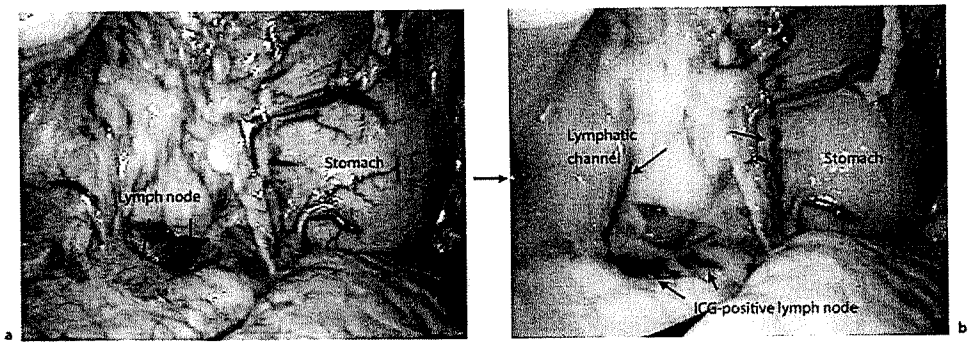

Fig. 1. a Serosal side of the stomach during laparoscopic surgery (ordinary light), 20 min after injection of ICG. **b** With IREE, lymph nodes (sentinel nodes) and lymphatic vessels positive for ICG can be identified easily.

section, such as massive submucosal invasion of cancer cells. In a large retrospective study including 445 patients who received endoscopic mucosal resection for treatment of early gastric cancer, 86 patients (19%) subsequently underwent additional surgery because of incomplete resection, local recurrence, or unclear margins in endoscopic mucosal resection specimens caused by piecemeal resection or by the electrocautery [2].

The incidence of lymph node metastasis of early gastric cancer in submucosal invasion has been reported to be about 20%, and that of metastasis to second-level lymph nodes, those around the left gastric, common hepatic, and celiac artery, has been reported to be about 4% [4]. As a result, in Japan, standard gastrectomy with D2 lymph node dissection has been advocated for early cancer with invasion to the submucosal layer, although this strategy is not accepted in Western countries. Standard gastrectomy with dissection of all first- and second-level lymph nodes (D2) is also recommended for patients who received endoscopic mucosal resection for early gastric cancer that involved the submucosal layer on subsequent histopathological examination.

However, many patients without lymph node metastasis are submitted to unnecessary extensive lymph node dissection, possibly leading to increases in postoperative morbidity and length of hospital stay. For several years, we have performed sentinel node navigation surgery using infrared ray electronic endoscopy (IREE) combined with indocyanine green (ICG) injection for patients with cT1N0 or cT2N0 gastric cancer [5], and then sentinel node navigation surgery was used for patients undergoing salvage operation after endoscopic mucosal resection. The aim of this study was to retrospectively evaluate the efficacy of sentinel node navigation surgery using IREE combined in such patients.

Patients and Methods

Among the 205 patients who underwent sentinel node navigation surgery for cT1N0 and cT2N0 gastric cancer between 2000 and 2007, 14 patients who received endoscopic resection (endoscopic mucosal resection and endoscopic submucosal dissection) as initial treatment were included in this study. The study was approved by the Ethics Committee for Biomedical Research of the Jikei Institutional Review Board, and all patients gave their written informed consent.

The methods for sentinel node mapping for gastric cancer have been previously described [5]. Briefly, each of the four submucosal sites surrounding the gastric cancer was injected with 0.5 ml ICG (5 mg/ml; Diagnogreen®; Dai-Ichi Pharm, Tokyo, Japan) through an endoscope using an endoscopic puncture needle during open or laparoscopic surgery. After 20 min, sentinel nodes stained with ICG were observed intraperitoneally around the serosa and surrounding fat tissue from the serosal side. IREE (Olympus Optical, Tokyo, Japan) was used to illuminate regional lymph nodes from the serosal side.

Positive staining was identified by at least 4 team members involved in sentinel node navigation surgery during the operation (fig. 1). The sentinel nodes were immediately examined by frozen section with hematoxylin and eosin staining. If possible, CAM5.2 (Becton Dickinson, San Jose, Calif., USA) was used for immunohistochemical staining with anticytokeratin antibody. During surgery, two sections were examined by both HE and immunohistochemical staining.

When modified gastrectomy was considered feasible for patients with sentinel nodes that stained positive for ICG (preoperative diagnosis of depth after endoscopic treatment is submucosal invasion <0.5 mm), dissection of the ICG positive lymphatic area (lymphatic basin dissection) [6, 7] was performed to remove possible micrometastases in the lymphatic area.

Barium radiographs, endoscopy, abdominal ultrasonography, and computed tomography were performed for preoperative staging and postoperative surveillance.

Results

Patient details and the reasons for additional surgery after endoscopic resection are shown in tables 1 and 2, respectively. All patients were treated within 2 months after endoscopic resection.

The operative procedures are listed in table 3. D1+α or β dissection was performed for distal or proximal gastrectomy. In addition to wedge resection of the stomach or segmental gastrectomy, lymphatic basin dissection was performed in 6 patients in whom the sentinel nodes were examined intraoperatively and were shown to have no metastasis. The extent of lymph node dissection in lymphatic basin dissection is the level of the root of feeding artery (left gastric artery, right gastric artery, left gastric artery, right gastroepiploic artery). As described in figure 2, D2 dissection was performed in 2 patients. Sentinel node detection rates and the incidence of metastasis to sentinel nodes are shown in table 4.

Mapping of sentinel nodes in 3 patients with lymph node metastasis is shown in figure 2. Case 1 (33-mm M signet ring cell carcinoma, ly1, v0) underwent standard distal gastrectomy with D2 dissection because frozen sections (hematoxylin and eosin staining) showed that the No. 4d node (sentinel node) was infiltrated by cancer cells during open surgery. Case 2 (12-mm SM1 moderately differentiated adenocarcinoma including poorly differentiated component in the invasive front, ly1, v0) was converted from laparoscopic wedge resection to open distal gastrectomy with D2 dissection because frozen sections showed the No. 6 node (sentinel node) to be involved. Case 3 (8-mm M well-differentiated adenocarcinoma, ly0, v0) underwent open segmental gastrectomy with lymphatic basin dissection of the left gastric artery area because frozen section examination during surgery showed no sentinel node metastasis. However, postoperative immunohistochemical staining with anticytokeratin antibody revealed micrometastases in the No. 3 node (sentinel node). Micrometastasis could be assessed by CAM5.2 immunohistochemistry in only three nodes of this case 3 among 14 patients after endoscopic treatment.

After a median follow-up of 32 (3–78) months, no patients had tumor recurrence. As to case 3 who underwent only segmental gastrectomy with lymphatic basin dissection, recurrence is not observed after 42 months.

Table 1. Patient details

Age, years	62.2 ± 15.16 (30–82)
Sex ratio, M:F	12:2
Histologic type	
Well-differentiated	4
Moderately differentiated	8
Signet ring cell carcinoma	2
Tumor size, mm	18.0 ± 9.66 (6–33)
Depth of invasion	
m	4
sm	10
Lymphatic invasion	
Positive	6
Negative	8
Venous invasion	
Positive	2
Negative	12

Table 2. Reasons for additional surgery after endoscopic resection

Reasons	Patients
Incomplete resection	4
Submucosal invasion	8
Local recurrence after endoscopic resection	1
Unclear cut margin	1

Table 3. Operative procedures

Type of operation	
Open gastrectomy	7
Laparoscopy-assisted gastrectomy	7
Extent of resection	
Proximal gastrectomy	3
Distal gastrectomy	5
Segmental gastrectomy	3
Wedge resection	3
Extent of dissection	
D1+α	4
D1+β	2
D2	2
Lymphatic basin dissection	6

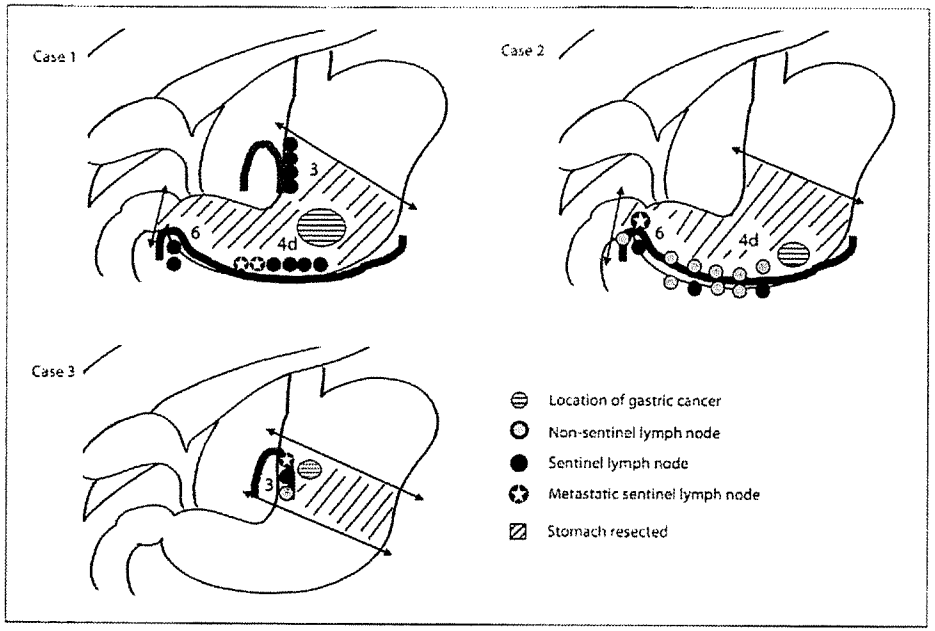

Fig. 2. Lymphatic drainage of patients with metastatic lymph nodes. Case 1 had a 33-mm M signet ring cell carcinoma in the anterior wall (lymphatic invasion: ly1, venous invasion: v0), which had two lymphatic basins (LGA, RGEA), and sentinel nodes in Nos. 3, 4d and 6. Metastatic lymph nodes were located in No. 4d, all of which were sentinel nodes. Case 2 had a 12-mm SM1 moderately differentiated adenocarcinoma (including poorly differentiated component in the invasive front) in the greater curvature (lymphatic invasion: ly1, venous invasion: v0), which had one lymphatic basin (RGEA), and sentinel nodes in Nos. 4d and 6. A metastatic lymph node was located in No. 6, which was a sentinel node. Case 3 had an 8-mm M well-differentiated adenocarcinoma in the lesser curvature (lymphatic invasion: ly0, venous invasion: v0), which had one lymphatic basin (LGA), and sentinel nodes in No. 3. A metastatic lymph node was included in No. 3 (sentinel node).

