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Real-time in vivo optical biopsy using confocal laser endomicroscopy to evaluate distal margin in situ and determine surgical procedure in low rectal cancer

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Abstract

Background In low rectal cancer, a negative distal margin (DM) is necessary for R0 radical resection, and therefore, the choice of surgical procedure is dependent on whether the planned transection rectum has residual cancer or not. Currently, surgeons choose surgical procedures according to intraoperative in vitro DM frozen sections. This study aimed to investigate the feasibility of real-time in vivo optical biopsy using confocal laser endomicroscopy (CLE) to evaluate DM in situ and determine the surgical procedure in low rectal cancer.

Methods Optical biopsy using CLE was performed when the rectum was dissected at the levator ani plane and rectum transection was ready. For negative DM, the surgical procedure of low anterior resection (LAR) was chosen. For positive DM, the surgical procedure of abdominoperineal resection (APR) was chosen. The specimen at the site of the planned transection rectum underwent intraoperative frozen section and routine pathological procedures.

Results Eighteen patients underwent real-time in vivo optical biopsy using CLE in surgery. Eleven patients' CLE images of DM showed a regular, round crypt, and round luminal opening covered by a simple layer of columnar epithelial cells and goblet cells. LAR was then performed. Pathology revealed that the 11 DMs were negative, and the median length of the DMs was 2.0 cm. The remaining seven patients' CLE images of the planned transection rectum showed the loss of crypt architecture and irregular epithelial layer with loss of goblet cells. APR was then performed. Pathology confirmed cancer invasion, and the median distance from tumor to dentate line was 1.0 cm. The sensitivity, specificity, and accuracy of CLE optical biopsy of DM were 85.71%, 100%, and 94.44%, respectively.

Conclusions It is feasible to perform real-time in vivo optical biopsy using CLE to evaluate DM in situ and determine the surgical procedure in low rectal cancer.

Keywords Optical biopsy · In vivo · Distal margin · Surgical procedure · Confocal laser endomicroscopy · Low rectal cancer

In low rectal cancer, a negative distal margin (DM) is necessary for R0 radical resection, and therefore, choosing a surgical procedure depends on whether the planned transection rectum has residual cancer or not. Currently, there are

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two major surgical procedures, low anterior resection (LAR) and abdominoperineal resection (APR), for low rectal cancer [1, 2]. When the rectum is dissected at the levator ani plane and rectum transection is ready, LAR is performed when low rectal cancer can be completely resected with negative DM. Otherwise, APR is required. Surgeons choose surgical procedures according to intraoperative in vitro DM frozen sections. In the operating room, surgeons finish anastomosis and submit the stapler distal "doughnut" to intraoperative frozen section to determine whether the DM has a residual tumor or not. There are two disadvantages of intraoperative frozen section. First, it is in vitro, which means that pathological diagnosis can only be made after rectum resection and anastomosis. Second, it is time-consuming, usually taking more than 30 min to acquire the final diagnosis. Once the intraoperative frozen section confirms positive margin, anal resection should be implemented to ensure radical treatment. In this situation, anastomosis is performed and expensive staplers are used, which means that both time and money are wasted. Therefore, if a real-time in vivo examination to evaluate DM in situ is available, it will be very helpful to determine the surgical procedure and achieve a real "tailored surgery" for low rectal cancer.

Confocal laser endomicroscopy (CLE) is a novel endoscopic technique that integrates confocal microscopy into the distal tip of a conventional white light endoscopy (WLE) [3]. WLE is able to observe the gross morphology of digestive tract mucosa, and CLE is able to perform high-resolution imaging in vivo and provide real-time information on living cell morphology and tissue architecture [4]. Therefore, it has been applied to observe the mucosal layer of the digestive tract and make "optical biopsy" of digestive mucosal diseases, such as Barrett's esophagus [5], inflammatory bowel disease [6], gastric intestinal metaplasia [7], and colorectal neoplasia [8]. Although CLE had been used in the endoscopic field for diagnosis, it is seldom applied in the surgical field, especially in low rectal cancer, to make optical biopsy for DM and help surgeons make a decision regarding surgical procedure. We hypothesized that CLE could make a real-time in vivo optical biopsy for DM in low rectal cancer surgery and help surgeons choose the surgical procedure. Thus, we performed this study to prove this hypothesis. The purpose of this study was to investigate the feasibility of real-time in vivo optical biopsy using CLE to evaluate DM in situ and determine the surgical procedure in low rectal cancer.

