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NEW TECHNOLOGY

Indocyanine green fluorescence-guided laparoscopic surgery, with omental appendices as fluorescent markers for colorectal cancer resection: a pilot study

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Abstract

Background Currently, we lack tools that can reliably guide laparoscopic surgeons to a target anatomical destination for dissection. We aimed to develop and evaluate a fluorescent destination marker (FDM), composed of a resected omental appendix injected with indocyanine green (ICG), for real-time navigation in laparoscopic surgery for colorectal cancer in this pilot study.

Methods This study included ten patients diagnosed with colorectal cancer. To prepare FDMs, we laparoscopically harvested omental appendices attached to the colon we planned to resect. The harvested appendices were injected with diluted ICG, and a gauze tag was attached. The FDMs were placed at target intra-abdominal sites with a ligation clip.

Results Patient diagnoses included 1 cecal, 2 ascending colon, 3 transverse colon, 2 sigmoid colon, and 2 rectal cancers. No conversion to open surgery was required and no intraoperative complications occurred. We created 12 sets of FDMs, which were placed at a total of 13 sites in abdominal cavities. FDM fluorescence was successfully detected in all cases. Furthermore, FDMs could be detected earlier than the gauze tags at 12 points, and they were detected at the same time at 1 point.

Conclusions All FDMs facilitated laparoscopic surgery by allowing the surgeon to find the tissue to be dissected, particularly in procedures that required the dissection of lymph nodes around middle colic vessels and mobilization of the splenic flexure. FDMs showed potential for guiding the laparoscopic surgeon to a target anatomical destination. This marker represents a contribution to the evolution of real-time navigation surgery.

Keywords Laparoscopic surgery · Navigation · Colorectal cancer · Indocyanine green

Although indications for laparoscopic surgery in colorectal cancer resections have been expanding in the last two decades, this procedure is regarded technically challenging, even today. The laparoscopic technique is particularly challenging for resections of transverse colon cancer, rectal cancer, colorectal cancers with adjacent organ invasion, and in cases with severe intra-abdominal adhesions. Generally, the conventional "open surgery" approach has been considered appropriate in difficult cases, for several reasons. One critical reason is that open surgery provides an understanding of the anatomical relationships, due to the wide field of view and information gained from the surgeon's tactile sensation. However, in laparoscopic settings, operative working-space and tactile sensation are limited. Therefore, alternative means of understanding anatomical relationships are required during laparoscopic surgery. For example, a gauze or sponge can be placed as a marker in the target space to indicate the site of dissection during laparoscopic surgery. When approaching from a different direction, the laparoscopic surgeon can detect the marker as a bulge, which indicates the target anatomical destination [1–4]. Placing a gauze intraoperative landmark is useful, safe, convenient, and inexpensive, but it has some drawbacks. At times, the gauze silhouette is too vague to pinpoint the destination of interest; other times, the gauze cannot be detected, particularly when overlaying tissue is thick; and sometimes, the gauze marker moves during the manipulation of surrounding

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organs. An ideal marker could guide the laparoscopic surgeon to the dissection site precisely, despite overlaying tissues and nearby disturbances. Currently, we lack tools that fit those criteria. To address this problem, we developed a fluorescent marker based on autologous omental appendices injected with indocyanine green (ICG). We reasoned that this marker could be attached to a target site and detected with near-infrared fluorescence imaging.

In this pilot study, we assessed whether an ICG-labeled omental appendix, which we designated a fluorescent destination marker (FDM), could be used to identify a target anatomical destination during laparoscopic colorectal surgery.

Methods

Patients

Eligible patients were aged 20 or above with histopathologically proven colorectal cancer, no distant metastasis, and an ECOG performance status of 0–2. All patients were scheduled for laparoscopic surgery and capable of giving informed consent. Patients were excluded when they had iodine hypersensitivity, another primary cancer in organs other than the colon and rectum, or a severe mental disease.

In the present study, we enrolled ten consecutive patients with colorectal cancer that met the all eligibility criteria. Written informed consent was obtained from all patients. The primary endpoint was the detection rate of FDM compared to that of a gauze tag tied to the FDM. The study protocol was approved by the institutional review board of Toyonaka Municipal Hospital. This pilot study was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry, Identification Number: UMIN00031755 (http://www.umin.ac.jp/ctr/index.htm).