Table 4. Identification of sentinel node and lymph node metastasis

	Open surgery (n = 7)	Laparoscopic surgery (n = 7)	Total (n = 14)
Sentinel node identification	7 (100%)	7 (100%)	14 (100%)
Lymph node metastasis	2[1]	1[2]	3
Accuracy for rapid pathology for sentinel node metastasis	86% (6/7)	100% (7/7)	93% (13/14)

[1] Cases 1 and 3 in figure 2.
[2] Case 2 in figure 2.

Discussion

Although subsequent gastrectomy after endoscopic treatments should be intended to completely remove residual tumor, there is a large gap between endoscopic resection and open standard gastrectomy with extensive lymph node dissection. Limited surgery has to be considered for patients who received endoscopic resection as an initial treatment for early gastric cancer.

There is growing evidence that sentinel node navigation surgery could be safely used in patients with early gastric cancer and could reduce the extent of gastrectomy and lymph node dissection without increasing tumor recurrence rates [8–11]. According to a recent report [12] of 80 patients, 61 with negative sentinel node metastases underwent a less extensive, function-preserving gastrectomy. In that study, the false-negative rate on sentinel node biopsy was 23% for frozen section and 7% for postoperative pathology, both of which are comparable with the results of the present study. However, the appropriateness of sentinel node navigation surgery for patients who received inappropriate endoscopic treatment for early gastric cancer has been poorly investigated. The concern is that disorganized lymphatic channels caused by endoscopic resection may disturb the lymphatic flow necessary for detecting sentinel node.

In the current protocol for sentinel node mapping for gastric cancer in a university hospital where a high detection rate (292/301) of sentinel node for gastric cancer is achieved, patients who had previous endoscopic treatments are excluded as candidates for sentinel node navigation surgery [13]. However, in a recent report involving 6 patients who underwent laparoscopy-assisted distal gastrectomy after endoscopic mucosal resection, 20 sentinel nodes and 85 non-sentinel nodes were obtained, and the lymphatic tissue after endoscopic mucosal resection was shown to be normal [14].

In the present study, sentinel nodes for gastric cancer could be detected using IREE combined with ICG in all 14 patients who had previously received endoscopic resection during open and laparoscopy-assisted gastric resection. Two of the 14 patients were found to have sentinel node metastases on rapid frozen section pathological examination during surgery, and they underwent standard gastrectomy with D2 lymph node dissection according to the current recommendations. In another patient (case 3, fig. 2), postoperative immunohistochemical staining revealed sentinel node micrometastases that could not be detected at surgery. He underwent segmental gastrectomy with lymphatic basin dissection, leading to eradication of tumor cells (however, if micrometastasis was intraoperatively detected, we think that standard gastrectomy with D2 dissection should be performed in principle until the significance of the micrometastasis becomes definitively determined). For the remaining 11 patients with sentinel nodes free from tumor invasion, less extensive lymph node dissection and/or limited resection of the stomach could be performed.

None of the metastatic cases had indications for endoscopic mucosal resection according to Japanese gastric cancer treatment guidelines [1]. Furthermore, we emphasize the high rate sentinel node metastasis (3/14; 21%) even after endoscopic treatment. Because the whole resected specimen was not evaluated, we cannot show the histological difference of lymphatics between the normal gastric wall structure and the structure of this series. However, we injected the ICG into the normal submucosa away from the scar after endoscopic resection; we consider that this method reproduces the original lymphatic flow from a primary gastric tumor.

Limited surgery with or without sentinel node navigation is still considered partially experimental, technical issues in sentinel node mapping need to be resolved, and the significance of lymph node micrometastases still needs to be confirmed [15]; nevertheless, sentinel node navigation surgery appears to be useful for tailoring treatments for patients with early gastric cancer, even after endoscopic treatments.

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Jikeikai Med J 2009; 56: 11-7

Sentinel Node Navigation Surgery for Early Malignant Tumor of the Duodenum

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ABSTRACT

Introduction: Pancreaticoduodenectomy is a standard operation for duodenal malignant tumor but is associated with a high incidence of postoperative morbidity and impaired quality of life. We report on patients who successfully underwent limited surgery for early duodenal tumors using sentinel node navigation surgery and infrared ray observation.

Methods: Indocyanine green (ICG) was injected submucosally around the tumor through an endoscope. An infrared ray laparoscopy system was used to identify sentinel nodes. These nodes were stained with hematoxylin and eosin intraoperatively or with cytokeratin immunohistochemical staining postoperatively and examined for evidence of metastasis.

Results: In case 1, 1 retropancreatic lymph node (No. 13, Japanese classification) and 1 prepancreatic lymph node (No. 17) were ICG-positive. In case 2, 1 right gastroepiploic lymph nodes (No. 6) and 2 No. 13 lymph nodes were ICG-positive. In case 3, 1 right gastroepiploic lymph node (No. 6) was ICG-positive. These lymph nodes showed no metastasis on frozen-section examination. In case 1, wedge resection of the second part of duodenum and jejunal patch operation were performed, and in cases 2 and 3, wedge resection of the duodenal bulb was performed.

Conclusion: Sentinel lymph nodes in cases of early duodenal cancer could be easily detected with an infrared ray laparoscopy system, which seems to be useful for limited surgery for duodenal malignancy. (Jikeikai Med J 2009; 56: 11-7)

Key words: sentinel node navigation surgery, malignant duodenal tumor

INTRODUCTION

Pancreaticoduodenectomy is a standard operation for duodenal malignant tumor but is associated with a high incidence of postoperative morbidity and impaired quality of life. We report patients who successfully underwent limited surgery for early duodenal tumors using sentinel node navigation surgery (SNNS) with infrared ray observation.

METHODS AND PATIENTS

SNNS with infrared ray observation was performed in 3 patients with early duodenal tumors. Indocyanine green (ICG, 5 mg/ml, Diagnogreen®, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan) was injected submucosally (0.5 ml per injection) with a 23-gauge endoscopic puncture needle through an endoscope at 4 quadrants around the tumor. Because the duodenal wall was thin, physiological saline was injected into the submucosal layer before ICG was injected.

Received for publication, December 11, 2008
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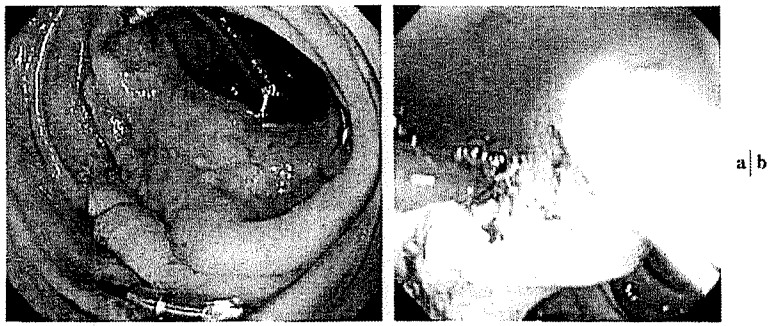


Fig. 1. Endoscopic findings (case 1). a. Early cancer in the second part of the duodenum. b. Intraoperative endoscopy after ICG injection.

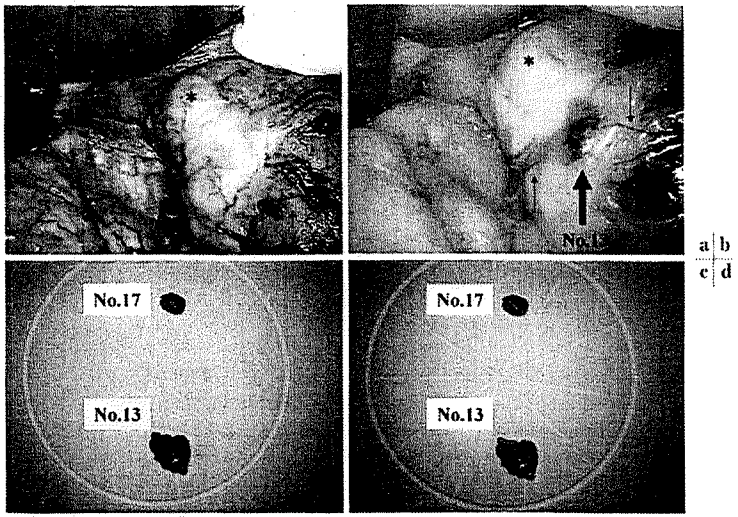


Fig. 2. SNNS (case 1). a. Observation with white light. ICG (+) LNs could not be detected. *; pancreas, †; duodenum (primary lesion). b. Observation with IRLS. ICG (+) lymphatic vessels and LNs were confirmed. Narrow arrows indicate ICG (+) lymphatic vessels. The thick arrow indicates an ICG (+) LN. c. Sentinel LNs. They could not be recognized as green nodes with the naked eye. d. Sentinel nodes. The ICG (+) LNs appeared as black nodes with IRLS.

Twenty minutes after the ICG injection, an infrared ray laparoscopy system (IRLS; Olympus Medical Systems Co., Tokyo, Japan) was used to identify ICG-positive [ICG (+)] lymphatic vessels and LNs through examination of the fatty tissue on the serosa of the duodenum and pancreas. We defined ICG (+) LNs as sentinel nodes. After observation, lymphatic basin dissection was performed. Thereafter, ICG (+) LNs were preserved by freezing, subjected to hematoxylin and eosin staining or with cytokeratin immunohistochemical staining (CAM5.2; BD Biosciences, San Jose, CA, USA), and examined for evidence of metastasis.

