は98.1%(558/569病変)と良好であった。しかし，このデータは精密検査として行った場合の結果であり，大腸癌検診として行った場合には，描出率は低下することが予想される。

1．大腸3D-CT画像の種類

大腸の三次元表示方法として，CT enema (CT air-contrast enema, air image，注腸類似画像)，CT colonoscopy (ヴァーチャル内視鏡像)，CT enemaにCT angiography (3D angiography)を組み合わせた画像，そして大腸展開画像(大腸切除標本類似画像，virtual gross pathology)などがあげられる(図1A～1D)。さらに，二次元画像ではあるが，MPR (multiplanar reformation，多断面再構成)画像がMDCT撮影により得られ，これにより任意の断面の二次元表示が可能となる(図1E)。われわれは，原則として術前の全症例(腸閉塞症例を除く)でCT enema像を作成し，大腸癌の部位診断および深達度診断を行っている。以下に，これら3D-CT画像の役割と有用性をそれぞれ詳述する。

2．大腸癌の部位診断における有用性

日本を含めた先進国では現在，進行癌を含めた大腸癌に対する腹腔鏡手術が急速に広まりつつある[11]。当センターでも，2001年4月から2004年12月までの期間に，原発性大腸癌507例のうち，312例(61.5%)で腹腔鏡下大腸切除術が行われた。

腹腔鏡手術では触覚が欠如するため，部位診断に基づいてトロッカー(スコープや操作用鉗子を挿入するための孔)の挿入位置，小開腹創の部位と長さ，腸切除の長さ，ストマ位置などを決定する。American Society of Colon and Rectal Surgeons(米国大腸外科医学会)では6.5%の外科医が，腹腔鏡下大腸切除術の際に誤った部位の腸管を切除したため，開腹術に移行し，追加腸切除した経験があると報告している[12]。腹腔鏡手術では従来の開腹手術と比較して，病変の部位診断が特に重要となるが，大腸内視鏡検査による部位診断では，誤診率が14～22%にのぼると報告されている[13)14)]。一方，CT enema検査による病変の部位診断能は，97.3%と報告されており，術前情報として非常に有用であると考えられる[14]。特に，直腸癌では仙骨・尾骨をCT enema像に重ね合わせることで，解剖学的な病

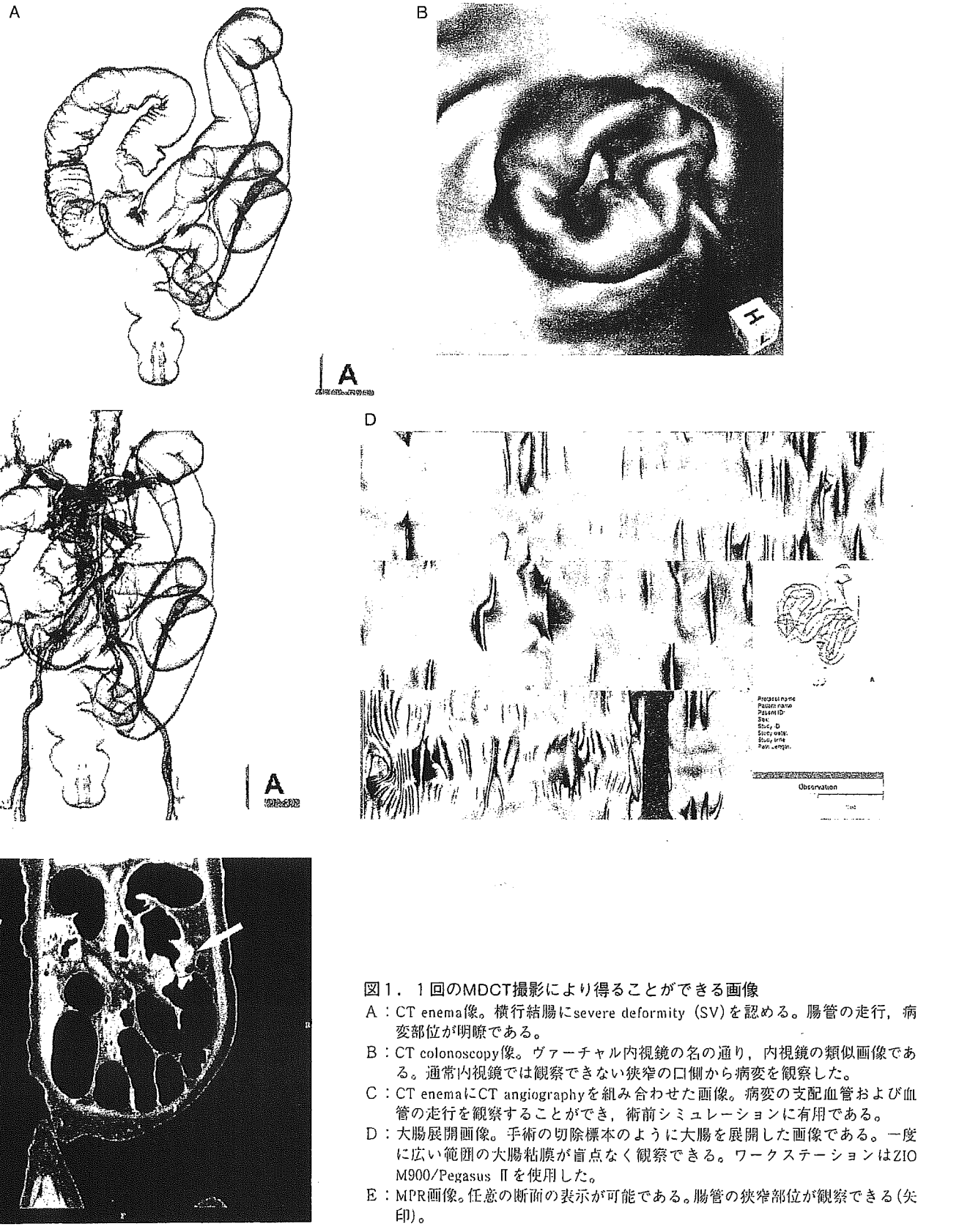

図1．1回のMDCT撮影により得ることができる画像

A：CT enema像。横行結腸にsevere deformity (SV)を認める。腸管の走行，病変部位が明瞭である。

B：CT colonoscopy像。ヴァーチャル内視鏡の名の通り，内視鏡の類似画像である。通常内視鏡では観察できない狭窄の口側から病変を観察した。

C：CT enemaにCT angiographyを組み合わせた画像。病変の支配血管および血管の走行を観察することができ，術前シミュレーションに有用である。

D：大腸展開画像。手術の切除標本のように大腸を展開した画像である。一度に広い範囲の大腸粘膜が盲点なく観察できる。ワークステーションはZIO M900/Pegasus Ⅱを使用した。