Preparation of FDM

Each FDM was prepared at the beginning of surgery (Fig. 1, Supplementary Video 1). First, after confirming that a curative resection could be performed, we laparoscopically harvested omental appendices that were attached to the colon, near the site we planned to resect, but located distant from the tumor border (> 5 cm). For the rectal cancer resections, we harvested omental appendices attached to the oral colon. The maximum number of omental appendices harvested was limited to 3 appendices in the study protocol. Next, a stock solution of ICG (Diagnogreen; Dai-Ichi Pharmaceuticals, Tokyo, Japan) was prepared by dissolving 25 mg of powdered ICG in 10 mL of sterilized water. This solution was then diluted 100- to 400-fold with saline. Then, we injected 0.1–0.5 mL diluted ICG into each omental appendix with a 26-gauge needle, which is the off-label use of ICG. The puncture site was tied securely with a silk thread to avoid spilling the injected ICG. A small loop was created in the silk thread adjacent to the omental appendix. The time required to prepare a set of FDMs was measured from the start of harvesting omental appendices to the confirmation that the fluorescence of the created FDM

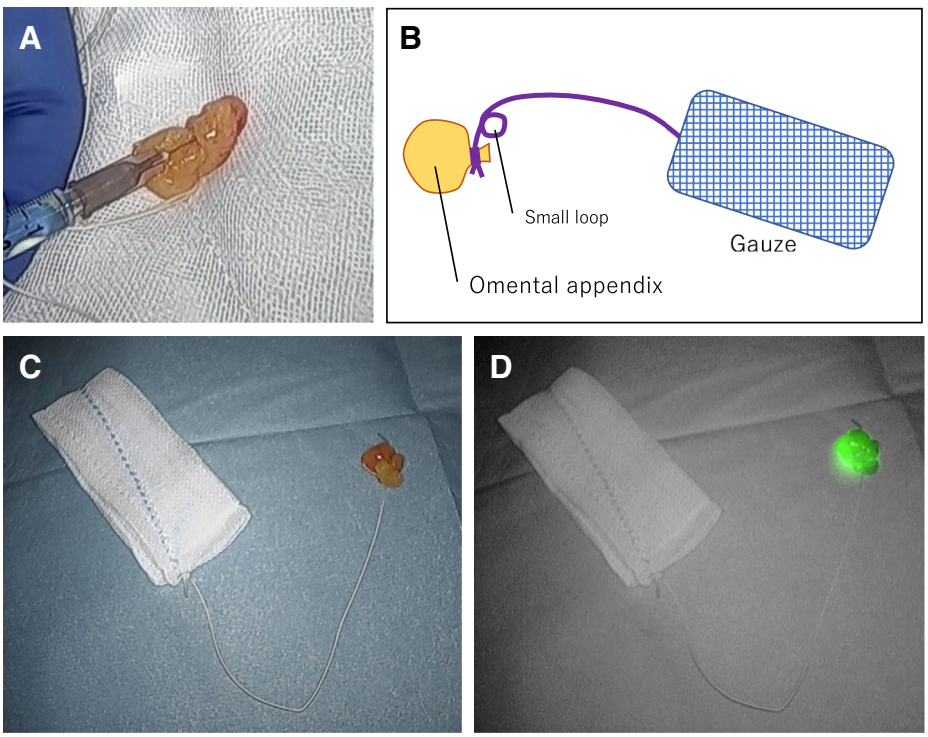

Fig. 1 Preparation of the FDM. **A** Diluted indocyanine green is injected into an omental appendix. **B** Schematic of the FDM used in this study, with the gauze tag attached. The small loop holds the clip for attaching the FDM to the target tissue. **C, D** The FDM with a connected gauze tag is shown **C** under white light imaging and **D** under near-infrared fluorescence imaging. FDM, fluorescent destination marker