Materials and methods

Patients

A prospective study was performed between July 2016 and November 2017. Patients with low rectal cancer, confirmed by preoperative endoscopic biopsy, were recruited to participate in this prospective study. This study was approved by the institutional review board of Nanfang Hospital, and written informed consent was obtained prior to study participation. Inclusion criteria were as follows: 18–70 years of age, body mass index (BMI) less than 30, American Society of Anesthesiologists (ASA) class 1–3, rectal cancer confirmed pathologically by endoscopic biopsy, the distance from lower edge of tumor to the dentate line was less than 5 cm, single low rectal cancer, and planned radical resection. Exclusion criteria were past history of allergies, previous abdominal surgery, pregnant woman, emergency patients with obstruction or perforation, T4b cancer evaluated by CT or MRI or endoscopic ultrasonography, distant metastasis, and planned local excision or Hartmann procedure. Eighteen patients were enrolled in this study.

Real-time in vivo optical biopsy using CLE in surgery

In this study, fluorescein sodium made by Guangzhou Baiyunshan Ming Hing Pharmaceutical Company (3 ml: 0.6 g/ampule, H44023401) was used as the exogenous contrast agent. Each patient received a fluorescein sodium hypersensitive test before CLE examination. Then, 0.1 ml fluorescein sodium was diluted with 0.9% sodium chloride solution to 1 ml and injected through peripheral intravenous under intraoperative monitoring. Then, doctors observed the skin and vital signs for 5 min to determine whether the patient was allergic to fluorescein sodium. If there was no skin rash and vital signs were stable, the contrast agent was administered 1 min before CLE examination. The specific usage and dose were as follows: 3 ml of fluorescein sodium was diluted with 0.9% sodium chloride solution to 6 ml and intravenously injected in the periphery.

Optical biopsy using CLE was performed when the rectum was dissected at the levator ani plane and rectum transection was ready. The surgeon closed the rectum transversely with a laparoscopic long forcep at the levator ani plane. Then, real-time in vivo optical biopsy using CLE was performed trans-anally to exam the rectal mucosa at the levator ani plane. The CLE (ISC-1000) produced by Pentax Company (Tokyo, Japan) was used, which is not only able to observe the rectum's gross morphology but can also observe mucosal microarchitecture at a resolution of 0.7 μm. In this study, we used an argon laser excitation light at 488 nm wave length. Endomicroscopic examination was performed at a scanning speed of 0.8 frames/s. CLE produced confocal images with a field of a view of 1024×1024 pixels. The surgeon and endoscopist analyzed the CLE images during examination and made a real-time evaluation of rectal mucosa in the transverse section at the levator ani plane. If the CLE images showed normal mucosa architecture, which is regular with a round crypt and round luminal opening

covered by a simple layer of columnar epithelial cells and goblet cells, DM was determined to be negative. If the CLE images showed abnormal mucosa architecture and cancer cells, such as the loss of crypt architecture and irregular epithelial layer with loss of goblet cells, DM was determined to be positive. For negative DM in planned transection rectum, the surgical procedure of LAR was chosen. For positive DM in planned transection rectum, an endoscopic clip was used to mark the planned transection location for further pathological sampling and final hematoxylin–eosin (H–E) diagnosis. After the clip was marked, the surgical procedure of APR was chosen. DM at the site of the planned transection rectum underwent intraoperative frozen section, and the specimen was subjected to routine pathological procedures. The CLE images were compared with the H–E staining images.

Sample size determination

In our hospital, between January 2016 and June 2016, the time of intraoperative diagnosis by frozen section is 30 ± 10 min. We hypothesized that the average time of intraoperative diagnosis by CLE is 15 min, after which 18 cases were determined. With this number of cases, the study would have 90% power to detect a 50% decrease in the time of intraoperative diagnosis (two-sided type I error = 0.05).