E：MPR画像。任意の断面の表示が可能である。腸管の狭窄部位が観察できる(矢印)。

変位置の把握が明確になり，術式決定に有用である(図2)。

3．大腸癌深達度診断における有用性

われわれは，牛尾らの注腸二重造影像による側面変形分類を応用し[15]，CT enemaでも大腸癌深達度の補助診断が可能であることを報告してきた[14](図3，表)。CT enemaは，検査施行者の技量の差が少なく，より客観的，かつ再現性が高いこと，病変側面像の描出が非常に容易であること，体位変換が少ないこと，撮影時間が短く済むこと，腸管前処置を内視鏡検査と共有できることなどが従来の注腸検査に比べた長所であると考えている。

4．内視鏡不通過症例における有用性

病変による狭窄が強く通常大腸内視鏡が不通過の場合には，3D-CT検査が有用である。MPR像やCT colonoscopy像も追加作成することで，狭窄部位より口側腸管の観察を行い，副病変の検索を行うことが可能である。高度狭窄を伴う多発大腸癌の症例数はそれほど多くはないが，従来の多くの検査が無力であったことから，3D-CT検査から得られる情報は非常に有用である[16][17]。

また，内視鏡挿入困難例では，注腸検査が同日に追加されることが多い。しかし，苦痛を訴えて内視鏡検査を断念した症例に，さらに体位変換の多い注腸検査を追加した場合，患者の負担は著しく大きくなる。代わりに3D-CT検査を追加することで，患者の負担を軽減することが可能である。

5．術前シミュレーションの有用性

腹腔鏡手術での血管処理が比較的困難な横行結腸癌あるいは結腸再建を必要とする食道癌手術症例などでは，必要に応じてCT enemaにCT angiography (3D angiography)を組み合わせた画像構築を行っている(図1C)。

6．低侵襲

経肛門的にバリウムなどの造影剤を注入する従来の注腸検査と比較して，空気を注入するだけの3D-CT検査は低侵襲であり，狭窄症例でも比較的安全に施行できると考えられる[18]。しかし，最近，大腸3D-CT検査での穿孔例が散見されており，十分な注意は必要である[19][20]。

7．大腸癌検診とその将来性

大腸展開像(図1D)などを用いることにより，今後，3D-CT検査が本邦でも大腸癌検診における有用な選択肢の1つとなりえる可能性がある。しかし，実際の臨床応用には，第Ⅰ章で述べたような問題点が山積しており，今後の解決が待たれる。その一方で，人間ドックでは通常内視鏡検査や注腸検査を希望しない場合のオプションなどとして，現状でも部分的な臨床応用は可能であると考える[18]。

Ⅲ．大腸癌3D-CT検査における腸管前処置法と画像構築方法

大腸癌に対する良好な3D-CT画像を得るためには腸管前処置が必要であり，便や液状残渣の存在が病変の描出能に大きく関与する。残便を少なくするために polyethylene glycol electrolyte (PEG)が使用されることが多いが，PEGを用いることで液状残渣が多くなるという問題がある。このため，2体位での3D-CT撮影が一般的に行われることが多い。われわれは液状残渣の問題を解決し，大腸内視鏡検査と共通する前処置法として，PEGに造影剤(contrast-medium)を含めたpolyethylene glycol solution plus contrast-