Case 1: The patient was a 65-year-old woman with a 50×30-mm tumor of the second part of the duodenum (c0IIa) pathologically diagnosed as well-differentiated adenocarcinoma in adenoma (Fig. 1 and 3).

Case 2: The patient was an 81-year-old woman with a 10-mm-diameter carcinoid of the anterior wall of the duodenal bulb (Fig. 4a). Endoscopic ultrasonography revealed that the carcinoid had reached the submucosa (Fig. 4b).

Case 3: The patient was a 59-year-old man with a 10-mm-diameter carcinoid of the anterior wall of the duodenal bulb. Endoscopic ultrasonography revealed that the carcinoid had reached the submucosa.

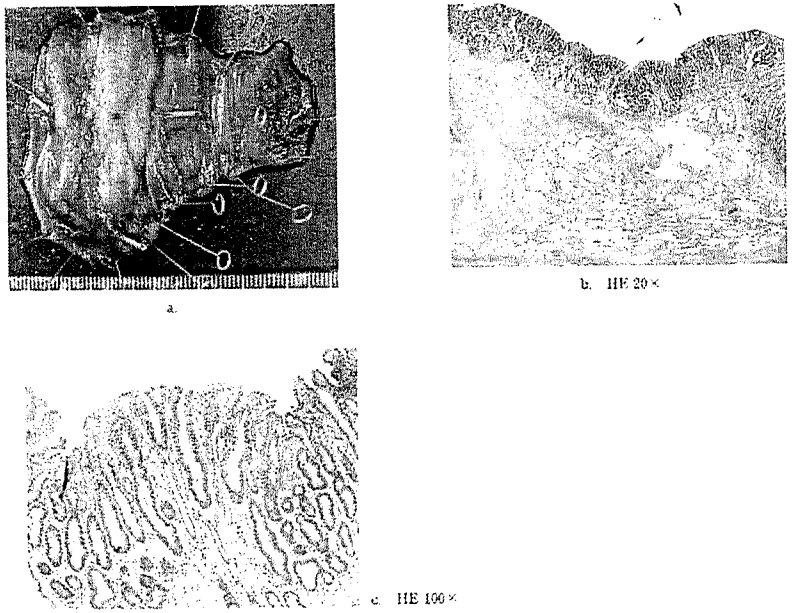


Fig. 3. Surgical specimen and pathological findings (case 1). a. Macroscopic appearance of surgical specimen of a duodenal tumor. b, c. Microscopic examination of the duodenal tumors showed well differentiated adenocarcinoma in adenoma (b: hematoxylin and eosin, 20×; c: hematoxylin and eosin, 100×).

Results

Case 1: One retropancreatic LN (No. 13) and 1 prepancreatic LN (No. 17) were ICG (+) (Fig. 2). Although ICG (+) LNs could not be identified with the naked eye (Fig. 2a), such ICG (+) No. 13 LNs appeared black on IRLS (Fig. 2b). The ICG (+) LNs, which were about 5 mm in diameter, were removed (Fig. 2c, d). Intraoperative frozen-section examination revealed no LN metastasis. Wedge resection of the duodenum and jejunal patch reconstruction were performed. Pathological examination showed that the cancer was confined to the mucosa and measured 43×

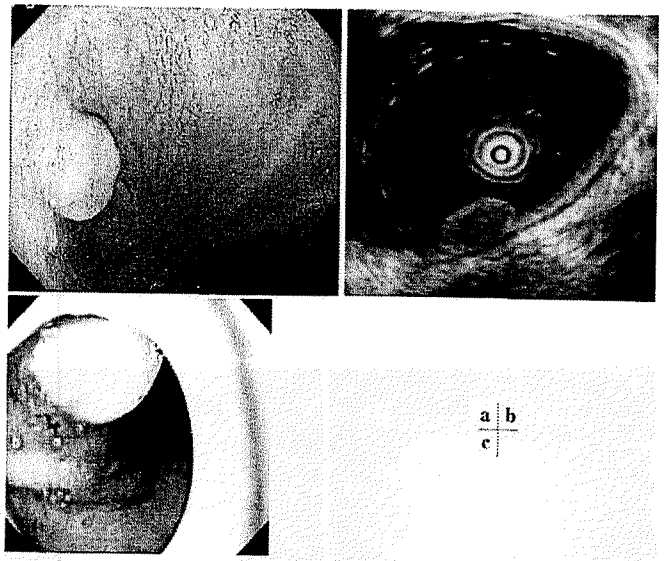


Fig. 4. Endoscopic findings (case 2). a. Duodenal carcinoid in the anterior wall of the bulb. b. Endoscopic ultrasonography. The tumor was confined to the submucosal layer. c. Intraoperative endoscopy after ICG injection.

36 mm (Fig. 3).

Case 2: Although conventional observation failed to identify any ICG (+) LNs (Fig. 5a), 1 ICG (+) right gastroepiploic LN (Fig. 5b) and 2 No. 13 ICG (+) LNs were detected with IRLS and then removed. Intraoperative examination of frozen sections demonstrated no LN metastasis. Wedge resection of the duodenal bulb was performed. Pathological examination showed that the carcinoid was confined to the submucosa and measured 8×7 mm (Fig. 6).

Case 3: One ICG (+) No. 6 LN was detected with IRLS, whereas conventional observation failed to identify any ICG (+) LNs. Intraoperative examination of frozen sections demonstrated no LN metastasis. Wedge resection of the duodenal bulb was performed. Pathological examination showed that the carcinoid was confined to the submucosa and was 5 mm in diameter.

Preoperative abdominal computer tomography of these 3 cases revealed no LNs around the duodenum. The 3 patients are alive without recurrence.

Lymph node (LN) stations were modified from those of the Japanese Classification of Gastric Carcinoma[1].

Discussion

Duodenal tumors are extremely rare in the gastrointestinal tract. Yokoyama et al. have reported that from 1985 through 1991 duodenal adenomas accounted for 0.04% (17 of 39,169 tumors) of gastrointestinal tumors at the National Cancer Center, Japan, and that primary duodenal cancers, excluding cancers of the papilla of Vater, accounted for only 0.01% of tumors (3 of 39,169 tumors)[2]. Primary duodenal carcinoids account for only 2.6% of all carcinoid tumors[3]. Because of the rarity of duodenal cancers and carcinoids, to our knowledge the incidence of LN metastasis of early duodenal cancers has not been reported. A previous report from Japan found that 13% of small (less than 1 cm) duodenal carcinoid tumors are associated with regional LN metastasis[4]. In the United States, Burke et al. have reported that 16 of 77 carcinoid tumors (21%) of the duodenum had LN metastases[5]. Features associated with an increased risk of LN metastasis include involvement of the muscularis propria, size greater than 2 cm, and the presence of mitotic figures. Nevertheless, Mullen et al. have reported that LN metastases were identified in surgical specimen from 7 of 13 patients (54%), including specimens from 2 patients with tumors smaller than 1 cm and confined to the submucosa[6]. Therefore, endoscopic resection alone is inadequate for the treatment of duodenal carcinoids. Moreover, a large-diameter duodenal mucosal cancer, like that in case 1, is difficult to treat with only endoscopic mucosal resection. However, because pancreaticoduodenectomy is major surgery with high rates of morbidity and mortality[6], accurate intraoperative diagnosis of LN metastasis with SNNS would allow a less invasive operation, such as wedge resection of the duodenum.

Since reported by Morton et al. in 1992, the concept of sentinel lymph nodes in melanoma and breast cancer has been validated, and SNNS is now widely performed[7,8]. Recently, several reports of SNNS for gastric cancer and colonic cancer have been published[9-16]. Since May 2000, we have been using our own technique, IRLS, for gastric cancer; we have obtained excellent results and have reported that SNNS with IRLS is useful for limited gastric surgery for early gastric cancer without compromising curability[17,18].

In conclusion, IRLS can be used to detect sentinel LNs in early duodenal malignant tumors, and SNNS is useful for limited duodenal resection.

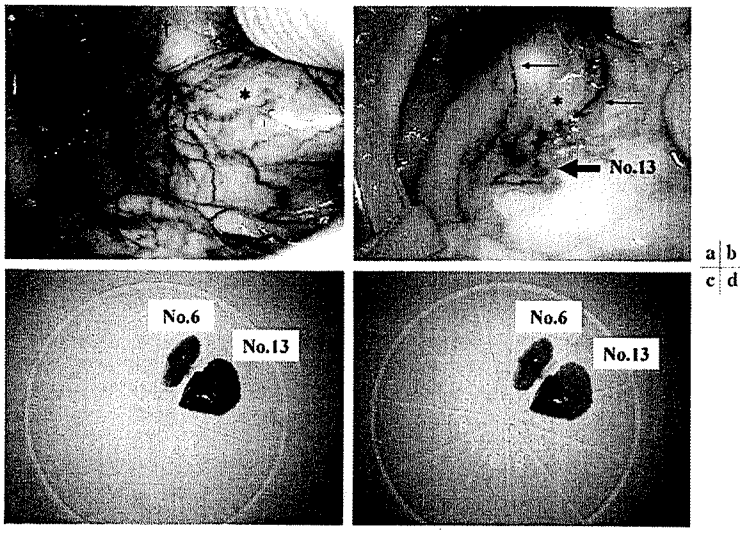
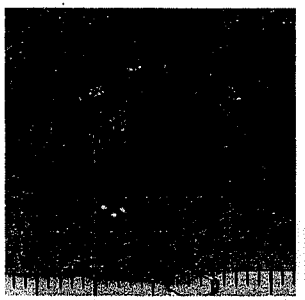
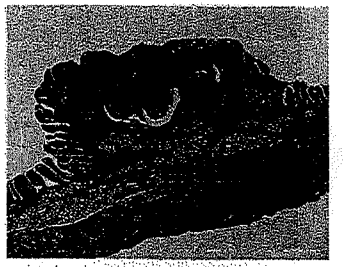


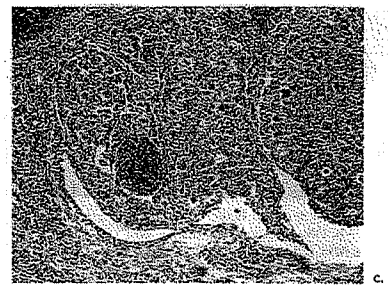
Fig. 5. SNNS (case 2). a. Observation with white light. ICG (+) LNs could not be detected. *, pancreas; †, duodenum (primary lesion). b. Observation with IRLS. ICG (+) lymphatic vessels and LNs could be clearly detected. Narrow arrows indicate ICG (+) lymphatic vessels. The thick arrows indicate ICG (+) LNs. c. Sentinel nodes. These could not be recognized as green nodes with the naked eye. d. Sentinel nodes. These ICG (+) LNs could be recognized as black nodes on infrared ray observation.



a.



b. HE 20×



c. HE 100×

Fig. 6. Surgical specimen and pathological findings. a. Macroscopic appearance of a resected surgical specimen, which contained a yellowish submucosal tumor. b. Microscopic findings of the duodenal carcinoid tumor of the submucosal layer (hematoxylin and eosin, 20×). c. Histological examination showed small, round cells that were characteristic of carcinoid (hematoxylin and eosin, 100×).