Results

Eighteen patients with low rectal cancer participated in this prospective study. All patients underwent real-time in vivo optical biopsy using CLE in surgery. The patient demographics and cancer characteristics are summarized in Table 1. During the operation, optical biopsy using CLE was performed when the rectum was dissected at the levator ani plane and rectum transection was ready. The surgeon closed the rectum transversely with a laparoscopic long forcep at the levator ani plane. Then, real-time in vivo CLE optical biopsy was performed trans-anally to exam the rectal mucosa at the levator ani plane (Fig. 1). The surgeon and endoscopist analyzed the CLE images during CLE examination and made a real-time diagnosis of DM in the planned transection rectum at the levator ani plane. The average time of CLE examination was 12 min. The CLE diagnostic features of negative DM and positive DM are shown in Table 2.

Eleven patients' CLE images of DMs in the planned transection rectum showed normal mucosa architecture and cell morphology, which is regular with round crypts and round luminal opening covered by a simple layer of columnar epithelial cells and goblet cells (Fig. 2A). DMs were determined to be negative, and the procedures of LAR were then performed (Fig. 2B). Pathology revealed that the 11 DMs

Table 1 Patient demographics and cancer characteristics (18 cases)

Variable	
Age (years): median (range)	59 (41–70)
Gender (male/female)	11/7
BMI (kg/m^2): median (range)	24.74 (18.52–29.53)
Tumor size (cm): median (range)	3.5 (1.8–5.5)
Distance from tumor to dentate line (cm): median (range)	3.5 (0.5–5.0)
ASA (I/II/III/IV)	2/12/3/0
Neoadjuvant therapy (yes/no)	6/12

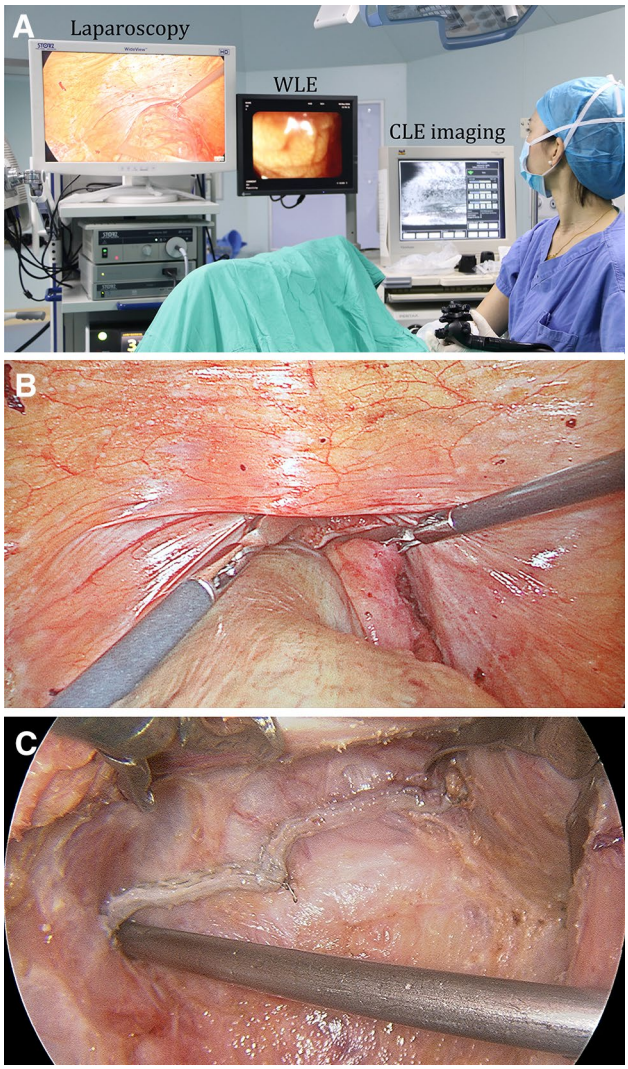

Fig. 1 Intraoperative CLE examination. **A** During the laparoscopic surgery, optical biopsy using CLE was performed when the rectum was dissected at the levator ani plane and rectum transection was ready. CLE optical biopsy was performed trans-anally to make sure whether the planned transection rectum had residual cancer or not. **B** The surgeon closed the rectum transversely with a laparoscopic long forcep at the levator ani plane. **C** Low rectum transection was finished at the levator ani plane when CLE imaging showed negative margin