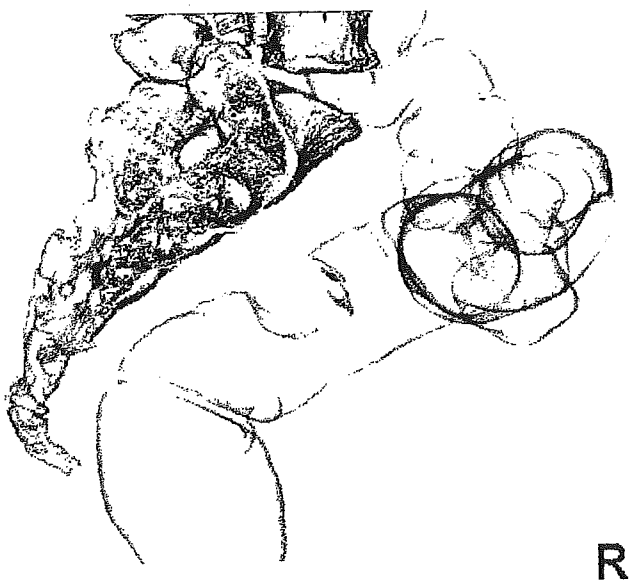

図2．CT enemaに仙骨・尾骨像を重ね合わせた画像

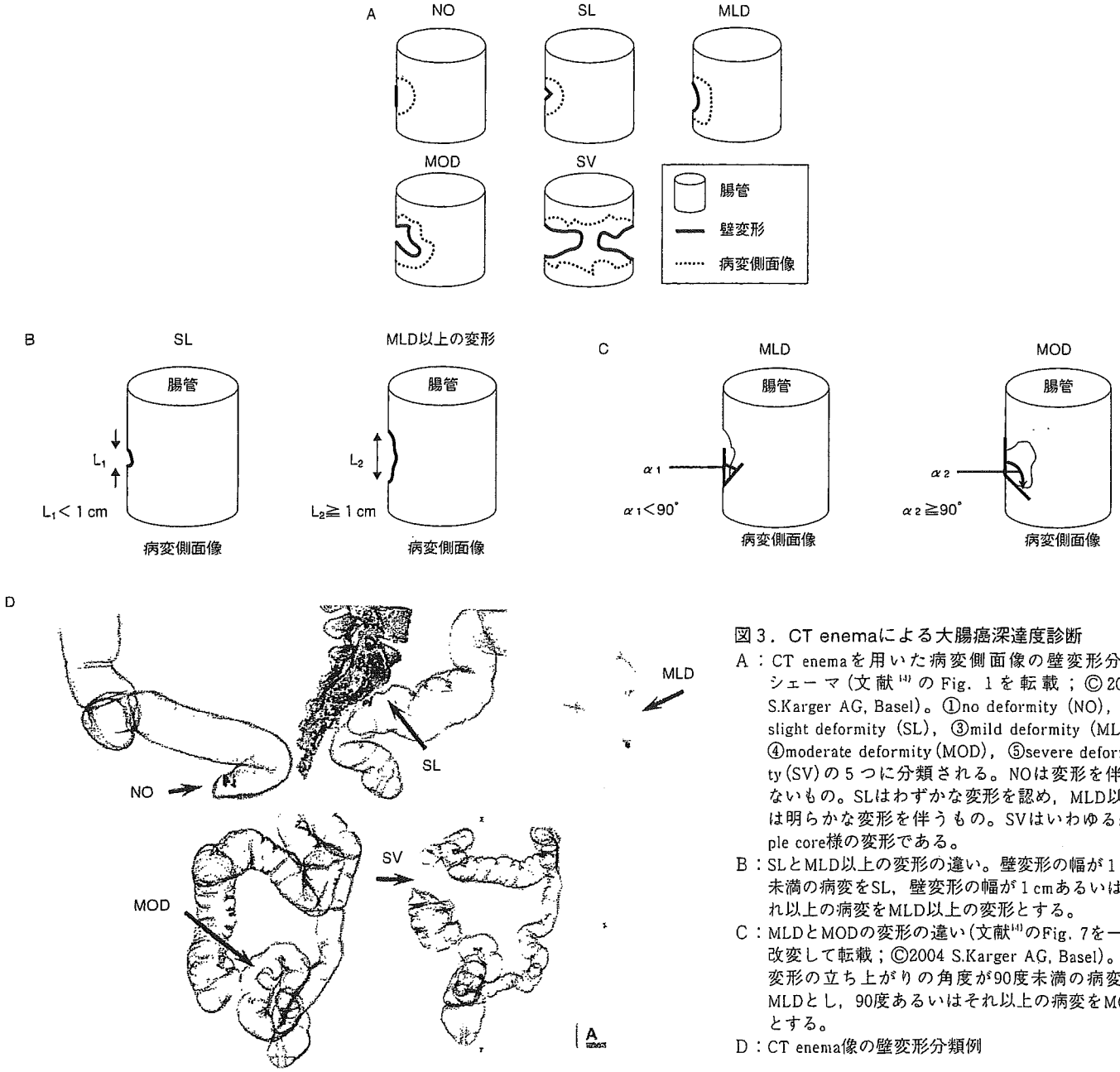

図3．CT enemaによる大腸癌深達度診断

A：CT enemaを用いた病変側面像の壁変形分類シェーマ（文献[4]のFig. 1を転載；©2004 S.Karger AG, Basel）。①no deformity (NO)，②slight deformity (SL)，③mild deformity (MLD)，④moderate deformity (MOD)，⑤severe deformity (SV)の5つに分類される。NOは変形を伴わないもの。SLはわずかな変形を認め，MLD以上は明らかな変形を伴うもの。SVはいわゆるapple core様の変形である。

B：SLとMLD以上の変形の違い。壁変形の幅が1 cm未満の病変をSL，壁変形の幅が1 cmあるいはそれ以上の病変をMLD以上の変形とする。

C：MLDとMODの変形の違い（文献[4]のFig. 7を一部改変して転載；©2004 S.Karger AG, Basel）。壁変形の立ち上がりの角度が90度未満の病変をMLDとし，90度あるいはそれ以上の病変をMODとする。

D：CT enema像の壁変形分類例

表．病変側面像の壁変形分類と病変の壁深達度（T staging）の関係

	Tis	T1	T2	T3	T4	
NO	33	9	1			43病変
SL	11	52	2			65病変
MLD		32	52	37		121病変
MOD			19	80	4	103病変
SV			1	186	39	226病変

壁変形が高度になるにつれ（NO→SV），壁深達度が深くなる（Tis→T4）関係がある。
T staging（UICC, TNM classification）.
Kruscal-Wallis test：$p<0.0001$

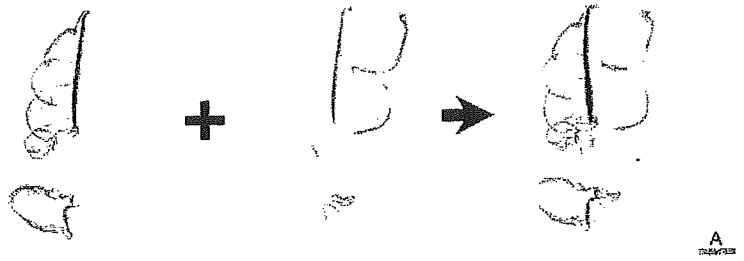

図4．Dual-contrast CT enema像
エア像と造影剤像の合成画像(図右)である。

medium bowel preparation (PEG-C法)を考案した[21]。PEG-C法は，体位変換の必要がなく，通常内視鏡と前処置を共有することが可能であり，病変描出能の点においても良好な成績を得ている(特許出願・公開中)[22]。前処置の方法は，PEG(ニフレック®)全量を水2Lに溶解し，このうちの1,620mLを内服後，残りをamidotrizoic acid，diatrizoic acid 76%，20mLを含むPEG-C溶液400mLとして最後に内服とする。3D-CT撮影後，エア像と造影剤像を構築し，その両方の画像データを合成することでDual-contrast CT enema画像が構築される(図4)。

おわりに

大腸癌診断における3D-CT検査は，検査上の注意や制限はあるものの，現状でも有用である。特にCT enema像は，患者負担の軽減や検査日程の短縮につながり，大腸癌術前検査の1つと数えることが可能である。