could be recognized extracorporeally. The jaw of a ligation clip was passed through this loop to attach the FDM to the target site. The ligation clips used were 10-mm LIGACLIP (Ethicon, Cincinnati, OH, USA), 5-mm ENDO CLIP III (Covidien, Norwalk, CT, USA), or Hem-o-lok (Teleflex Medical, NC, USA). To prevent losing the FDM, we tied a gauze tag to the opposite end of the thread. This tag was also placed at the site with the FDM (Fig. 1). The fluorescent FDM was detected with an endoscopic fluorescence imaging system (1588 AIM laparoscopes, Stryker, MI, USA). In our procedure, all team members that participated in the operation searched for both the fluorescence of the FDM and the silhouette of the attached gauze. The surgeon (AH, a specialist in colorectal surgery and the principal investigator of this study) was involved in all of the surgeries. Each placed FDM was detected by the fluorescence (green light). The gauze tag was detected by the bulge it caused. The FDMs were removed from the peritoneal cavity during surgery. Free FDMs were removed soon after opening the space where the FDM was placed. When the FDM was attached to the specimen to be resected, it was removed concomitant with the removal of the cancer specimen.

Procedure for laparoscopic surgery

We performed the standard surgical procedure for laparoscopic colorectal cancer resections established in our institution. Briefly, after inserting an initial port into the umbilicus, a pneumoperitoneum was established with carbon dioxide insufflations at 10 mmHg. Three to four additional ports were then inserted, and the operation continued, according to the multi-port technique. The selection of where to place the FDM was determined at the surgeon's discretion and recorded. After resecting the cancer specimen, an anastomosis was completed with the end-to-end double stapling technique, for sigmoid colon cancer or rectal cancer, or with a functional end-to-end anastomosis technique, for other colon cancers. Immediately before anastomosis, we used fluorescence angiography, with a 5-mg intravenous ICG injection, to assess the perfusion of the colon. Fluorescent angiography was also performed after detecting the FDM and after finishing the assessment of FDM fluorescence. We did not perform any evaluations of lymphatic flow with ICG. All surgeries were performed by two surgeons (AH and TT, both specialists in colorectal surgery). Other attending surgeons and surgical trainees participated in the surgeries as assistants.

Results

Patient background

Ten patients were included in this study. Patient diagnoses were 1 cecal cancer, 2 ascending colon cancers, 3 transverse colon cancers, 2 sigmoid colon cancers, and 2 rectal cancers (Table 1). The median (first quartile, third quartile) age of patients was 69 years (67 years, 75 years); 7 were male and 3 were female. No conversion to open surgery was required, and no intraoperative complications occurred. One patient had a history of ileocecal resection, due to severe acute appendicitis and liver cirrhosis (case number 10). This patient required a long operative time, due to an intraperitoneal adhesion and a susceptibility to hemorrhage. In one patient with rectal cancer (case number 2), Clavien–Dindo grade II dysuria occurred postoperatively. In another patient with transverse colon cancer (case number 6), a Clavien–Dindo grade II urinary tract infection occurred. In both cases, the patients recovered rapidly with conservative treatment. The other 8 patients were discharged uneventfully.

The detection of FDM

A total of 12 sets of FDMs were prepared, and they were placed at a total of 13 sites in the abdominal cavities (details in Table 1). The median (first quartile, third quartile) time to prepare a set of FDMs was 8 min (7 min, 9 min). We detected the fluorescent FDMs successfully at all sites. Furthermore, at all sites except one, the FDMs were detected with near-infrared fluorescence imaging earlier than the gauze tags were detected. In the one exception, the fluorescent FDM and the gauze silhouette were detected concurrently. ICG spillage was observed at 4 sites; however, all spills were limited to a trivial area.

Operative procedures facilitated with the FDM

We performed several procedures in our series and found that the FDM could be utilized efficiently, particularly in three specific procedures. First, in four cases, lymph nodes around the middle colic vessels were dissected for right-sided colon cancer resections, according to the central vascular ligation (CVL) procedure [5–8] (Fig. 2, Supplementary Video 2). The fluorescence of the FDM attached to the stump of the middle colic artery (MCA) could be detected from the anterior side of the transverse mesocolon, at the lower edge of the pancreas. The FDM was detected in all four cases, but the gauze attached to the FDM could not be detected in any of these cases. By creating an orifice that connected to the dissected space in front of the SMV, we could carry out surgical procedures with a medial-to-lateral approach. Second, the splenic flexure was mobilized in three cases, and the FDMs were placed on the Gerota fascia at the lower border of the pancreas, from the caudal side (Fig. 3). In all three cases, the fluorescent FDMs were detected successfully (before detecting the attached gauze), from the anterior side of the transverse mesocolon. This detection facilitated mobilization of the splenic flexure. Third, we performed a lateral