Table 2 The CLE diagnostic features of negative DM and positive DM

CLE imaging	Negative DM	Positive DM
Tissue architecture	Regular foveolar pattern with central, round crypt openings	Irregular epithelial layer with loss of crypts and goblet cells, no crypt architecture
Glands	Normal glands lined with epithelial cells and goblets cells	Irregular tubular structures, Ridged-lined irregular epithelial layer
Stroma	Normal stroma	Reduced stroma
Cell morphology	Normal simple layer of columnar epithelial cells	Cellular and nuclear pleomorphism
Goblet cells	Yes	No

were negative by final H–E staining images (Fig. 2C). The median distance from tumor to DM was 2.0 cm with a range of 0.5–3.5 cm. The remaining seven patients' CLE images of DMs in the planned transection rectum showed abnormal mucosa architecture and cell morphology, which included the loss of crypt architecture and irregular epithelial layer with loss of goblet cells (Fig. 2D); therefore, DMs were determined to be positive, and the surgical procedures of APR were performed (Fig. 2E). Pathology confirmed cancer invasion by final H–E staining images (Fig. 2F). The median distance from tumor to dentate line was 1.0 cm with a range of 0.5–1.5 cm. In these seven positive DMs, CLE imaging showed abnormal mucosa architecture. Six patients' CLE images were determined as cancer tissue, but one patient's CLE image was deemed "abnormal" because CLE imaging was full of fluorescein leakage, and the blurred image was difficult to diagnose. The surgeon and endoscopist faced difficulty in identifying the tissue microstructure and could