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JSAST 手術手技研究会記事

当番世話人
細川　正夫

指定研究発表

座長：夏越　祥次（鹿児島大学腫瘍制御学・消化器外科学）

主題Ⅱ　私の推奨する手術手技1，2

座長：幕内　博康（東海大学外科）
小川　純一（秋田大学呼吸器外科）

2009年5月16日ロイトン札幌にて行われた第63回手術手技研究会より収録。

指定研究発表
「胃癌センチネルリンパ節生検における リンパ管内の癌細胞検出とその臨床的 意義の検討」

竹内裕也（慶應義塾大学一般・消化器外科）：先生方すでにご存じかと思いますが，センチネルリンパ節というのは腫瘍原発巣からリンパ流を受ける最初のリンパ節のことです（**図122**）。すでに乳癌では術中にセンチネルリンパ節生検を行いまして，転移がなければ他の腋窩郭清を完全に省略するという治療が実地臨床として行われています。では，はたして胃癌に対してはどうなのかということで，sentinel node navigation surgery研究会が最近多施設共同研究を行いました。約400例の胃癌センチネルリンパ節生検の検討でセンチネルリンパ節同定率97.5%，転移検出感度93%，センチネルリンパ節を指標としたリンパ節転移正診率は99%ということで，きわめて良好な結果を示しております。

そこで，近い将来，早期胃癌に対してはセンチネルリンパ節生検をまず行って，術中に転移がないことがわかれば，腹腔鏡下にこのような縮小手術を行うことが期待されています（**図123**）。また，一方ではESDという治療が進歩して，ESD＋センチネルリンパ節生検というような新しい取り組みも期待されております。

そこで問題になってくる点が1つあります。乳癌ではセンチネルリンパ節をpick upで，つまりただセンチネルリンパ節だけを生検しておりますが，はたして胃癌でそれはよいことなのでしょうか（**図124**）。乳癌のようにセンチネルリンパ節だけのpick upでは，たとえば偽陰性症例といって，センチネルリンパ節に転移がないけれど，他のリンパ節に微小転移があったりするようなことも，頻度は少ないけれどあると言われております。また，原発巣とセンチネ

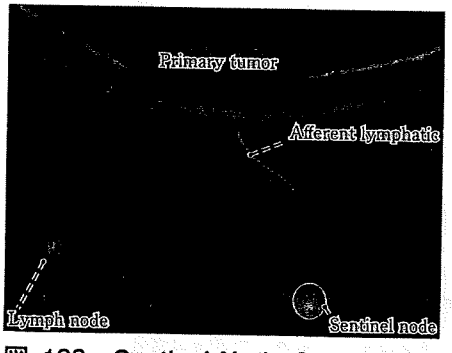

図 122　Sentinel Node Concept

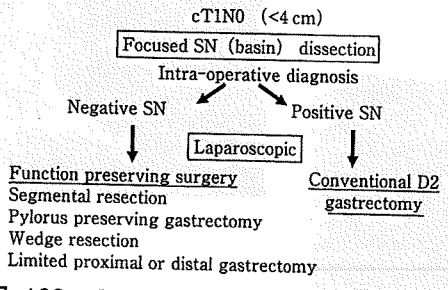

図 123　Current status of SN mapping for gastric cancer

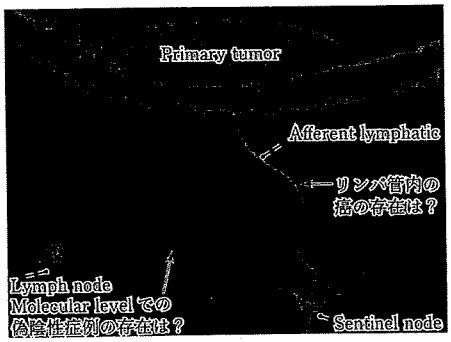

図 124　センチネルリンパ節だけの pick up による転移検索でよいのか

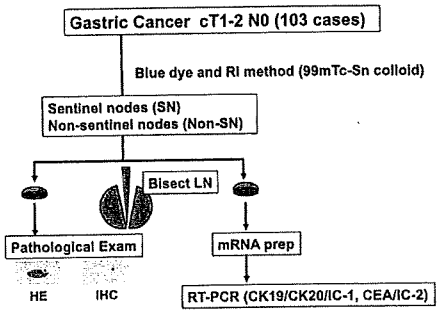

図 126　研究 1　胃癌センチネルリンパ節の微小転移診断

病理学的・分子生物学的手法を用いて①胃癌センチネルリンパ節理論の検証と，②胃癌原発巣からセンチネルリンパ節にいたる一次リンパ管内の癌細胞の存在を探る。

この結果から胃癌センチネルリンパ節生検における pick up 法の可否を検証することを目的とする。

図 125　本研究の主旨

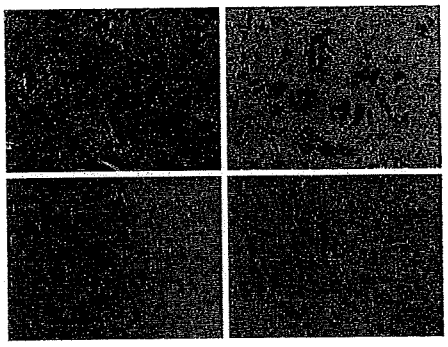

図 127

ルリンパ節を結ぶ一次リンパ管に癌がいてもおかしくないわけです。そのような疑問を持って，この研究を始めることにいたしました。

今回，病理学的，分子生物学的手法を用いて，胃癌センチネルリンパ節理論の検証と，胃癌原発巣からセンチネルリンパ節に至る一次リンパ管内の癌細胞の存在を探ります。この結果から，胃癌センチネルリンパ節生検における pick up 法の可否を検証することを目的としました（**図 125**）。

そのため，「センチネルリンパ節の微小転移診断」と「一次リンパ管内の癌存在診断」の 2 つの研究を行いました。

まず，胃癌センチネルリンパ節の微小転移診断法の開発とセンチネルリンパ節理論の検証です。当院では色素法と RI 法の両方を用いてセンチネルリンパ節を同定しております。その後，通常郭清も行っておりますので，センチネルリンパ節と非センチネルリンパ節をそれぞれ半割して，一方は病理で普通の HE 染色とサイトケラチンによる免染，それからもう半割したものを，メッセンジャー RNA をとって RT-PCR 法を行っております（**図 126**）。

図 127 は通常のセンチネルリンパ節の組織像ですが，このようにマッシブに転移があればサイトケラチン染色をしなくてもわかるのですが，たとえばこのような微小なマイクロメタや ITC と言われているようなものであれば，通常の HE 観察ではなかなか診断はむずかしいということになります。

そこで，私どもは Roche Diagnostics 社と共同開発して，術中診断可能な real time RT-PCR 法を開発しました。マーカーとしては CK19, CK20, CEA を用いております。Sensitivity ではメッセンジャー RNA 10 コピーぐらいで検出できることになっております（**図 128**）。

cT1/T2 N0 103 例の結果です。まず病理組織学的所見と PCR の結果を比較検討しました。病理組織学的に陽性であった 13 例は全例 RT-PCR 陽性でした。一方，病理組織学的に陰性

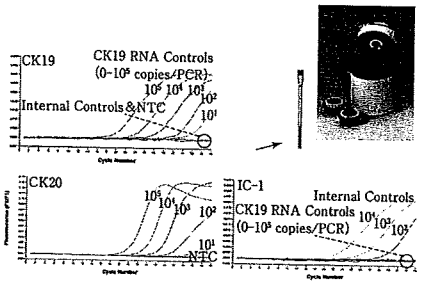

図 128　Real time RT-PCR on LightCycler

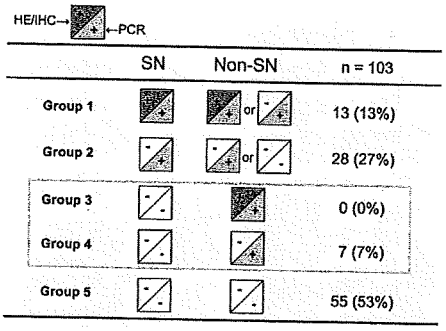

図 130　Results

SN status	RT-PCR +	RT-PCR -
H&E/IHC +	13 (13%)	0 (0%)
H&E/IHC -	28 (27%)	62 (60%)

Sensitivity 100% (13/13)
31% (28/90) upstaged

図 129　Results (n=103)

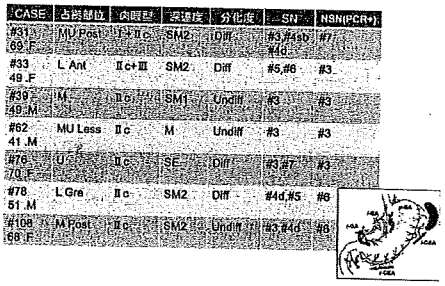

図 131　Molecular level での偽陰性7例
(Kinami et al. Int J Clin Oncol, 2008)