Table 1 Patient backgrounds and details of the fluorescent destination marker

Case #	Gender	BMI (kg/m^2)	Disease	Procedure	Mobilization of splenic flexure	Conversion to open surgery	Intraoperative complication	Operative duration (min)	Blood loss (mL)	Length of hospital stay (days)	Number of created FDMs	Size of FDM (mm)	Dilution ratio of ICG	Injection volume of diluted ICG (μL)	FDM attachment site	The marker detected first	Residual fluorescence due to ICG spillage
1	Female	22.8	Cecal cancer	Ileocecal resection	No	No	No	222	0	6	1	15	400	150	Gerota fascia on the lateral side of the duodenum	FDM	Yes
2	Female	23.3	Lower rectal cancer	Very low anterior resection, bilateral pelvic lymph node dissection, and Ileostomy creation	No	No	No	635	210	17	1	15	250	200	Retroperitoneum, behind the descending colon	FDM = gauze	Yes
															Levator ani muscle, caudad to S4 nerve	FDM	No
3	Female	24.1	Ascending colon cancer	Right hemicolectomy	No	No	No	245	5	7	1	8	250	150	The stump of the middle colic artery	FDM	No
4	Male	22.2	Sigmoid colon cancer	Sigmoidectomy	Yes	No	No	238	45	9	1	20	250	200	Gerota fascia at the lower border of pancreas	FDM	Yes
5	Male	18.7	Lower rectal cancer	Intersphincteric resection and Ileostomy creation	Yes	No	No	425	85	11	2	10	280	150	Gerota fascia at the lower border of pancreas	FDM	Yes
												15	280	200	Anterior lower rectal wall, intraluminal	FDM	No

Table 1 (continued)

Case #	Gender	BMI (kg/m^2)	Disease	Procedure	Mobilization of splenic flexure	Conversion to open surgery	Intraoperative complication	Operative duration (min)	Blood loss (mL)	Length of hospital stay (days)	Number of created FDMs	Size of FDM (mm)	Dilution ratio of ICG	Injection volume of diluted ICG (μL)	FDM attachment site	The marker detected first	Residual fluorescence due to ICG spillage
6	Male	19.5	Transverse colon cancer	Right hemicolectomy	No	No	No	301	10	7	1	12	250	100	The stump of the middle colic artery	FDM	No
7	Male	21.8	Transverse colon cancer	Subtotal colectomy	Yes	No	No	437	60	10	2	10	250	100	Gerota fascia at the lower border of pancreas	FDM	No
			Ascending colon diverticulitis									10	250	100	The stump of the middle colic artery	FDM	No
8	Male	20.4	Transverse colon cancer	Right hemicolectomy	No	No	No	390	65	14	1	8	250	100	The stump of the middle colic artery	FDM	No
9	Male	19.8	Ascending colon cancer	Right hemicolectomy	No	No	No	200	5	11	1	8	250	100	Gerota fascia on the lateral side of the duodenum	FDM	No
10	Male	17.8	Sigmoid colon cancer	Sigmoidectomy	No	No	No	428	255	7	1	15	250	200	Prehypogastric fascia, left posterior to the rectum	FDM	No

BMI body mass index, *FDM* fluorescent destination marker, *ICG* indocyanine green