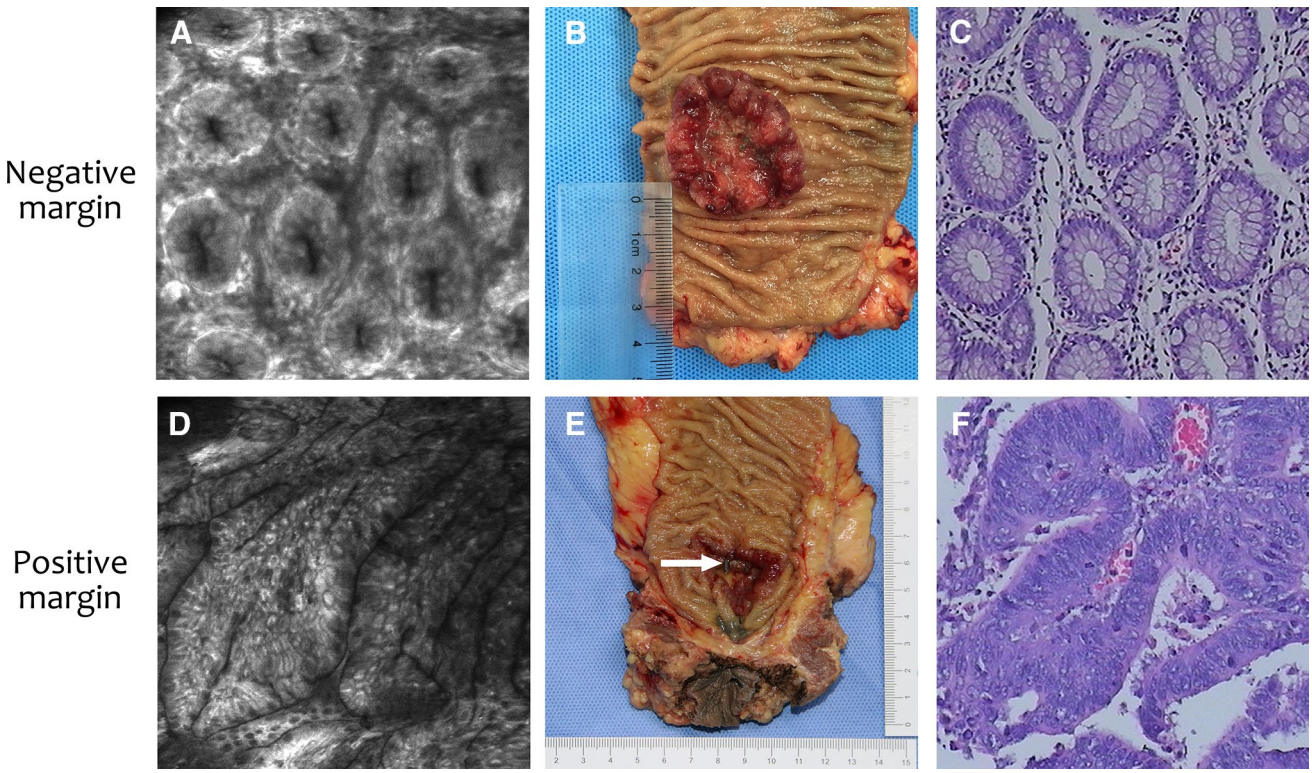

Fig. 2 Real-time in vivo optical biopsy using CLE to evaluate DM in situ and determine the surgical procedure in low rectal cancer. **A** CLE showed normal mucosa architecture, which has regular crypt and round luminal opening covered by a simple layer of columnar epithelial cells and goblet cells, in planned transection rectum. **B** DM was determined to be negative, and LAR was then performed. **C** H–E staining image confirmed negative DM. **D** CLE showed abnormal mucosa architecture, which included the loss of crypt and irregular epithelial layer with loss of goblet cells, in the planned transection rectum. **E** DM at the planned transection rectum was determined to be positive, and APR was then performed. The arrow shows an endoscopic clip at the planned transection rectum. **F** H–E staining image confirmed cancer invasion in the planned transection rectum

not determine whether the abnormal structure represented cancer, necrosis, or inflammation. In this case, CLE imaging could not make a cancer diagnosis for DM in the planned transection rectum. Since a negative margin was not found, intraoperative biopsy was performed, and frozen section confirmed residual tumor and tissue necrosis after neoadjuvant radiotherapy and chemotherapy. Then, the surgical procedures of APR were performed. Postoperative H–E staining pathology showed a residual tumor close to the dentate line.

All specimens underwent routine pathological procedures, and CLE optical diagnosis was compared with the final H–E diagnosis. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of CLE optical biopsy were 85.71% (6/7), 100% (11/11), 94.44% (17/18), 100% (6/6), and 91.66% (11/12), respectively (Table 3). The time of CLE examination was 12 ± 5 min, and the time of intraoperative frozen section was 30 ± 10 min. There was a significant difference between the time of CLE examination and the time of intraoperative frozen section ($p < 0.05$). There was no fluorescein sodium allergy occurred in this study. All patients had intraoperative yellow-staining urine, which did not need any treatment and disappeared automatically 6 h after surgery. No patient had any side effects of fluorescein sodium. The surgical outcome and pathological results are shown in Table 4. The median followup time was 6 months, and no cancer recurrence was found. No patient died in this study.

Discussion

In this study, we used CLE imaging to perform real-time in vivo optical diagnosis for DM in the planned transection rectum and found that CLE imaging could clearly show tissue architecture and cell morphology while also identifying negative or positive DM in situ with sensitivity,

Table 3 Accuracy of CLE biopsy for DM

$N = 18$	Pathological diagnosis		
	Cancer (N1 = 7)	No cancer (N2 = 11)	
CLE diagnosis			
Cancer (N3 = 6)	6	0	PPV = 100% (6/6)
No cancer (N4 = 12)	1	11	NPV = 91.66% (11/12)
	Sensitivity = 85.71% (6/7)	Specificity = 100% (11/11)	Accuracy = 94.44% (17/18)

PPV positive predictive value, *NPV* negative predictive value

Table 4 Surgical outcome and pathological results ($N = 18$)