文　献

1) Vining DJ, Gelfand DW：Noninvasive colonoscopy using helical CT scanning. 3D reconstruction and virtual reality. 23rd Annual Meeting of the Society of Gastrointestinal Radiologist (SGR) 70(abstr.), 1994
2) Gazelle GS, McMahon PM, Scholz FJ：Screening for colorectal cancer. Radiology 215：327－335, 2000
3) Rex DK：Screening colonoscopy：Can we drive? ed by Dennis JJ, 65th Annual Meeting of the American College of Gastroenterology. New York, 2000
4) 吉田広行：MDCTを用いたvirtual enema/virtual colonoscopyによる大腸癌検診 米国における位置づけを含めて．早期大腸癌 8：507－514, 2004
5) Pickhardt PJ, Choi JR, Hwang I, et al：Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. N Engl J Med 349：2191－2200, 2003
6) Rockey DC, Paulson E, Niedzwiecki D, et al：Analysis of air contrast barium enema, computed tomographic colonography, and colonoscopy：prospective comparison. Lancet 365：305－311, 2005
7) Van Gelder RE, Nio CY, Florie J, et al：Computed tomographic colonography compared with colonoscopy in patients at increased risk for colorectal cancer. Gastroenterol 127：41－48, 2004
8) Kawamura YJ, Sasaki J, Okamaoto H, et al：Clinical significance of virtual colonoscopy (CT colonography) with special reference to polyp morphology. Hepatogastroenterology 51：1686－1688, 2004
9) Izumiya T, Hirata I, Hamamoto N, et al：Usefulness of multidetector row computed tomography for detection of flat and depressed colorectal cancer. Dig Endosc 17：36－43, 2005
10) Gonzalez AB, Darby：Risk of cancer from diagnostic X-rays：estimates for the UK and 14 other countries. Lancet 363：345－351, 2004
11) 河村　裕，小西文雄：癌治療の手技としての内視鏡手術の適応と限界 大腸癌．Jpn J Cancer Chemother 31：1485－1488, 2004
12) Wexner SD, Cohen SM, Ulrich A, et al：Laparoscopic colorectal surgery-are we being honest with our patients? Dis Colon Rectum 38：723－727, 1995
13) Vignati P, Welch JP, Cohen JL：Endoscopic localization of colon cancers. Surg Endosc 8：1085－1087, 1994
14) Nagata K, Endo S, Kudo S, et al：CT air-contrast enema as a preoperative examination for colorectal cancer. Dig Surg 21：352－358, 2004
15) 牛尾恭輔，石川　勉，笹川道三，他：大腸癌のX線診断；深達度診断を中心に．消化器外科 6：1474－1493, 1983
16) 遠藤俊吾，永田浩一，田中淳一，他：virtual endoscopyとCT enemaにより術前診断が可能であったS状結腸癌，多発大腸ポリープの1例．胃と腸 37：1449－1454, 2002
17) Nagata K, Kudo S：Triple colon cancer successfully demonstrated by CT air-contrast enema. Dig Surg 21：10－11, 2004
18) 永田浩一，遠藤俊吾，工藤進英，他：新しい検診法の可能性 MDCTを用いたvirtual enema/virtual colonoscopyによる大腸癌検診 本邦における臨床応用の可能性と問題点．早期大腸癌 8：515－518, 2004
19) Kamar M, Portnoy O, Bar-Dayan A, et al：Actual colonic perforation in virtual colonoscopy：report of a case. Dis Colon Rectum 47：1242－1244, 2004
20) Coady-Fariborzian L, Angel LP, Procaccino JA：Perforated colon secondary to virtual colonoscopy：report of a case. Dis Colon Rectum 47：1247－1249, 2004
21) 永田浩一，遠藤俊吾，日高英二，他：CT colonography検査の新しい前処置法．日本大腸肛門病会誌 56：306－307, 2003
22) Nagata K, Endo S, Ichikawa T, et al：Preoperative evaluation by dual contrast CT enema in patients with neoplastic colorectal obstruction. Endoscopy 36(Suppl. I)：A163, 2004

ANTICANCER RESEARCH 25: 1237-1242 (2005)

Expression of Dihydropyrimidine Dehydrogenase, Thymidylate Synthase, p53 and p21 in Metastatic Liver Tumor from Colorectal Cancer after 5-Fluorouracil-based Chemotherapy

SHIGERU YAMAGISHI, HIROSHI SHIMADA, TAKASHI ISHIKAWA, SHOUICHI FUJII, KUNIYA TANAKA, HIDENOBU MASUI, SHIGEKI YAMAGUCHI, YASUSHI ICHIKAWA, SHINJI TOGO and HIDEYUKI IKE

Yokohama City University Graduate School of Medicine, Department of Gastroenterological Surgery, Yokohama, Japan

Abstract. *Background: The expression of genes thought to be related to 5-FU chemosensitivity has been extensively investigated. However, little data is available on the expression patterns of these genes after chemotherapy. Patients and Methods: We investigated the expression of four genes, DPD, TS, p53 and p21, in the metastatic liver lesions obtained from colorectal cancer patients who had been treated with hepatic arterial infusions of 5-fluorouracil(5-FU)-based chemotherapy. Results: Expression of DPD, TS and p53 in the metastatic liver lesions was significantly higher in the chemotherapy-response group than in the no response group. In the response group, viable cancer cell nests were seen in confined spaces surrounded by fibrous tissue. It was of interest that these cancer cells in the response group showed conspicuous immunoreactivity of DPD, TS and p53. Conclusion: An analysis of genes involved in 5-FU sensitivity revealed that surviving tumor cells exhibited resistance characteristics, indicating that the chemotherapy regimen should be altered, even in partially responding cases, unless the response is pathologically complete.*

Pharmacogenetic markers of tumor cells have been intensively investigated using molecular biology technologies to predict chemosensitivity to 5-fluorouracil (5-FU) in colorectal cancer patients. Among them, *dihydropyrimidine dehydrogenase* (DPD) and *thymidylate synthase* (TS) are the most promising genes that have been used clinically in gastrointestinal cancer treatment. However, none of these genes are absolute predictors of 5-FU sensitivity.

Neoadjuvant chemotherapy has been proposed as an alternative approach to conventional surgery as an initial management strategy with the aim of improving the outcome of cancer patients. Strategies aimed at downstaging large or multifocal tumors and the control of micrometastases to enable curative resection by neoadjuvant chemotherapy have attracted much attention. Recently, in the field of breast cancer research, a sub-analysis of the National Surgical Adjuvant Breast and Bowel Project B-18 (NSABP B-18) trial revealed that a pathological complete response was the only reliable marker for selecting cases that were sensitive to a specific drug, resulting in an improved survival period (1).

In our institution, the resection of liver metastases for colorectal cancer has been actively performed after 5-FU-based chemotherapy *via* hepatic artery infusion. Although the survival advantage of hepatic arterial infusion over systemic therapy has been debated, the efficacy of this treatment with regard to tumor reduction was shown to be advantageous. We have used this procedure to treat patients with primarily unresectable metastases confined to the liver.

In this study, we examined the expression of *DPD, TS, p53* and *p21* in surgical specimens of liver metastases obtained from patients after chemotherapy.

Patients and Methods

Samples. Surgical specimens of synchronous or metachronous bilobular multiple liver metastatic tumors from 12 patients were obtained at Yokohama City University, Japan. The patients comprised 5 males and 7 females with a median age of 57.8 years (range, 41-74 years) (Table I). Since none of the 12 patients exhibited metastases at sites other than the liver, surgical resection was performed after 5-FU-based chemotherapy *via* hepatic arterial infusion. The treatment regimen was as follows: an infusion of 5-FU (500 mg/body) or 5-FU (500 mg/ body) + l-Leucovorin (150 mg/

Correspondence to: Shigeru Yamagishi, M.D., Gastroenterological Center, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama, 232-0024 Japan. Tel: +81-45-261-5656 (Ext. 2155), Fax: +81-45-261-9492, e-mail: s-gishi@kmc.mhc.east.ntt.co.jp

Key Words: Colorectal cancer, liver metastasis, chemotherapy, gene.

Table I. Patient characteristics.