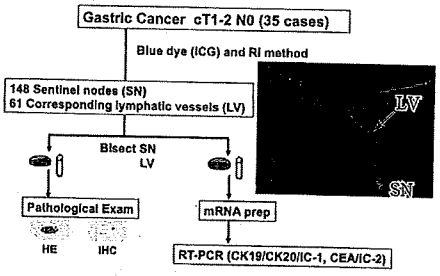

図 132　研究2　一次リンパ管内の癌存在診断

であった90例中28例（約30%）がRT-PCR陽性でした（図129）。

次にセンチネルリンパ節とそれ以外のノンセンチネルリンパ節で，転移状況によってグループ1〜5に分けてみました(図130)。このグループ1，2，5というのがセンチネルリンパ節理論が成り立つグループです。グループ3と4というのはいわゆる偽陰性症例で，すなわちセンチネルリンパ節の転移はないのですが，ノンセンチネルリンパ節に病理組織学的に，あるいはPCRで転移があるというものです。幸い病理組織学的には転移のあるものはなかったのですが，PCRで7例がノンセンチネルリンパ節に転移陽性ということでした。

この7例の内訳を見てみますと，腫瘍が比較的大きなもの，SEの症例，未分化なものなどありましたが，胃のリンパ流域は5領域あると言われていますが，センチネルリンパ節とPCRで陽性だったリンパ節が，7例ともそれぞれ同じリンパ領域に含まれることがわかりました（図131）。

次の研究ですが，一次リンパ管内の癌存在診断です（図132）。cT1/T2N0胃癌で，35例ですが，手術加刀時に術中内視鏡下にICG，色素を注入します。そうするとリンパ管が染め出されて，センチネルリンパ節まで染まってまいります。これをセットに摘出して，リンパ管，センチネルリンパ節，それぞれ半割して，病理学的に，あるいはRTPCRで転移検索を行いました。

図133はリンパ管がよく見えた症例です。たとえば，原発巣がこのあたり（図133矢印）

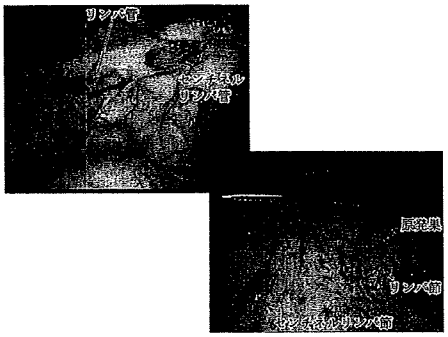

図 133

Parameter	
Gender	
Male/Female	20/15
Median age (yr)	59(27-79)
Tumor location	
Upper/middle/lower	6/26/3
Histologic type	
Differentiated/Undifferentiated	15/20
pT factor	
M/SM/MP/SS	17/13/4/1
ly+/ly-	10/25
v+/v-	5/30

図 135 Clinicopathologic Findings of 35 Patients With cN0 Gastric Cancer

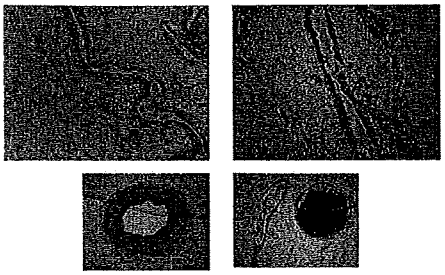

図 134 一次リンパ管

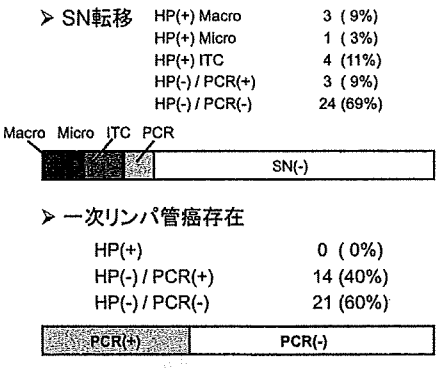

図 136

にあるのですが，リンパ管があってセンチネルリンパ節，ここにも緑色のリンパ管があります（図 133 矢印）。見える症例はあるのですが，見えない症例もあって，全例リンパ管がとれたというわけではありません。病理組織学的に，自分はこれまで太いリンパ管を気にして見たことがなかったのですが，病理の先生に伺うと，壁が比較的薄くて，平滑筋があまりない，蛇行がある，弱々しい弁が随所に見られる，血管ではありませんので，赤血球がないということで，比較的太いリンパ管であれば同定は可能です（図 134）。動脈，静脈との鑑別は容易で，リンパ節のように細胞が詰まっているわけではないので，中の癌細胞の同定は比較的容易ではあるということです。

35 例の内訳をお示しします（図 135）。まずセンチネルリンパ節のほうの結果ですが，病理組織学的にマクロの転移陽性であったものが 3 例（9%）ありました。2 mm 以下のマイクロメタであったのが 1 例（3%），0.2 mm 以下の ITC が 4 例（11%），病理組織学的には検出できなかったのですが，PCR 陽性だったのが 3 例（9%）ありました。残りの 70%がセンチネルリンパ節完全転移陰性ということでした（図 136）。

一方，問題のリンパ管のほうですが，残念ながら，病理組織学的に 1 例も癌細胞を見つけることはできませんでした。一方，PCR で 40%の症例が陽性，60%が陰性でした。

そこで，一次リンパ管の PCR 陽性と既存の病理組織学的背景と何か相関がないかということで調べてみたのですが，いくつか面白いことがわかりました。

まず，センチネルリンパ節転移状況との相関を見てみますと，センチネルリンパ節転移があった 11 例中 10 例が一次リンパ管も PCR 陽性でした（図 137）。これは当たり前といえば当たり前かもしれません。リンパ管の先にセンチネルリンパ節があるわけですから，センチネ

n＝35		一次リンパ管	
		-	+
SN	-	20（83%）	4（17%）
	+	1（9%）	10（91%）

P＜0.0001

図 137　SN転移状況と一次リンパ管内癌存在は有意に相関する

n＝35		一次リンパ管	
		-	+
原発巣分化度	分化型	12（80%）	3（20%）
	未分化型	9（45%）	11（55%）

P＝0.046

図 138　原発巣分化度と一次リンパ管内癌存在は有意に相関する

n＝35		一次リンパ管	
		-	+
ly	-	18（72%）	7（28%）
	+	3（30%）	7（70%）

P＝0.05

図 139　原発巣ly因子と一次リンパ管内癌存在は有意に相関する

CASE	肉眼形	深達度	分化度	ly	v	腫瘍径
59 M	M Less	0 IIc	T1(M)	Diff	0	0	4.0
62 M	M Gre	0 IIc	T1(SM1)	Undiff	0	0	3.8
63 M	M Less	0 IIc	T1(SM2)	Diff	1	0	2.0
46 F	M Gre	0 IIc	T2(MP)	Diff	1	1	2.0

ly0　20例中2例（10%）が一次リンパ管陽性
ly1　4例中2例（50%）が一次リンパ管陽性
P=0.11

図 140　SN陰性/一次リンパ管陽性4例（17%）

⇒Molecular levelでもSNコンセプトは成立するが偽陰性例は存在する
⇒偽陰性例におけるPCR陽性リンパ節はSNと同じbasin内に含まれる可能性が高い
⇒SN転移陽性と一次リンパ管陽性は有意に相関する
⇒未分化型癌やly陽性と一次リンパ管陽性は有意に相関する
⇒SN陰性例でも一次リンパ管陽性例が約20%存在する可能性がある。とくにly陽性例は注意が必要である。

図 141　まとめ

ルリンパ節に転移があれば，その手前のリンパ管に癌細胞がいてもおかしくはないということです。一方，センチネルリンパ節が転移陰性であった24例中4例（17%）でPCR陽性だったのです。

その他，原発巣の分化度で見てみますと，分化型，未分化型で分けてみますと，未分化型で一次リンパ管PCR陽性の割合が有意に高いということがわかりました（**図138**）。また，原発巣のly因子で見てみますと，原発巣でly陽性だと，PCR陽性の割合が有意に高いことがわかりました（**図139**）。

さきほどのセンチネルリンパ節転移陰性24例中，一次リンパ管が陽性だったのが4例（17%）ありましたが，その内訳です（**図140**）。なにか特徴がないかと調べたのですが，ly陰性であった20例中，一次リンパ管陽性だったのは2例（10%）のみだったのですが，ly陽性例4例中2例が一次リンパ管陽性でした。これは有意差はないのですが，やはりセンチネルリンパ節が転移陰性であったとしても，原発巣のly因子が陽性である場合は，その先の一次リンパ管にも癌がいる可能性が高く注意が必要なことがわかりました。

以上まとめますと，PCRの検討でも胃癌センチネルリンパ節理論は成立しますが，数は少ないのですが偽陰性症例は存在していました。偽陰性例におけるPCR陽性リンパ節はセンチネルリンパ節と同じリンパ流域に含まれる可能性が高いことがわかりました（**図141**）。