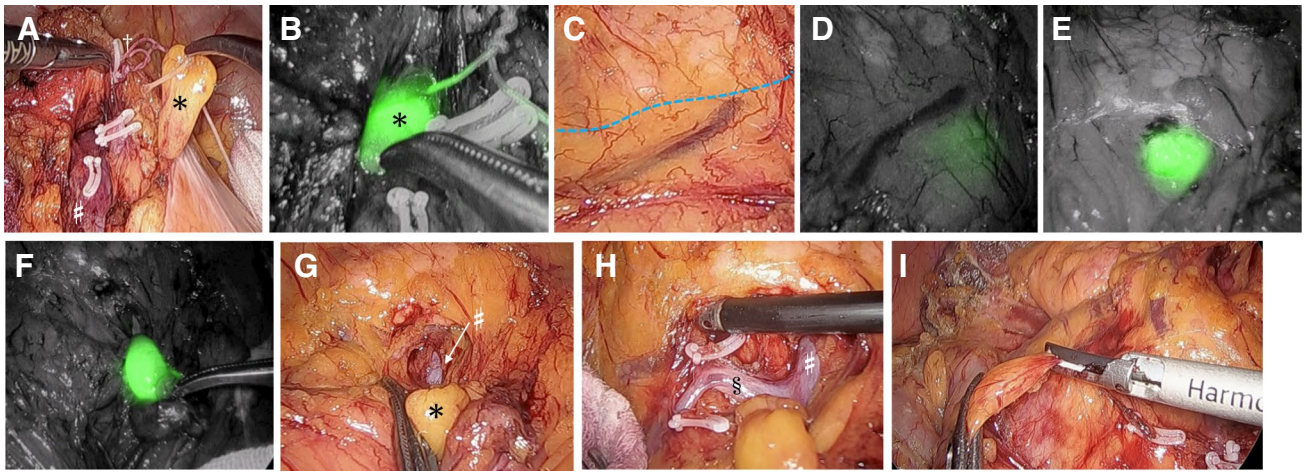

Fig. 2 Intraoperative images acquired during a laparoscopic right hemicolectomy. **A** The FDM (*) is attached to the stump of the middle colic artery, which was cut at its origin from the caudal side. **B** The FDM is placed on the dissected surface of the superior mesenteric vein (near-infrared fluorescence imaging). **C** Anterior view of the pancreas and transverse mesocolon. The broken line (blue) indicates the lower border of the pancreas. **D** The fluorescent FDM (near-infrared fluorescence imaging) could be detected through overlying tissue. Guided by the fluorescence of the FDM, we dissected the mesocolon along the lower border of the pancreas. **E** Fluorescent FDM visibility is enhanced gradually as overlying tissue was dissected. **F** The space is opened on the superior mesenteric vein, where the FDM was placed (near-infrared fluorescence imaging). **G** The superior mesenteric vein (# arrow) is exposed from an anterior approach. **H** After opening the space anterior to the superior mesenteric vein, the gastrocolic trunk (§) and its related veins are dissected and ligated with a medial-to-lateral approach. **I** Dissecting the mesocolon from the pancreas and duodenum could be performed with a medial-to-lateral approach. *FDM; †the stump of the middle colic artery; #superior mesenteric vein; §gastrocolic trunk; *FDM* fluorescent destination marker. (Color figure online)