Primary tumor	Age	Gender	Response	Depth	n	DPD pri	DPD meta	TS pri	TS meta	p53 pri	p53 meta	p21 pri	p21 meta
rectum	59	F	PD+NC	se	1	2	0	0	0	0	0	1	0
rectum	41	F	PD+NC	ss	4	2	0	0	0	4	0	0	0
colon	55	M	PD+NC	se	1	0	2	0	0	0	0	0	0
colon	45	M	PD+NC	se	1	2	0	2	0	6	0	0	0
colon	51	F	PD+NC	mp	1	-	3	-	1	-	3	-	0
colon	74	M	PD+NC	si	0	1	3	1	1	0	0	0	0
colon	67	F	PR	ss	2	2	6	1	4	0	0	2	2
colon	65	F	PR	se	4	0	4	0	2	12	8	0	0
colon	54	F	PR	ss	2	1	4	1	1	9	9	2	1
rectum	69	M	PR	ss	0	0	4	2	6	9	12	6	1
colon	59	M	PR	ss	2	0	9	1	1	9	6	1	0
colon	55	F	PR	ss	1	4	4	0	3	0	0	1	0

pri: primary lesion
meta: metastatic lesion

body) + Cisplatinum (10 mg/ body) was administered every day for 5 days, and this cycle was repeated every 2 weeks for up to 4 cycles. None of the patients had received any other chemotherapy or radiotherapy treatments prior to the hepatic arterial infusions of 5-FU. Paraffin-embedded archival samples of their primary colorectal lesions were also examined. The study was approved by the institutional review board of the Yokohama City University School of Medicine, Japan.

Clinical evaluation. The chemotherapy response was determined by comparing the volume of the liver metastases before and after chemotherapy. A CT scan was performed after the 4 treatment cycles had been completed, and the results were evaluated using the World Health Organization (WHO) criteria (2). A complete response (CR) was defined as the complete disappearance of all intrahepatic tumor formation, and a partial response (PR) was defined as a reduction in the tumor volume by 50% or more, measured as the sum of the products of the two largest perpendicular diameters of all visible lesions. No change (NC) was defined as a reduction in tumor volume of less than 50% or an increase of less than 25%. An increase in tumor volume of 25% or more or the appearance of new liver lesions was defined as progressive disease (PD).

Antibodies. Rabbit anti-recombinant human *DPD* polyclonal antibody (dilution=1:500; The Second Cancer Laboratory, Taiho Pharmaceutical Co, Saitama, Japan.), *TS* polyclonal antibody (RTSSA, dilution=1:800; The Second Cancer Laboratory, Taiho Pharmaceutical Co.), mouse monoclonal antibody against *p53* protein (DO-7, dilution = 1:100; DAKO, Glostrup, Denmark) and *p21* protein (OP64, dilution=1:100; Oncogene Research Products, Cambridge MA, USA) were used as the primary antibodies for the immunohistochemical staining.

Immunohistochemistry

(i) DPD. Tissue sections (4 μm thick) were cut from each block, deparaffinized in xylene, rehydrated with graded ethanol and immersed in TBS. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in distilled water for 15 minutes. *DPD* protein expression was evaluated using the avidin-biotin complex immunohistochemical technique and a rabbit polyclonal antibody to recombinant human *DPD*. To block the nonspecific binding of the primary antibody, a normal rabbit serum (DAKO X901) dilution in TBS was used for 20 minutes. After removing the blocking solution, the *DPD* antibody (2 mg/ml) was applied for 60 minutes in a humidified chamber at room temperature. The sections were then incubated with biotin-conjugated swine anti-rabbit immunoglobulins for 20 minutes (DAKO-E353), followed by avidin-biotinylated peroxidase complex for 30 minutes. After developing the color reaction product with a freshly prepared 3,3'-diaminobenzidine chromogen solution for 5 minutes, the sections were counterstained with light hematoxylin for 10 seconds, dehydrated in a series of ethanols, cleared in xylene, mounted and covered with glass coverslips. Positive and negative controls were included in each experiment.

(ii) TS, p53 and p21. Tissue specimens (4 μm thick) were fixed in formalin and embedded in paraffin wax. After dewaxing, the sections were treated with 3% hydrogen peroxidase solution in methanol for 20 minutes to block endogenous peroxidase activity. The sections were then heated in a 0.01 M citrate buffer (pH 6.0) for 3-minute periods in a microwave oven for antigen retrieval. Non-specific antibody bindings were blocked using 10% normal bovine serum in PBS at 37°C for 15 minutes for the *p53* and *p21* staining procedures and normal goat serum for the *TS* staining procedure. The sections were then incubated with the primary antibodies described in the previous section. The sections were incubated at room temperature for 10 minutes with a byotinylated anti-mouse IgG + IgA + IgM (for monoclonal primary antibody) for *p53* and *p21* staining, and with a biotinylated anti-rabbit IgG for *TS* staining. The sections were then incubated at room temperature with peroxidase conjugated streptavidin and Elite ABC solution, respectively. The peroxidase reaction was developed using a 3,3'-deaminobenzidine tetrahydrochloride solution (Sigma Chemical Co, St. Louis, MO, USA) and 0.03% hydrogen peroxide. The sections were counterstained with hematoxylin, dehydrated and mounted in a routine fashion. Positive controls and negative controls were always included in all experiments.