センチネルリンパ節転移陽性と一次リンパ管

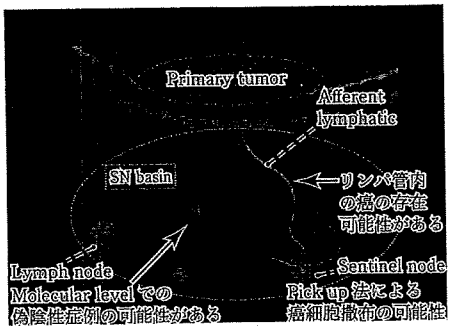

図 142　センチネルリンパ節だけの pick up による転移検索でよいのか

陽性は有意に相関していました。また，未分化型や ly 陽性と一次リンパ管陽性は相関していました。

センチネルリンパ節転移陰性例でも一次リンパ管陽性例が約 2 割存在しておりました。とくに ly 陽性例は注意が必要であると考えられました。

最初のクリニカルクエスチョンである，乳癌のようなセンチネルリンパ節だけの pick up による転移検索でよいのかという疑問に対しては，やはり偽陰性症例の可能性が少数ですがあるということや，リンパ管内の癌の存在の可能性が示唆され，とくにセンチネルリンパ節が転移陰性であっても，一次リンパ管の中に癌がいる可能性があります。もしそのような状況で，pick up 法によるセンチネルリンパ節生検を行った場合，リンパ管断端から癌細胞が撒布される可能性も指摘されます。たとえば乳癌のような悪性度が比較的高くないものであれば，臨床的には意味がないかもしれませんが，ことに消化器癌，胃癌のような場合は，現時点ではセンチネルリンパ節を含むリンパ流域をまず *en bloc* に切除して，バックテーブルでセンチネルリンパ節を同定して転移検索をするのが安全なのではないかというのが私の結論です（**図 142**）。ご清聴ありがとうございました。

夏越祥次（司会）：竹内先生ありがとうございました。私たちも，血中の遊離癌細胞がかなり早い時期から出ているものですから，リンパ管にもおそらくあるのだろうと思っておりましたが，先生にそれを明快に説明していただけました。今後はリンパ管の中の癌細胞が着床していくかどうかということも含めて，またご報告いただきたいと思います。本日はおめでとうございます。ありがとうございました。

主題Ⅱ-1
食道癌（開胸）—開胸食道癌手術におけるクリティカルポイント—〜手術野の展開 / 局所解剖の認識 / 血管・神経の温存〜

梶山美明（順天堂大学上部消化管外科学　教授）：食道癌手術が困難な理由には，この 3 つが主に考えられると思います（**図 143**）。手術野を得ることが困難である。局所解剖の認識が困難である。合併症が多い。これらに対してそれぞれ，手術野の展開，局所解剖の認識，血管神経の温存ということからお話しさせていただいて，10 分のビデオを提示させていただきます。

今回のテーマは「私の推奨する手術手技」ということですが，私自身が皆様に誇れるような手術ではございません。秋山先生，鶴丸先生から教えていただき，私なりに解釈した結果を本日提示させていただきたいと思います。

観念的になりますが，どんな手術も基本手技を着実に積み重ねていくということがコツである。あえて言うならば，そのように考えております。とくに食道癌手術では忍耐と冷静さが必要になります。1 つ 1 つ積み上げて，その集大成として手術が完成する。1 つ 1 つの基本手技が，どの 1 つのエレメントが崩れても手術は成

1. 良好な手術野を得ることが困難
　→「手術野の展開」
2. 局所解剖の認識が困難
　→「局所解剖の認識」
3. 合併症が多い
　→「血管・神経の温存」

図 143　食道癌手術が困難な理由は？

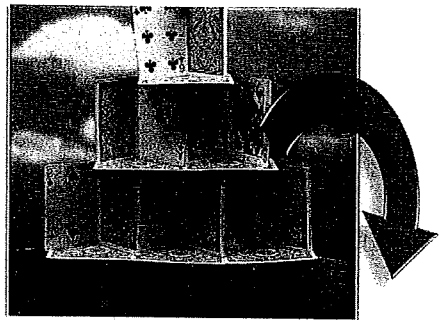

図 144　“手術は一つの作品であり，基本手技の集大成である”

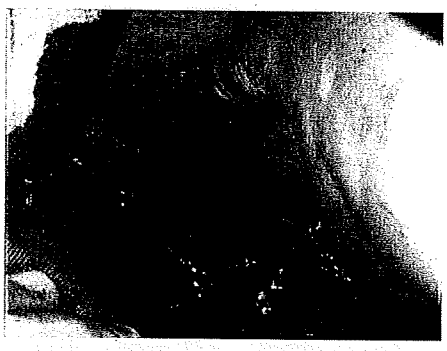

図 146　奥を広げる手術野の展開

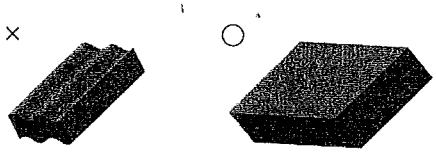

図 148　手術面の創出
“「線」ではなく「面」を創る”
＝どの方向にも等しい力で牽引する
力のひずみを生じさせない→損傷の防止

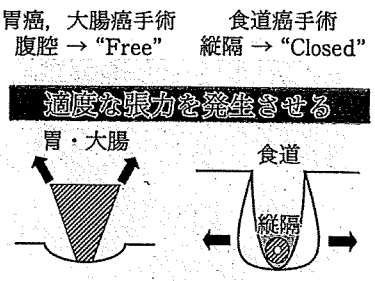

図 145　手術野の展開

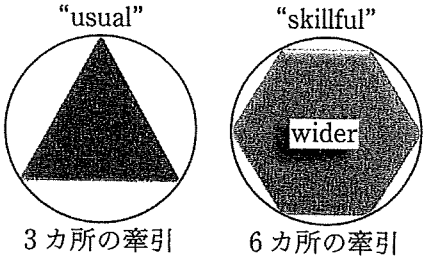

図 147　手術野の創出

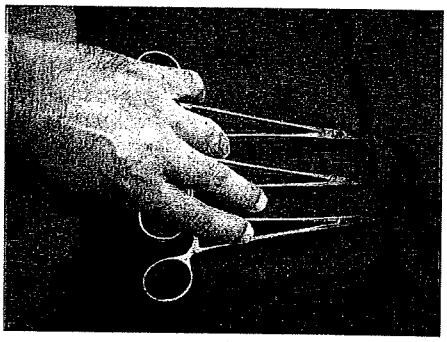

図 149　複数の鉗子をコントロールする

立しないと考えております（図 144）。

最初のテーマである「手術野の展開」ということですが，これは胃癌，大腸癌手術ではオープンな腹腔というスペースに対して，食道癌手術ではクローズドの縦隔という，非常に奥深いところの術野になります。したがって，すべての手術で共通なこととして，適度な張力を発生させて術野を作るということが大事ですが，とくに食道では，奥深くをいかに広げるかという術野の展開の工夫が必要になってまいります。胃癌，大腸癌などオープンの腹腔の手術では，手前に引き上げることによって術野を展開できますが，食道癌では，いかに奥を広げるかということが大事になるのです（図 145，146）。

あとは牽引の場所，数をできるだけ多くする（図 147）。それによって手術野の面積を広くできる。また，その手術野を展開する際には，このように線ができるような術野ではなく，きれいに，どの方向にも等しい力で牽引する（図 148），面を作ることが，オープンであろうが鏡視下手術であろうが，同じ重要なテーマであろうと思われます。

このためには，たとえば1つの手で複数の鉗子をコントロールするということも重要な基本手技の1つであると考えております（図 149）。

CCR7 and CXCR4 expression predicts lymph node status including micrometastasis in gastric cancer

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Received March 6, 2009; Accepted May 4, 2009

DOI: 10.3892/ijo_00000308

Abstract. The chemokine receptors CCR7 and CXCR4 play a major role in the mechanism of lymph node metastasis from primary tumor cells. We postulated that their expression in gastric tumor cells could predict lymph node status including lymph node micrometastasis (LNMM). We assessed CCR7 and CXCR4 expression in 93 resected gastric tumor specimens by immunohistochemistry. Dissected lymph nodes were examined by reverse transcription-polymerase chain reaction and immunohistochemistry using cytokeratin monoclonal antibody to detect LNMM in addition to hematoxylin-eosin (H&E) staining. Levels of CCR7 and CXCR4 expression were high in 26.9% (25/93) and in 32.3% (30/93), respectively of tumor cells and the levels significantly correlated with lymph node metastasis according to H&E staining (P=0.0212 and P=0.0115, respectively). We identified LNMM in 25 of 83 (30.1%) node-negative patients. Both CCR7 and CXCR4 expression significantly correlated with lymph node status including LNMM (P=0.0092 and P=0.0075, respectively). Furthermore, levels of combined CCR7 and CXCR4 expression significantly correlated with lymph node metastatic status (P=0.0021). Assessment of CCR7 and CXCR4 expression in gastric cancer is a useful tool for predicting lymph node metastatic status including LNMM.

Introduction

Chemokines are small secreted proteins that presently comprise subfamilies C, CC, CXC, and CX3C based on the arrangement of their cysteine residues in the NH_2-terminal (1). These chemokines act through their G-protein-linked receptors on target cells (2). Many chemokine receptors have been identified and their activation regulates cytoskeletal rearrangement, adhesion, and directional migration (3,4). Recent studies have demonstrated that chemokines and their receptors principally function as a signaling pathway in leukocyte trafficking and lymphocyte homing (5,6). Furthermore, these signaling pathways play an important role in tumor progression (7). Müller *et al* (8) reported that at least the chemokine receptors CCR7 and CXCR4 are highly expressed in human breast cancer cell lines and primary breast tumors and that lymph nodes, which are representative secondary metastatic sites of breast cancer, highly express their ligands CCL21 and CXCL12. These results indicate that CCR7 and CXCR4 expressed by breast tumor cells play a major role in the mechanism of lymph node metastasis from primary tumor cells.

Lymph nodes are the most common metastatic sites and nodal metastasis is recognized as an important prognostic factor in gastric cancer (9-11). Therefore, patients with lymph node metastasis have a poor prognosis, despite complete resection (R0). On the other hand, endoscopic mucosal resection (EMR) and endoscopic sub-mucosal dissection (ESD) without lymphadenectomy have been widely applied in Japan to treat mucosal gastric cancer (12-14). However, lymph node metastasis in mucosal and submucosal gastric cancer is pathologically detectable in 2-4 and 13-20%, respectively (15-19).