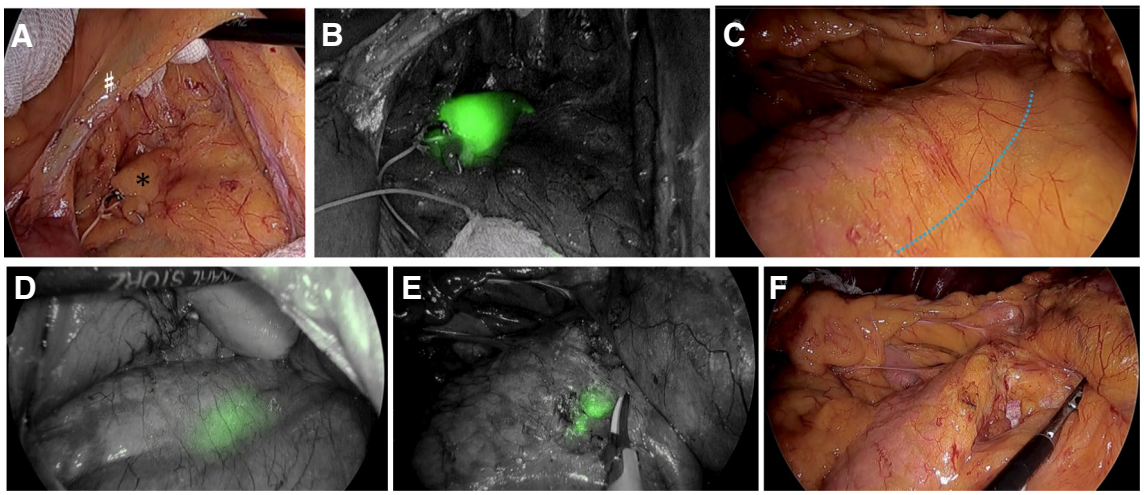

Fig. 3 Intraoperative images acquired during a laparoscopic sigmoidectomy with splenic flexure mobilization. **A** The FDM (*) is placed on the surface of Gerota fascia at the lower border of pancreas, just lateral to the inferior mesenteric vein (#). **B** Near-infrared fluorescence imaging of the FDM after placement. **C** Anterior view of the pancreas and transverse mesocolon. The broken line (blue) indicates the lower border of the pancreas. **D** The fluorescent FDM could be detected through the overlying tissue (near-infrared fluorescence imaging). Guided by the fluorescence of the FDM, we dissected the mesocolon along the lower border of the pancreas. **E** The fluorescent FDM visibility is enhanced gradually as overlying tissue was dissected. **F** The space is opened on the Gerota fascia, where FDM was placed. *FDM; #inferior mesenteric vein; *FDM* fluorescent destination marker. (Color figure online)

pelvic lymph node dissection in one patient with advanced lower rectal cancer (Fig. 4). In this procedure, we dissected along the surfaces of the internal obturator muscle and the pelvic floor muscle to create a passage to the upper edge of the anal canal. This procedure is typically difficult, due to the anatomical complexity in the deep pelvis [9]. The FDM

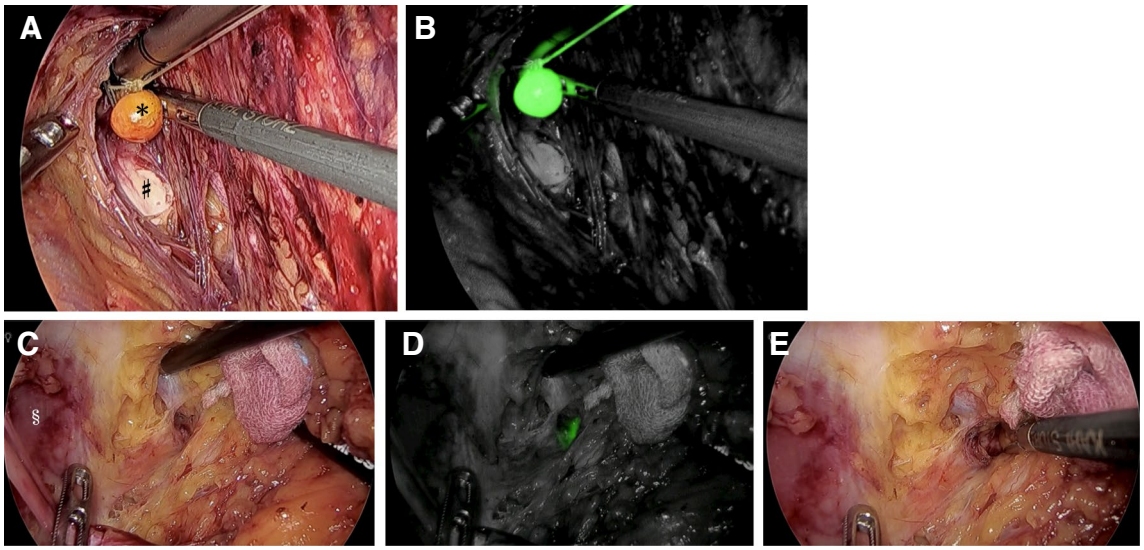

Fig. 4 Intraoperative images acquired during a laparoscopic lateral pelvic lymph node dissection on the left side. **A** The FDM (*) is placed on the fascia of the levator ani muscle (#) after mobilizing ureter and pelvic plexus to the medial side. **B** The FDM viewed under near-infrared fluorescence imaging. **C** Dissection along the surface of internal obturator muscle (§) where the gauze tag could not be detected. **D** The fluorescent FDM is visible at the depth of pelvic lateral cavity (near-infrared fluorescence imaging). **E** The passage to the upper edge of anal canal is dissected with guidance from the fluorescent FDM. *FDM; [#]levator ani muscle; [§]internal obturator muscle; *FDM* fluorescent destination marker

had been placed on the fascia of the levator ani muscle, just caudad to the S4 nerve. This FDM was detected from the lateral side, which facilitated performing the above-described dissection.

Discussion

In the present study, we provided proof-of-concept that the FDM could be used for intraoperative real-time navigation. We found that the detection rate for the FDM was 100%, which was substantially superior to that of the gauze tag. This high detection rate was mainly due to the special property of ICG fluorescence, which can transmit through biological tissue. Furthermore, the FDM guided the surgeon to each target anatomical destination accurately, without fluorescence blur or substantial ICG spillage. These results demonstrated that the FDM could serve as an efficient navigation tool, which might facilitate the surgeon's understanding of anatomical relationships in laparoscopic surgery. We concluded that the FDM could be useful, both in advanced laparoscopic surgery and in surgical training.