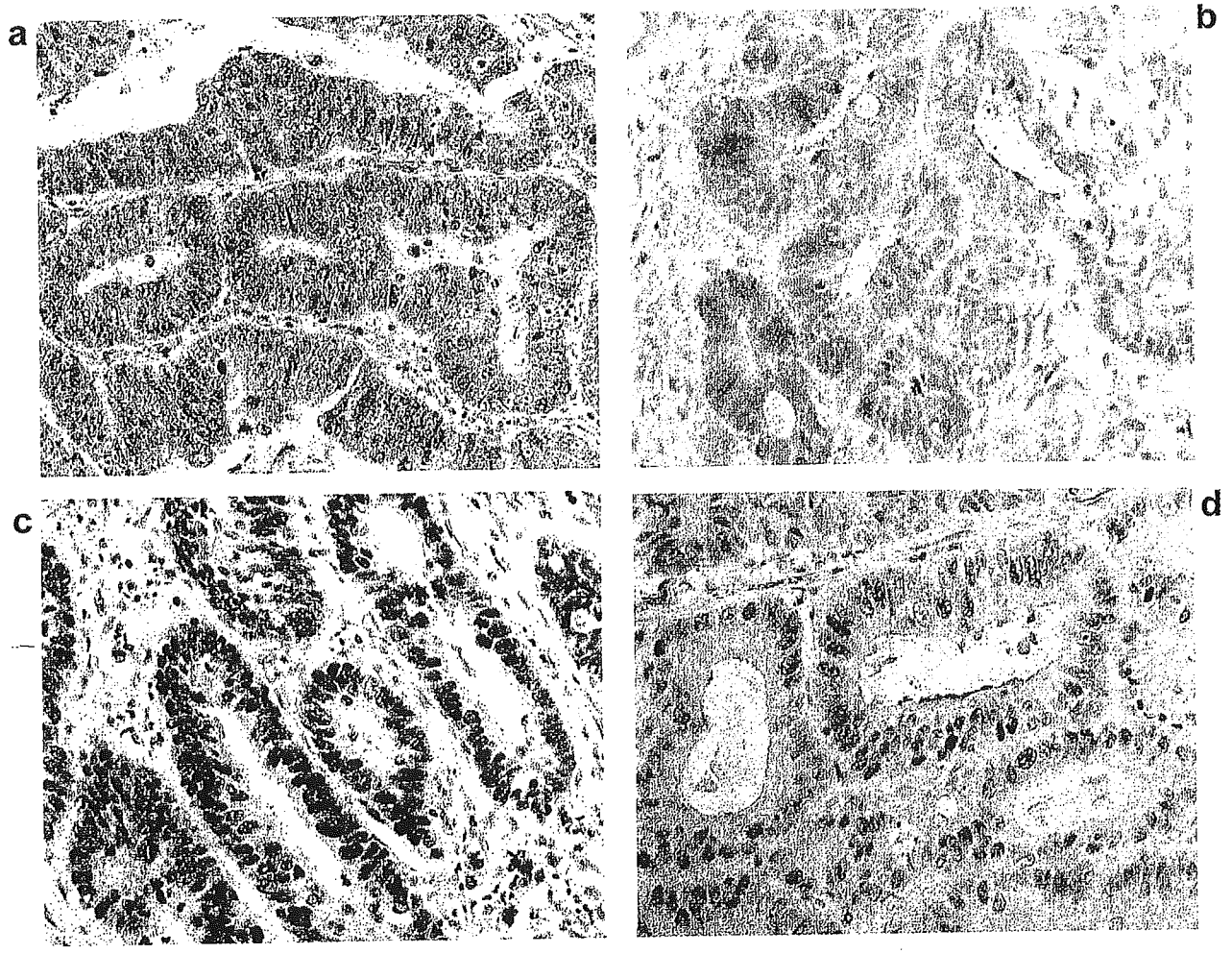

Figure 1. *Typical expression of DPD, TS, p53 and p21 genes after chemotherapy in surgical specimens of liver metastases. A: Expression of DPD, B: Expression of TS, C: Expression of p53, D: Expression of p21 (magnification, x400).*

(iii) Quantitation. Three representative fields were examined, more than 1000 tumor cells were randomly selected and the number of positive cells was counted at magnification x200. The expression of these proteins were evaluated according to the method described by Sinicrope *et al.* (3). In brief, positive-staining tumor cells were expressed as a percentage of the total number of tumor cells and assigned to one of the following five categories: class 0, ≤5%; class 1, 5% to 25%; class 2, 25% to 50%; class 3, 50% to 75%; and class 4, ≥75%. The intensity of the immunostaining was scored as follows: 1, weak; 2, moderate; 3, intense. These two scores were then multiplied. When heterogeneous levels of protein expression were found within a tumor (in multiple sections from different paraffin-embedded blocks of the same tumor), the highest protein expression score obtained for that lesion was used.

Statistical analyses. Dr. SPSS software for Windows was used for the statistical analyses; statistical significance was defined as $p<0.05$.

Results

Chemotherapy *via* hepatic arterial infusion was successfully performed in all 12 cases with no severe complications. The total 5-FU dosage was 3200±1500 mg (mean±SD). Clinical evaluations revealed that the liver tumors in 6 of the 12 patients partially responded to the treatment, although no complete responses occurred. In the remaining 6 cases, the tumors did not change in size or progressed after treatment (Table I).

Immunohistochemistry for the 4 genes was successfully performed in all 12 cases. *DPD* and *TS* were clearly observed in the cytoplasm of the cancer cells, while *p53* and *p21* were observed in the nuclei of the cancer cells. Representative cases are shown in Figure 1. Since half of the patients exhibited partial responses to the treatment,

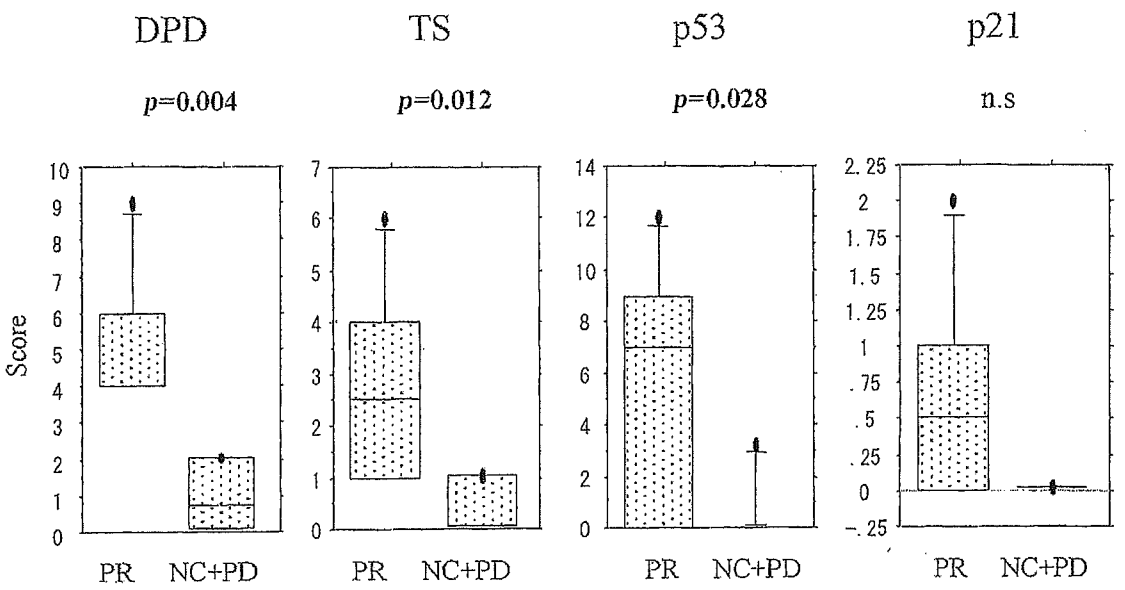

Figure 2. *Comparison of metastatic liver lesions in the PR group and the NC+PD group. The expression levels of DPD, TS and p53 were significantly higher in the PR group than in the NC+PD group ($p<0.05$).*