We previously reported that lymph node micrometastasis (LNMM) can be identified by immunohistochemistry (IHC) and by reverse transcription-polymerase chain reaction (RT-PCR) assays in patients with gastric cancer who are pathologically node-negative (pN0) according to conventional hematoxylin-eosin (H&E) staining (20,21). The clinical significance of LNMM in gastric cancer is controversial (22). However, 92% of gastric tumor cells within LNMM are Ki-67 positive (23), indicating the potential proliferative activity of LNMM in gastric cancer. Accordingly, part of the strategic surgical approach to treating gastric cancer is to detect lymph node metastasis including LNMM. However, to preoperatively assess LNMM using methods such as computed tomography and ultrasound is difficult. To date, no reports have revealed the relationship between LNMM and CCR7 and CXCR4 expression in gastric cancer. Additionally, no better biomarkers

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Key words: CCR7, CXCR4, lymph node micrometastasis, gastric cancer

for predicting lymph node status including LNMM have been identified in gastric cancer.

The present study investigates CCR7 and CXCR4 expression in gastric tumors and examines the relationship between such expression and lymph node status including LNMM.

Materials and methods

Gastric cancer cell lines. We constructed standard curves for RT-PCR assays using the gastric cancer cell line, MKN-45, which was cultured in RPMI-1640 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal calf serum (Mitsubishi Kasei, Tokyo, Japan) and 100 U/ml each of penicillin and streptomycin at 37°C in a humidified 5% CO_2 atmosphere as described (20,21).

Patients. We enrolled 93 patients (65 men and 28 women; age range, 41-84 years; average 64 years) with gastric cancer who underwent curative gastrectomy with lymphadenectomy at Kagoshima University Hospital between 2003 and 2005. Patients who had undergone preoperative radiation therapy or chemotherapy were excluded from the study. Tumors were classified and staged based on the Japanese classification of gastric carcinoma (24). Fourteen, 51 and 28 tumors were located in the upper, middle and lower thirds of the stomach, respectively. Eighty-one and 12 patients had T1 (invasion of mucosa or submucosa) and T2 (invasion of muscularis propria or subserosa)/T3 (penetration of serosa) tumors, respectively, that were histopathologically classified as differentiated (n=55; papillary, well and moderately differentiated tubular adenocarcinomas) and undifferentiated (n=38; poorly differentiated adenocarcinoma, mucinous adenocarcinoma, and signet-ring cell carcinoma). Paraffin-embedded archival tissue (PEAT) specimens obtained from these resected primary tumors were histopathologically confirmed by a surgical pathologist. All specimens were collected from the patients after informed consent had been obtained in accordance with the institutional guidelines of our hospital.

Lymph nodes. We examined 2,415 lymph nodes from 93 patients with gastric cancer (range 2-69 nodes; average 26 nodes). The negative controls for LNMM for our RT-PCR assays comprised 30 normal lymph nodes from 14 patients without cancer (gall bladder stone, n=6; gastric adenoma, n=4; gastric ulcer, n=3; Crohn's disease, n=1). The lymph nodes were cut into 2 blocks at the plane of the largest dimension. One block was suspended in 1 ml of Isogen (Nippon Gene, Toyama, Japan) and immediately stored at -80°C. The other block was fixed in 10% formaldehyde, embedded in paraffin, and then cut into 3 μm sections for H&E staining and IHC using a monoclonal antibody (mAb) to cytokeratin (CK).

RNA extraction. Thawed lymph nodes were homogenized in FastPrep (Qbiogene, Inc., Carlsbad, CA, USA) and then total RNA was extracted, isolated and purified using phenol-chloroform as described (20,21). The concentration and purity of the total RNA were determined using a GeneQuant pro UV/Vis spectrophotometer (Amersham Pharmacia Biotech, Cambridge, UK).

Primers and probes. Primer and probe sequences of carcinoembryonic antigen (CEA) and glyceraldehyde-3-phosphatase dehydrogenase (GAPDH) were designed for RT-PCR assays as described (20,21). The forward primers, donor and acceptor probe sequences, and reverse primers for CEA and GAPDH were as follows: CEA (forward), 5'-TGTCGGCATCATGAT TGG-3'; (donor and acceptor), 5'-CCTGAAATGAAGAA ACTACACCAGGGC-3'-fluorescein and 5'-LC-Red640-GCTATATCAGAGCAACCCCAACCAGC-3'-phosphorylation; (reverse), 5'-GCAAATGCTTTAAGGAAGAAGC-3'; GAPDH (forward), 5'-TGAACGGGAAGCTCACTGG-3'; (donor and acceptor), 5'-TCAACAGCGACACCCACTCCT-3'-fluorescein and 5'-LC-Red640-CACCTTTGACGCTGGG GCT-3'-phosphorylation; (reverse), 5'-TCCACCACCCTGT TGCTGTA-3'. The integrity of the RNA was confirmed by RT-PCR assays using GAPDH.

RT-PCR assay. Contamination with genomic DNA was avoided using DNase-I (Invitrogen, Life Technologies, Foster City, CA, USA) and complementary DNA (cDNA) was synthesized using the Advantage RT-for-PCR kit (Clontech Laboratories, Inc., Palo Alto, CA, USA) as described (20,21). All RT-PCR assays were performed using the LightCycler system (Roche Diagnostics, Mannheim, Germany). The reaction mixtures contained cDNA, primers, fluorescent and LC-Red probes, $MgCl_2$, LightCycler FastStart DNA Master hybridization probes (Roche) and anti-Taq DNA polymerase antibody (TaqStart antibody, Clontech Laboratories). The amplification profile consisted of 95°C for 10 min followed by 35 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 15 sec, and extension at 72°C for 5 sec. All primers and probes were synthesized and purified by reverse-phase high-performance liquid chromatography and the optimal reagent concentrations and PCR cycling conditions were established at the Nihon Gene Research Laboratories (Sendai, Japan). Standard curves for each assay were generated using a threshold cycle of serially diluted MKN-45 cells as described (20,21). Quantitative data were analyzed using LightCycler software (Roche). All RT-PCR assays included positive (gastric cancer cell line), negative (normal lymph nodes from patients without cancer) and reagent controls (reagents without cDNA). Our RT-PCR assay system was optimized and established for detecting LNMM as described (20,21).

Immunohistochemical staining. We assessed LNMM in all dissected lymph nodes by IHC staining using a CK AE1/AE3 mAb (Dako Corp., Carpinteria, CA, USA) as described (20,23). The PEAT sections were deparaffinized in xylene and rehydrated in ethanol, and then endogenous peroxidase activity was blocked by 5-min incubation in methanol containing 3% hydrogen peroxide. The sections were then immersed in proteinase K (Dako Corp.) to activate the antigen and incubated with CK mAb diluted 1:200 for 30 min. The sections were washed with phosphate-buffered saline (PBS) and CK was stained using the ABC method (Vectastain ABC kit, Vector Laboratories, Inc., Burlingame, CA, USA) (25) and visualized using diaminobenzidine tetrahydrochloride (DAB). The negative control sections were processed identically but without the primary antibody. The positive controls were PEAT

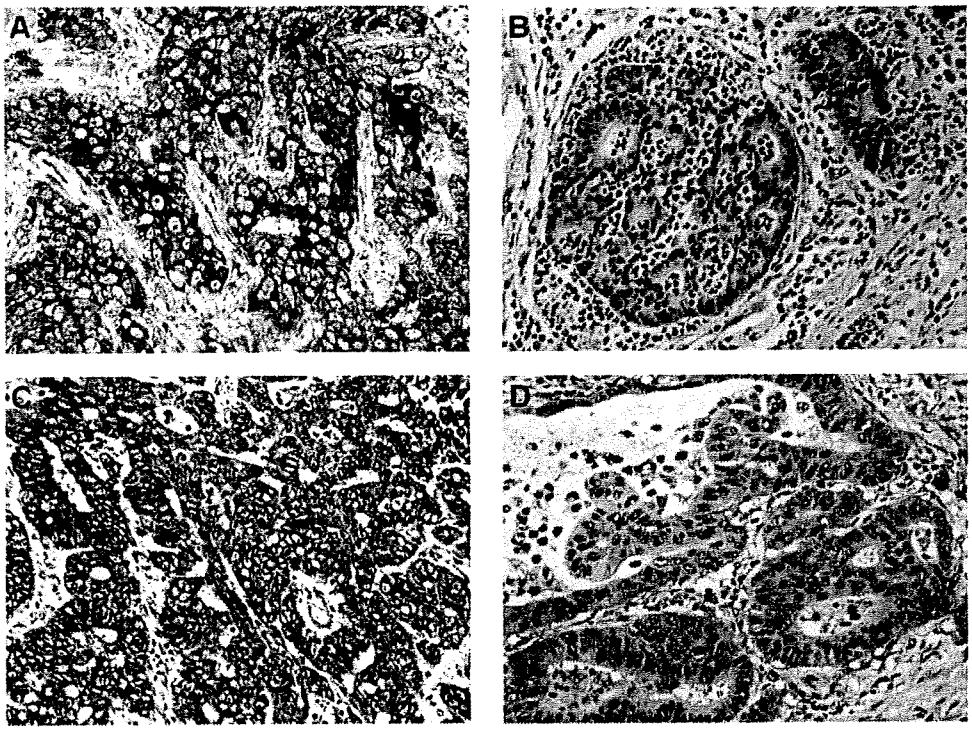

Figure 1. Representative IHC of CCR7 and CXCR4 expression in gastric cancer tissues. Tumor cells with: (A), high; and (B), low expression of CCR7; and (C), high; and (D), low expression of CXCR4. Original magnification x400.

sections of normal gastric mucosa that were consistently positive for CK.

The PEAT sections (3-μm thick) with resected primary tumors were incubated on slides at 50°C overnight, deparaffinized with xylene, and then rehydrated with a graded series of ethanol. The sections were immersed in methanol containing 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase, washed three times for 5 min each with PBS, and then non-specific binding was blocked with 1% bovine serum albumin in PBS at room temperature for 30 min. The sections were incubated at 4°C overnight with anti-CCR7 (BD Biosciences, San Jose, CA, USA) and CXCR4 mAbs (R&D Systems, Minneapolis, MN, USA) diluted 1:100 in PBS. After three 5-min washes in PBS, the reactions for CCR7 and CXCR4 were developed using the ABC method (Vectastain ABC kit, Vector Laboratories) (25) and visualized using DAB. Negative controls were treated with PBS without primary antibodies under the same conditions.