Navigation in surgery has evolved to enhance the safety and promote the progress of surgery, beginning in the field of neurosurgery [10]. The major advancements in intraoperative navigation have been based on the development of equipment for stereotactic surgery. Recently, stereotactic technology has been indicated for pelvic surgeries, including rectal cancer resections, and it has proven to be an effective approach [11–13]. However, there are several barriers that impede integrating this type of navigation into clinical use. First, the equipment required for stereotactic surgery is expensive. Second, stereotactic surgery is difficult to apply in treating diseases that arise in soft organs, which readily shift positions in response to pressure or gravity.

An alternative type of intraoperative navigation was introduced with real-time fluorescence imaging guidance. This type of navigation has been increasingly employed for colorectal surgery, and ICG is the most frequently used fluorescent agent for intraoperative guidance [14]. Fluorescence angiography with ICG is used for assessing perfusion in the gastrointestinal tract. This approach was demonstrated to be effective in reducing the rate of anastomotic leakage [15–17]. In addition to fluorescence angiography, various other uses of ICG have been demonstrated, including ureter visualization [18]; endoscopic tattooing of colorectal lesions [19, 20]; lymphatic mapping and sentinel lymph node identification [21, 22]; detection of colorectal peritoneal carcinomatosis [23–25]; and guidance for safe transanal total mesorectal excision [26]. In all these applications, ICG was shown to be a powerful tool, because ICG fluorescence could be visualized directly during surgery, even when tissues covered the targeted organ.

In this study, we developed the FDM, which comprised omental appendices injected with ICG. The FDM served as a destination marker to assist navigation during surgery. Omental appendices are oval-shaped, small peritoneal processes attached to the taenia coli of the large intestine. To

emit the fluorescence, the ICG reacts with the abundant proteins contained in omental appendices [27]. Our method efficiently utilized omental appendices that otherwise would be discarded after a resection. Furthermore, the FDM has other benefits; the peritoneal surface of the omental appendix can confine the injected ICG, which could blur when spilled into surrounding tissues; the FDM is safe, because it derives from the patient's own tissue, without any special medical treatment; and the small size (1–2 cm) of omental appendices makes them easy to manipulate during surgery, and provides an accurate target for marking an anatomical destination. In this study, although the maximum number of omental appendices harvested was limited to three appendices in the study protocol, in fact, we only used one FDM in most cases. We consider that a set of FDMs, two at most, would be sufficient during surgery, because one FDM can be used several times, and difficult surgical procedures that might require placement of many FDMs are rare. We could not confirm the efficacy of the FDM in obese patients, because no obese patients were enrolled in this study. However, we believe that the FDM might be an effective approach in cases of obesity, because in these cases, anatomical orientation can be difficult to understand during surgery.

In making the FDM, some pitfalls should be taken into consideration. The puncture site where the ICG is injected must be tied securely; otherwise, a small amount of ICG might spill into the tissue where it is placed. This might disturb the pinpoint accuracy of detecting the FDM. In addition, it is also important to select an appropriate-sized omental appendix for harvesting; appendices that are too large are not appropriate for pinpoint detection. We found that appendices 1 cm long were the best size for FDMs.

In our opinion, although the FDM has several uses, it is most effective as a marker in transverse colon cancer resections. For this surgery, the middle colic vessels have to be ligated at their origin to accomplish a CVL. The anatomic complexity around this area makes the CVL a technically difficult procedure, particularly in laparoscopic settings [28]. In the present study, we performed lymph node dissections around the middle colic vessels in four patients. In these cases, our CVL procedure was as follows (Fig. 5): first, the transverse colon was flipped upward (cephalad), followed by a dissection along the surface of the SMV; the MCA was then ligated, from a caudal approach, and cut. Then, the FDM was attached to the resected stump of the MCA and placed on the surface of the SMV. Next, the transverse colon was pulled caudally, thereby fanning out the mesocolon. At this point, we could readily and accurately detect the stump of the MCA, due to the fluorescent signal emitted by the attached FDM, which transmitted through the thick transverse mesocolon. This marker allowed the surgeon to find the tissue to be dissected from a cranial approach, and the lymph node dissection around the MCA was performed efficiently. After the lymph node dissection, in a medial-to-lateral fashion, the vessels originating from the SMV or the gastrocolic vein (i.e., the middle colic vein or the