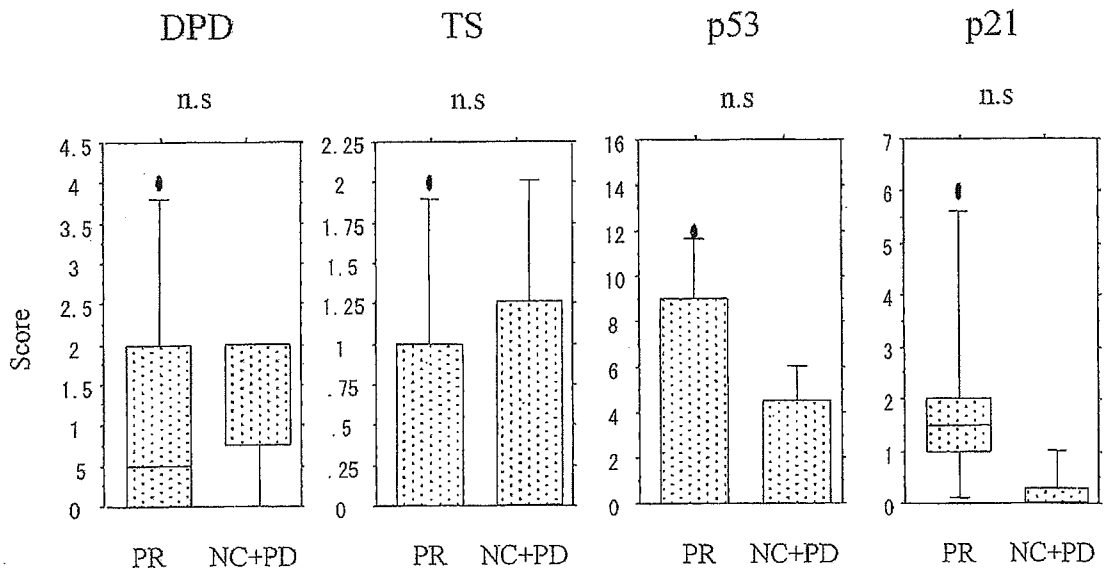

Figure 3. *Comparison of primary lesions in the PR and NC+PD groups. No significant differences in the expression levels of the 4 genes were seen between the PR and NC+PD groups.*

we compared the expression levels of these 4 genes in the chemotherapy response and no response groups. The mean scores for *DPD, TS* and *p53* immunoreactivity were significantly higher in the response group than in the no response group. All of the differences were statistically significant, as shown in Figure 2. However, the scores for *p21* were not significantly different between the two groups.

In the response group, viable cancer cell nests were seen in confined spaces surrounded by fibrous tissue. It was of interest that these cancer cells in the response group showed conspicuous immunoreactivity of *DPD, TS* and *p53*.

When the primary colorectal lesions from the archival samples were examined, no significant differences in the scores for any of the 4 genes were found between the response and no response groups (Figure 3).

Discussion

The expression of genes thought to be related to 5-FU chemosensitivity has been extensively investigated in the hope that methods for predicting 5-FU sensitivity might be established. However, little data is available on the expression patterns of these genes after chemotherapy. In our institute, the hepatic arterial infusion of 5-FU-based chemotherapy is routinely performed in patients with multiple liver metastases to improve the curative resection rate. Using surgical specimens, we examined the expression of 4 genes in liver tumors obtained from patients after chemotherapy. Although the number of cases in this study was limited, the expression rates of *DPD, TS* and *p53* were significantly higher in the response group than in the no response group. These findings suggest that the surviving tumor cells have both malignant and 5-FU-resistant characteristics.

DPD is the initial, rate-limiting enzyme in the catabolism of fluoropyrimidines, through which more than 80% of administered 5-FU is eliminated. Thus, the activity of this enzyme limits the efficacy of 5-FU treatment and is associated with tumor resistance to 5-FU (4,5). The intratumoral expression level of *TS* is considered a prognostic factor for survival in patients with colorectal cancer (6-8), although the ability of this marker to predict 5-FU chemosensitivity is controversial (9). The main pathway by which anticancer drugs induce apoptosis is a *p53*-dependent pathway (10,11). Normal *p53* protein has tumor-suppressing properties, and mutations in the *p53* gene result in the disruption of critical growth-regulating mechanisms (12-14). *p53* is also related to the malignancy of tumors and/or tumor resistance to chemotherapy. We previously reported that *p21* expression was correlated with the inhibiting activity of 5-FU (15), suggesting that *p21* may be a marker of 5-FU sensitivity.

These findings suggest two hypotheses: i) cells that are sensitive to 5-FU undergo apoptosis, but those that are resistant survive after chemotherapy, and ii) 5-FU exposure induces a mechanism that leads to drug resistance. The first hypothesis is feasible, but no direct evidence has been obtained to support this idea. However, Michael *et al.* examined *TS* expression in colorectal liver metastases after chemotherapy and found that previous fluorouracil exposure seemed to increase the resistance of the tumor cells to regional floxuridine *via TS* up-regulation (16). Nishiyama *et al.* performed an *in vitro* study on 5-FU exposure to examine changes in the expression of various genes, including *TS, DPD* and *MRP*. Although the results were very complicated, making their interpretation difficult, *DPD* and *TS* expression tended to increase in 5-FU-resistant cell lines after exposure to 5-FU (17). The mechanism of 5-FU chemoresistance is impossible to explain using the results of the present study alone.

However, the present findings may support the data obtained by the NSABP B-16 study on preoperative chemotherapy in patients with breast cancer (1). The outcome of chemotherapy was better in women whose tumors showed a pathological complete response than in women whose tumors exhibited a clinical partial response or a clinical no-response (relapse-free survival rates, 85.7%, 76.9% and 63.9%, respectively). Unless the cancer cells are totally killed by the drugs, remnant tumor cells survive and the prognosis of the patient does not improve. These findings strongly suggest that the initial chemotherapy treatment should be changed to one with a different mechanism, such as switching anthracycline to taxane, in patients with breast cancer who exhibit anything but a pathological complete response.

We also examined the expression of the 4 genes in the primary colorectal cancers obtained from this patient series, because liver metastases specimens obtained before chemotherapy were not available. No significant differences in the immunoreactivity of the 4 genes were seen between the response and the no response groups. Some differences in gene expression between the primary colorectal tumor cells and the metastatic liver tumor cells may exist. Therefore, it is not clear whether the 5-FU administration altered the expression of the 4 genes in the metastatic liver tumors after chemotherapy in the 2 groups.

Cancer chemotherapy has gradually improved with the production of new drugs exhibiting unique mechanisms and modifications. Hepatic resection after chemotherapy provides useful information enabling second-line chemotherapy treatments to be optimized. The results of this study suggest that a partial response may not be sufficient to improve the prognosis of colorectal cancer. Single-drug use limits the efficacy of treatment, while combination or sequential usage, like modified 5-FU, camptothecin and taxane regimens, may improve the prognosis of colorectal cancer patients.

References

1 Wolmark N, Wang J, Mamounas E, Bryant J and Fisher B: Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. J Natl Cancer Inst Monogr *30*: 96-102, 2001.