Two independent investigators (T. Arigami and S. Natsugoe) blinded to the clinicopathological data of the patients evaluated the IHC staining for CCR7 and CXCR4. High expression was defined as the presence of CCR7 and CXCR4 immunoreactivity in over 30% of the cancer cells (26,27). Expression of CCR7 and CXCR4 was evaluated in 10 fields each containing 100 cells using light microscopy (magnification x200).

Statistical analysis. Data were statistically compared using the χ^2 and Fisher's exact tests. All statistical calculations were performed using SAS statistical software (SAS Institute Inc., Cary, NC). A $P<0.05$ was considered statistically significant.

Results

CCR7 and CXCR4 expression in gastric tumors. Both CCR7 and CXCR4 were expressed in the cell membrane and/or cytoplasm of gastric tumors. CCR7 and CXCR4 were highly expressed in 25 (26.9%) and in 30 (32.3%) of 93 patients with gastric cancer, respectively (Fig. 1).

Correlation between CCR7 and CXCR4 expression and clinicopathological factors. CCR7 expression significantly correlated with depth of tumor, lymphatic and venous invasion, and lymph node metastasis (P=0.0024, 0.0004, <0.0001 and 0.0212, respectively), and the CXCR4 expression significantly correlated with histological type, tumor size, lymphatic and venous invasion, and lymph node metastasis (P=0.0238, 0.0162, 0.0009, 0.0005 and 0.0115, respectively; Table I).

Correlation between CCR7 and CXCR4 expression and lymph node status including LNMM. We used immunohistochemical staining with CK mAb and RT-PCR to assess LNMM in lymph nodes diagnosed as pN0 by H&E staining. We detected LNMM in 25 of 83 patients (30.1%) with such pN0 gastric cancers.

To assess the relationship between CCR7 and CXCR4 expression and lymph node status including LNMM, we classified the patients according to LNMM status as node-negative/LNMM-negative, node-negative/LNMM-positive and node-positive (Table II). Lymph node metastatic status including LNMM was significantly higher among patients with high levels, than with low levels of CCR7 and CXCR4 expression (P=0.0092 and 0.0075, respectively).

Table I. Relationship between CCR7/CXCR4 expressions and clinicopathological factors in 93 patients with gastric cancer.

	CCR7 expression			CXCR4 expression		
Clinicopathological factors	Low n=68 (%)	High n=25 (%)	P-value	Low n=63 (%)	High n=30 (%)	P-value
Gender						
Male	48 (73.8)	17 (26.2)	0.8039	44 (67.7)	21 (32.3)	>0.9999
Female	20 (71.4)	8 (28.6)		19 (67.9)	9 (32.1)	
Tumor location						
Upper	9 (64.3)	5 (35.7)	0.6415	9 (64.3)	5 (35.7)	0.2746
Middle	39 (76.5)	12 (23.5)		38 (74.5)	13 (25.5)	
Lower	20 (71.4)	8 (28.6)		16 (57.1)	12 (42.9)	
Histological type						
Differentiated	36 (65.5)	19 (34.5)	0.0578	32 (58.2)	23 (41.8)	0.0238
Undifferentiated	32 (84.2)	6 (15.8)		31 (81.6)	7 (18.4)	
Depth of tumor invasion						
pT1	64 (79.0)	17 (21.0)	0.0024	58 (71.6)	23 (28.4)	0.0506
pT2-pT3	4 (33.3)	8 (66.7)		5 (41.7)	7 (58.3)	
Tumor size						
<30 mm	34 (75.6)	11 (24.4)	0.6463	36 (80.0)	9 (20.0)	0.0162
≥30 mm	34 (70.8)	14 (29.2)		27 (56.2)	21 (43.8)	
Lymphatic invasion						
Negative	60 (82.2)	13 (17.8)	0.0004	56 (76.7)	17 (23.3)	0.0009
Positive	8 (40.0)	12 (60.0)		7 (35.0)	13 (65.0)	
Venous invasion						
Negative	64 (82.1)	14 (17.9)	<0.0001	59 (75.6)	19 (24.4)	0.0005
Positive	4 (26.7)	11 (73.3)		4 (26.7)	11 (73.3)	
Lymph node metastasis[a]						
Negative	64 (77.1)	19 (22.9)	0.0212	60 (72.3)	23 (27.7)	0.0115
Positive	4 (40.0)	6 (60.0)		3 (30.0)	7 (70.0)	

pT1, invasion of mucosa or submucosa; pT2, invasion of muscularis propria or subserosa; pT3, penetration of serosa. [a]Lymph node metastasis was identified based on hematoxylin-eosin staining.

Table II. Relationship between CCR7/CXCR4 expressions and lymph node status.

	pN(-)/LNMM(-) n=58	pN(-)/LNMM(+) n=25	pN(+) n=10	P-value
CCR7 expression				
Low (n=68)	48 (82.8)	16 (64.0)	4 (40.0)	0.0092
High (n=25)	10 (17.2)	9 (36.0)	6 (60.0)	
CXCR4 expression				
Low (n=63)	45 (77.6)	15 (60.0)	3 (30.0)	0.0075
High (n=30)	13 (22.4)	10 (40.0)	7 (70.0)	

LNMM, lymph node micrometastasis.

Table III. Relationship between CCR7 and CXCR4 expression.

	CXCR4 expression (%)		
CCR7 expression	Low (n=63)	High (n=30)	P-value
Low (n=68)	55 (59.1)	13 (14.0)	<0.0001
High (n=25)	8 (8.6)	17 (18.3)	

Relevance of CCR7 and CXCR4 expression in predicting lymph node status. Expression of CCR7 and CXCR4 were significantly correlated (P<0.0001; Table III). Based on the status of CCR7 and CXCR4, all patients were assigned to groups with low, intermediate or high expression (low expression of both CCR7 and CXCR4, low expression of either CCR7 or CXCR4 and high expression of both CCR7

Table IV. Relationship between lymph node status and expression patterns of CCR7 and CXCR4.

Expression patterns of CCR7 and CXCR4	pN(-)/LNMM(-) n=58	pN(-)/LNMM(+) n=25	pN(+) n=10	P-value
Low (n=55)	40 (69.0)	12 (48.0)	3 (30.0)	0.0021
Intermediate (n=21)	13 (22.4)	7 (28.0)	1 (10.0)	
High (n=17)	5 (8.6)	6 (24.0)	6 (60.0)	

LNMM, lymph node micrometastasis.

and CXCR4, respectively; Table IV). We found that lymph node metastatic status including LNMM was significantly higher among patients in the group with high levels, than with low levels of CCR7 and CXCR4 expression (P=0.0021).

Discussion

The chemokine receptors CCR7 and CXCR4 are expressed in tumor cells of breast cancer and malignant melanoma, as well as pancreatic, colorectal and esophageal and gastric cancers (8,28-33). The CCL21 and CXCL12 ligands for CCR7 and CXCR4 are abundant in lymph nodes (8). Wiley *et al* (34) reported that CCR7 expression enhances the metastasis of murine melanoma cells to draining lymph nodes in mice. Furthermore, Müller *et al* (8) reported that injection of an anti-CXCR4 antibody significantly reduced the metastasis of breast cancer cells to regional lymph nodes in immunodeficient mice. Thus, the CCR7 and CXCR4 signaling pathway might play crucial roles in the mechanism of lymph node metastasis from primary tumor cells. Therefore, we examined the correlation between CCR7 and CXCR4 expression and lymph node status.

We initially investigated CCR7 and CXCR4 expression in PEAT sections of primary gastric tumors and the relationships with clinicopathological factors. We found that CCR7 and CXCR4 expression in gastric tumor cells visualized and identified by IHC correlated with depth of tumor invasion, lymphatic invasion, venous invasion and lymph node metastasis determined by H&E staining. These findings indicated a close relationship between tumor progression and expression of both CCR7 and CXCR4.

We previously demonstrated the importance of LNMM when selecting therapeutic strategies for patients with gastric cancer (20,21,35). Therefore, lymph node metastatic status, including LNMM should be predicted. We postulated that CCR7 and CXCR4 expression correlated with lymph node metastatic status including LNMM in gastric cancer. Our findings showed that CCR7 and CXCR4 expression significantly correlated with lymph node status including LNMM. We then examined whether CCR7 and CXCR4 expression could predict lymph node status. We demonstrated that lymph node status, including LNMM, was more closely correlated with expression of both CCR7 and CXCR4 than with either alone. Assessment of CCR7 and CXCR4 expression in preoperative biopsy specimens might thus yield valuable information for predicting preoperative lymph node status including LNMM.

An antagonist of CXCR4 can suppress tumor migration, invasion, and lung metastasis in an animal model (36-40). Several CXCR4 antagonists, such as AMD3100, 4F-benzoyl-TE14011 and TN14003, are currently available (36-40). We showed here that expression of CCR7 and CXCR4 in gastric tumors significantly correlated with lymph node metastatic status, lymphatic and venous invasion. Therefore, CCR7 and CXCR4 antagonists might represent novel therapeutic agents that could regulate distant metastases, including those to lymph nodes in patients with advanced gastric cancer.

In conclusion, we demonstrated that CCR7 and CXCR4 are both expressed in gastric tumor cells and that their expression correlates with tumor progression and lymph node metastatic status including LNMM. Therefore, CCR7 and CXCR4 are potential markers for predicting lymph node metastatic status in patients with gastric cancer. The evaluation of CCR7 and CXCR4 expression might also serve as a useful means of predicting the presence or absence of LNMM in patients with early gastric cancer who will undergo less invasive surgery such as EMR and ESD. Furthermore, future studies on biological behavior of the gastric tumor cells expressing CCR7 and CXCR4 may allow the development of new immunotherapy inhibiting these signaling pathways for patients with gastric cancer.

Acknowledgements

This study was supported in part by grants-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture, Japan.

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