Variable	CLE negative DM ($n = 11$)	CLE positive DM ($n = 7$)
Surgical procedure	LAR	APR
Operative time (min): median (range)	200 (150–250)	270 (240–330)
Estimated blood loss (ml): median (range)	20 (10–100)	50 (10–200)
Fluorescein sodium allergy (no/yes)	11/0	7/0
Intraoperative complications (no/yes)	11/0	7/0
CLE diagnosis of planned transection rectum	Normal	Abnormal
H–E diagnosis of planned transection rectum	No cancer	Cancer
Distance from tumor to distal margin (cm): median (range)	2.0 (0.5–3.5)	
Distance from tumor to dentate line (cm): median (range)		1.0 (0.5–1.5)
Tumor size (cm): median (range)	2.5 (0–5.0)	3.0 (2.0–6.0)
Tumor differentiation: no cancer/high/moderate/poor/mucinous/signet ring cell	1/0/5/1/4/0	0/0/4/1/1/1
T stage (T0/T1/T2/T3/T4)	1/0/1/2/7	0/0/1/1/5
N stage (N0/N1/N2)	9/0/2	4/2/1
M stage (M0/M1)	11/0	7/0
AJCC/UICC stage (no cancer/I/II/III/IV)	1/1/7/2/0	0/1/3/3/0

CLE confocal laser endomicroscopy, *DM* distal margin, *LAR* low anterior resection, *APR* abdominoperineal resection, *AJCC* American Joint Committee on Cancer, *UICC* Union for International Cancer Control

specificity, and accuracy up to 85.71%, 100%, and 94.44%, respectively. In negative DM, CLE demonstrated normal mucosa architecture, which included regular crypt and round luminal opening covered by a simple layer of columnar epithelial cells and goblet cells, after which the surgical procedure of LAR was chosen. In contrast, in positive DM, CLE showed abnormal mucosa architecture and cancer morphology, such as cellular pleomorphism and irregular epithelial layer with the loss of crypts and goblet cells, after which the surgical procedure of APR was chosen. The CLE images were compared to gold-standard H–E staining images. This study showed that real-time in vivo optical biopsy using CLE was able to evaluate DM in situ and helpful in determining the surgical procedure in low rectal cancer. To the best of our knowledge, this is the first study using CLE to make real-time in vivo optical diagnosis to evaluate DM in situ and determine the surgical procedure in low rectal cancer.

In low rectal cancer, which is less than 5 cm from anal verge, the National Comprehensive Cancer Network (NCCN) guideline (Version 2.2015) recommended that negative DM of 1–2 cm may be acceptable, which must be confirmed as tumor-free using frozen section [9]. In China, resection for low rectal cancer requires the distance from tumor to DM to be at least 2 cm, and if the distance is shorter than 2 cm, frozen section is recommended to confirm the absence of tumor [10]. Some studies reported that the length of DM less than 1 cm was also acceptable, regardless of whether it was with or without neoadjuvant therapy [11–15]. From these data, we found that frozen section is currently necessary and routine for DM less than 1–2 cm. However, as we mentioned above, there are two disadvantages of intraoperative frozen section, including its in vitro and time-consuming nature. In our study, we used CLE to make real-time in vivo optical diagnosis for DM and then chose the proper surgical procedure. Eleven negative DMs and seven positive DMs in the planned transection rectum were identified by intraoperative CLE. In these 11 negative DMs, the median distance from tumor to DM was 2.0 cm with a range of 0.5–3.5 cm, and the final pathology confirmed that the DMs were tumor-free. Therefore, real-time in vivo optical diagnosis using CLE for DM could provide a "tailored surgery" for low rectal cancer.

A limitation of this study is that it is not a multicenter randomized controlled trial. Therefore, we have registered a study in Clinicaltrials to perform a multicenter prospective randomized controlled trial, which has been approved by the ethics committee of Nanfang Hospital. Currently, this multicenter prospective randomized controlled trial is ongoing.

In conclusion, real-time in vivo optical biopsy using CLE to evaluate DM in situ is feasible, which is very helpful for surgeons in choosing an optimal surgical procedure to provide a "tailored surgery" for low rectal cancer patients.

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Compliance with ethical standards

Disclosures Drs. Zhangyuanzhu Liu, Xiaobei Luo, Wei Jiang, Dexin Chen, Weisheng Chen, Kai Li, Xiumin Liu, Ziming Cui, Zhiming Li, Zelong Han, Side Liu, Guoxin Li Chris Xu, and Jun Yan have no conflicts of interest or financial ties to disclose.