2 World Health Organization: WHO Handbook for Reporting Results of Cancer Treatment. WHO Offset Publication No. 48. Geneva, World Health Organization, 1979.

3 Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ and Levin B: Bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res *55*: 237-241, 1995.

4 Heggie GD, Sommadossi JP, Cross DS, Huster WJ and Diasio RB: Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer Res *47*: 2203-2206, 1987.

5 Diasio RB: The role of *dihydropyrimidine dehydrogenase* (DPD) modulation in 5-FU pharmacology. Oncology *12*: 23-27, 1998.

6 Johnston PG, Fisher ER, Rockette HE, Fisher B, Wolmark N, Drake JC, Chabner BA and Allegra CJ: The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol *12*: 2640-2647, 1994.

7 Lenz HJ, Hayashi K, Salonga D, Danenberg KD, Danenberg PV, Metzger R, Banerjee D, Bertino JR, Groshen S, Leichman LP and Leichman CG: p53 point mutations and thymidylate synthase messenger RNA levels in disseminated colorectal cancer: an analysis of response and survival. Clin Cancer Res *4*: 1243-1250, 1998.

8 Aschele C, Debernardis D, Casazza S, Antonelli G, Tunesi G, Baldo C, Lionetto R, Maley F and Sobrero A: Immunohistochemical quantitation of thymidylate synthase expression in colorectal cancer metastases predicts for clinical outcome to fluorouracil-based chemotherapy. J Clin Oncol *17*: 1760-1770, 1999.

9 Findlay MP, Cunningham D, Morgan G, Clinton S, Hardcastle A and Aherne GW: Lack of correlation between thymidylate synthase levels in primary colorectal tumours and subsequent response to chemotherapy. Br J Cancer *75*: 903-909, 1997.

10 Takeda A, Nakajima K, Shimada H, Imaseki H, Takayama W, Hayashi H, Suzuki T, Ochiai T and Isono K: Clinical significance of serum p53 antibody detection on chemosensitivity assay in human colorectal cancer. J Surg Oncol *71*: 112-116, 1999.

11 Levine AJ: The p53 tumor-suppressor gene. N Engl J Med *326*: 1350-1352, 1992.

12 Chang F, Syrjanen S, Tervahauta A and Syrjanen K: Tumourigenesis associated with the p53 tumour suppressor gene. Br J Cancer *68*: 653-661, 1993.

13 Vogelstein B and Kinzler KW: p53 function and dysfunction. Cell *70*: 523-526, 1992.

14 el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B: WAF1, a potential mediator of p53 tumor suppression. Cell *75*: 817-825, 1993.

15 Hosaka N, Ichikawa Y, Ishikawa T, Nagashima Y, Kunisaki C, Takahashi M, Moriwaki Y, Akiyama H, Yamaguchi S, Ota M, Ooki S, Ike H and Shimada H: Correlation of immunohistochemical p53 labeling index with inhibition rate in chemosensitivity test in gastric and colon cancer. Anticancer Res *21*: 229-235, 2001.

16 Davies MM, Johnston PG, Kaur S and Allen-Mersh TG: Colorectal liver metastasis thymidylate synthase staining correlates with response to hepatic arterial floxuridine. Clin Cancer Res *5*: 325-328, 1999.

17 Nishiyama M, Yamamoto W, Park JS, Okamoto R, Hanaoka H, Takano H, Saito N, Matsukawa M, Shirasaka T and Kurihana M: Low-dose cisplatin and 5-fluorouracil in combination can repress increased gene expression of cellular resistance determinants to themselves. Clin Cancer Res *5*: 2620-2628, 1999.

Received October 18, 2004
Accepted January 27, 2005

特集　最新　直腸癌手術

内腸骨動・静脈合併切除を伴う神経非温存側方リンパ節郭清

加藤知行*　平井　孝*
金光幸秀*　小森康司*

はじめに

直腸癌に対する側方リンパ節郭清は本邦でその有用性が確かめられた術式であるが，手術時間がかかり，また出血量が増加して手術侵襲が大きいこと，必ずしも術式が容易ではなく確実な郭清を行わないとその効果が得られないこと，などの理由でどの病院でも行われているわけではない[1]。その適応は下部直腸癌で壁深達度が固有筋層よりも深く浸潤したものとする[2]。近年は自律神経を温存した側方リンパ節郭清が基準であるが，リンパ節転移が進行した症例では内腸骨血管合併切除を伴った側方リンパ節郭清が行われる。その適応は一般に側方リンパ節に転移を認めたものとされる。

I. 側方リンパ節郭清の手順

明らかに側方リンパ節に転移がある場合を除いて，通常は直腸を摘除してから大動脈リンパ節→大動脈分岐部リンパ節→右側の総腸骨リンパ節（No. 273）→外腸骨リンパ節（No. 293）→閉鎖（No. 282），内腸骨（No. 272），中直腸動脈根部（No. 262）の側方リンパ節を郭清し，続いて左側も同様にして系統的に両側の側方リンパ節の郭清を行う。郭清を進めている間に側方のリンパ節に転移を認めれば，転移がある側の内腸骨動・静脈と骨盤神経叢およびその分枝を合併切除して郭清することになる。合併切除の有無，神経温存の有無にかかわらずリンパ節郭清では各リンパ節群の範囲（図1）を正しく同定して，リンパ節を含む脂肪組織をfascia（血管鞘と筋膜）で包み込んで摘除するようにする。

直腸のリンパ節を含む腔所（図2）は直腸固有筋膜内（第1腔所），自律神経，血管を含む脈管神経誘導路内（第2腔所），閉鎖腔（第3腔所）に分けられる。第1腔所は直腸を摘除するときに同時に郭清されるので，内腸骨動・静脈合併切除，自律神経非温存側方リンパ節郭清は第2，3腔所を同時に郭清する術式である。

II. 術　式

直腸を摘除してからの内腸骨血管を合併切除した側方リンパ節郭清術式について解説する。

1. 傍大動脈リンパ節の郭清

側方リンパ節転移を認める症例では傍大動脈リンパ節も郭清している。

腎筋膜前葉を切開して右尿管と精巣（卵巣）動・静脈を遊離し，尿管にベッセルループをかけたのち，右大腰筋の外縁から筋膜を剝離して右大腰筋を露出し，下大静脈の右背側に到達する（図3a）。下大静脈の血管鞘を大静脈から剝離し，これを左側へ進め大動・静脈間リンパ節（図3b），ついで腹部大動脈の血管鞘を大動脈から剝離し，左大腰筋筋膜を大腰筋から剝

* Tomoyuki KATO et al.　愛知県がんセンター中央病院消化器外科部（〒464-8681　愛知県名古屋市千種区鹿子殿1-1）