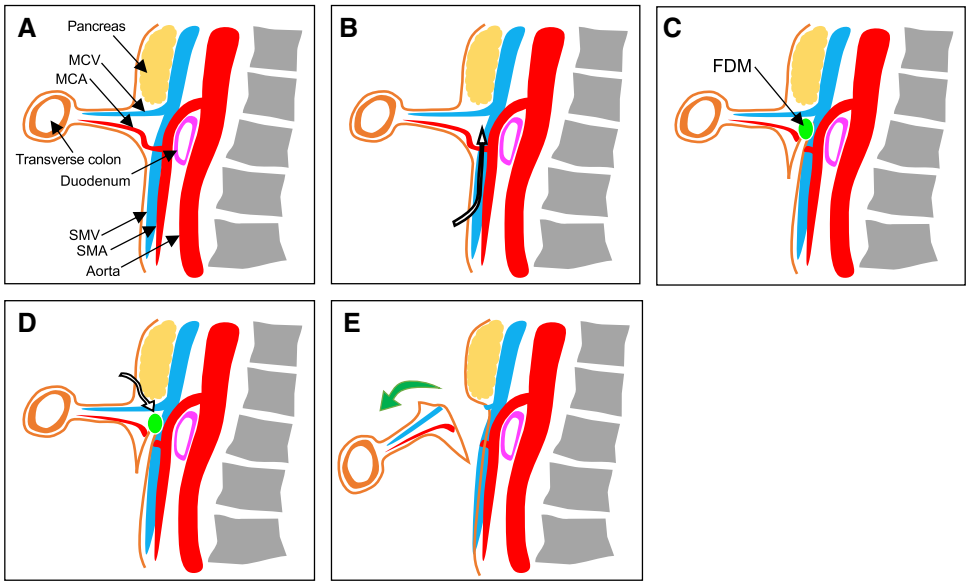

Fig. 5 Surgical procedure for dissecting lymph nodes around the middle colic vessels with FDM guidance. **A** Sagittal view of the anatomy around the middle colic vessels. **B** The dissection along the surface of the SMV. **C** The MCA is ligated from a caudal approach and cut. The FDM is attached to the resected MCA stump and placed on the surface of the SMV. **D** Detection of the root of the MCA from an anterior approach, with guidance from the fluorescent FDM. **E** Lymph node dissection can be performed after ligating the MCV. *SMV* superior mesenteric vein, *SMA* superior mesenteric artery, *MCV* middle colic vein, *MCA* middle colic artery, *FDM* fluorescent destination marker

accessory right colic vein) were ligated; the mesocolon was dissected from the pancreas or duodenum; and finally, the hepatic flexure was mobilized. In colorectal surgery, the efficiency of laparoscopic surgery can be maximized when the medial-to-lateral approach is adopted, due to the nature of laparoscopic surgery [29–31]. In performing the procedure described above, the direction of dissection and the longitudinal direction of the laparoscopic forceps were almost the same. This feature suggested that the FDM might be the ideal approach for this procedure. To date, laparoscopic surgery for transverse colon cancer has been regarded technically demanding, and therefore, it has been excluded from many clinical trials. The current results suggested that the use of an FDM might facilitate this procedure.

This study had several limitations. First, our results were based on a small number of patients; therefore, the significance of the FDM should be verified prospectively in further studies involving a larger cohort and which patients might benefit most with an FDM approach should be clarified. Second, the FDM might not suffice as the only reliable marker for guidance to an anatomic destination. Surgeons must cut or dissect the tissue with careful attention to other information, in addition to the signal emitted by the FDM. Third, it might require some time to prepare a set of FDMs.

In conclusion, we developed an FDM that showed potential for guiding surgeons to a target anatomic destination during laparoscopic surgery. This invention represents an advance in the evolution of real-time navigation surgery.

Compliance with ethical standards

Disclosures Atsushi Hamabe, Takayuki Ogino, Tsukasa Tanida, Shingo Noura, Shunji Morita, and Keizo Dono have no conflicts of interest or financial ties to disclosure.

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