References

1. Monastyrska E, Hagner W, Jankowski M, Glowacka I, Zegarska B, Zegarski W (2016) Prospective assessment of the quality of life in patients treated surgically for rectal cancer with lower anterior resection and abdominoperineal resection. Eur J Surg Oncol 42:1647–1653
2. McLeod RS (2001) Comparison of quality of life in patients undergoing abdominoperineal extirpation or anterior resection for rectal cancer. Ann Surg 233:157–158
3. Neumann H, Kiesslich R, Wallace MB, Neurath MF (2010) Confocal laser endomicroscopy: technical advances and clinical applications. Gastroenterology 139:388–392
4. Kiesslich R, Goetz M, Vieth M, Galle PR, Neurath MF (2005) Confocal laser endomicroscopy. Gastrointest Endosc Clin N Am 15:715–731
5. Johnson EA, De Lee R, Agni R, Pfau P, Reichelderfer M, Gopal DV (2012) Probe-based confocal laser endomicroscopy to guide real-time endoscopic therapy in Barrett's esophagus with dysplasia. Case Rep Gastroenterol 6:285–292
6. Chapman CG, Konda VJ (2016) Confocal laser endomicroscopy in inflammatory bowel disease: achieving new depths in mucosal healing. Gastrointest Endosc 83:792–794
7. He XK, Liu D, Sun LM (2016) Diagnostic performance of confocal laser endomicroscopy for optical diagnosis of gastric intestinal metaplasia: a meta-analysis. BMC Gastroenterol 16:109
8. Kiesslich R, Burg J, Vieth M, Gnaendiger J, Enders M, Delaney P, Polglase A, McLaren W, Janell D, Thomas S, Nafe B, Galle PR, Neurath MF (2004) Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer in vivo. Gastroenterology 127:706–713
9. Benson AB 3rd, Venook AP, Bekaii-Saab T, Chan E, Chen YJ, Cooper HS, Engstrom PF, Enzinger PC, Fenton MJ, Fuchs CS, Grem JL, Grothey A, Hochster HS, Hunt S, Kamel A, Kirilcuk N, Leong LA, Lin E, Messersmith WA, Mulcahy MF, Murphy JD, Nurkin S, Rohren E, Ryan DP, Saltz L, Sharma S, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb

E, Willett CG, Gregory KM, Freedman-Cass D (2015) Rectal cancer, version 2.2015. J Natl Compr Cancer Netw 13:719–728 (**quiz 728**)
10. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J (2016) Cancer statistics in China, 2015. CA 66:115–132
11. Bujko K, Rutkowski A, Chang GJ, Michalski W, Chmielik E, Kusnierz J (2012) Is the 1-cm rule of distal bowel resection margin in rectal cancer based on clinical evidence? A systematic review. Ann Surg Oncol 19:801–808
12. Kuvshinoff B, Maghfoor I, Miedema B, Bryer M, Westgate S, Wilkes J, Ota D (2001) Distal margin requirements after preoperative chemoradiotherapy for distal rectal carcinomas: are < or = 1 cm distal margins sufficient? Ann Surg Oncol 8:163–169
13. Leo E, Belli F, Miceli R, Mariani L, Gallino G, Battaglia L, Vannelli A, Andreola S (2009) Distal clearance margin of 1 cm or less: a safe distance in lower rectum cancer surgery. Int J Colorect Dis 24:317–322
14. Moore HG, Riedel E, Minsky BD, Saltz L, Paty P, Wong D, Cohen AM, Guillem JG (2003) Adequacy of 1-cm distal margin after restorative rectal cancer resection with sharp mesorectal excision and preoperative combined-modality therapy. Ann Surg Oncol 10:80–85
15. Rutkowski A, Bujko K, Nowacki MP, Chmielik E, Nasierowska-Guttmejer A, Wojnar A (2008) Distal bowel surgical margin shorter than 1 cm after preoperative radiation for rectal cancer: is it safe? Ann Surg Oncol 15:3124–3131