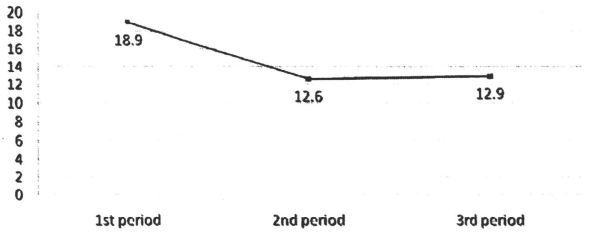

Fig. 1. Learning curve of procedure time per unit area of specimen (min/cm^2).

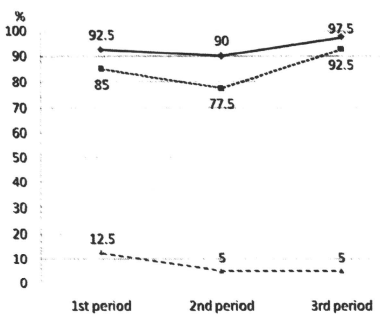

Fig. 2. Learning curve of the (◆) en-bloc resection rate, (■) en-bloc and R0 resection rate and (▲) perforation rate.

Fig. 2. The en-bloc resection rates of the 1st, 2nd and 3rd periods were 92.5% (37/40), 90% (36/40) and 97.5% (39/40), respectively. The en-bloc and R0 resection rates of the 1st, 2nd and 3rd periods were 85% (34/40), 77.5% (31/40) and 92.5% (37/40), respectively. The rates of finding of positive lateral margins for the 1st, 2nd and 3rd periods were 12.5% (5/40), 20% (10/40) and 7.5% (3/40), respectively. The perforation rates for the 1st, 2nd and 3rd periods were 12.5% (5/40), 5% (2/40) and 5% (2/40), respectively. Cases with perforation were treated conservatively with an endoscopic closure using endo-clips. Only one patient in the 2nd period underwent emergency surgery due to a perforation.

DISCUSSION

En-bloc resection is ideal for large colorectal tumors such as LST. ESD was developed for large en-bloc resections of colorectal tumors, as for early gastric cancers (EGC).[9–11] Due to the high curability rate and tolerable complications, ESD for EGC has been widely accepted as an appropriate approach for endoscopic resections in Japan[30] and in other Asian countries.[31] The learning curves for EGC were evaluated in several studies.[18,19] Choi *et al.* demonstrated that after 40 procedures, the en-bloc resection rate increased and after 20 procedures, the perforation rate decreased.[18] Kakushima *et al.* found that a reduction in the procedure time was a marker of proficiency in this skill.[19] To the best of our knowledge, the present study is the first report evaluating the learning curve for ESD of colorectal tumors.

When ESD for colorectal tumors were attempted in large referral centers, the result demonstrated high en-bloc resection rates and low local recurrence rates.[12–15] The perforation rates were varied in these reports ranging from 1.4 to 10%.[12–15] In general, the perforation rate with ESD tended to be higher than with conventional EMR. Saito *et al.* reported that the perforation rates with conventional EMR and ESD for colorectal tumors larger than 20 mm were 1.3 and 6.2%, respectively.[16] However, the long procedure time remained an important problem associated with ESD that undetermined its widespread adoption and standardization.[16,17] Several electrosurgical knives were developed and tested for clinical applications.[11,20,25–29] We initially used the hook knife for mucosal incisions and submucosal dissections.[25,27–29] During the study period, new fluids for submucosal fluid injections for creating sufficient cushioning to avoid perforations were investigated by several groups.[10,24,25,32] Initially, we used sodium hyaluronate solutions based on the method described by Yamamoto *et al.*[10,24,25]

In the present study, we used a novel measurement for evaluating the learning curve: the procedure time per unit area of specimen (min/cm^2). This measure eliminated confounding differences in lesion size. With this measure, we found that the proficiency was significantly improved after 40 procedures. From previous large-scale reports[12–15,33,34] (Table 2), we derived target levels of en-bloc resection rates, en-bloc and R0 resection rates and perforation rates: these targets were 90, 80, and 5%, respectively. Based on an analysis of our outcomes, we achieved the target en-bloc and R0 resection rate after 80 cases and the target perforation rate after 40 cases. The majority of R1 resections were due to positive lateral margins; thus, the R0 resection rate may be improved by carefully ensuring that sufficient safety margins are imposed during marginal mucosal incisions. We have been careful of strategy in treating lesions and the efficiency of submucosal dissection to achieve good outcomes. Most of the perforations occurred during submucosal dissection, so we paid particular attention to the process of submucosal dissection.

The limitations of the present study include the retrospective design and the fact that the ESD procedure

Table 2. Previous reports of endoscopic submucosal dissection for colorectal tumors

Author	Journal	*n*	En bloc resection	En bloc and R0 resection	Perforation
Saito *et al.*	*Gastrointest. Endosc.* 2009[33]	1111	88%	–	5.2%
Saito *et al.*	*Gastrointest. Endosc.* 2007[12]	200	84%	83%	5%
Fujishiro *et al.*	*Clin. Gastroenterol. Hepatol.* 2007 [13]	200	91.5%	71%	6%
Tamegai *et al.*	*Endoscopy* 2007[14]	71	98.6%	95.6%	1.4%
Tanaka *et al.*	*Gastrointest. Endosc.* 2007[15]	70	–	80%	10%
Hurlstone *et al.*	*Br. J. Surg.* 2007[34]	42	78.6%	74%	2.4%

changed in each period. We cannot rule out the possibility that the new pediatric colonoscope with a built-in water jet system and the CO_2 insufflations system might have influenced the procedure times and outcomes. Saito *et al.* reported that CO_2 insufflations reduced the dose of drugs required for sedation, but did not shorten the procedure time.[23] However, we assume that the technical improvements implemented during this study contributed to the improved learning curve.

Selection of the scope might also influence the outcome. In the 1st period we used a pediatric colonoscope with a handmade water-jet system, so problems with the water jet sometimes occurred. After 2nd period, we could use the pediatric scope with the built-in water jet system, so no similar problems occurred.

Several factors are considered important in the learning curve for the ESD procedure. One is a good coach that has experienced many cases and who teaches well. Another is a good textbook, including technical books and videos. ESD for early gastric cancer was considered easy and safe compared with colonic ESD. There are many early gastric cancers in Japan and other Asian countries, so most beginners experienced ESD of gastric cancers before colonic lesions. However, in Western countries in which there are few early gastric cancers, beginners should train with another method. For these beginners, the use of a pig stomach model is helpful in learning to use electrosurgical knives and high-frequency generator units. Additionally, a live demonstration is a fascinating way to learn from experts in a short time. We scheduled a live demonstration with specialists for ESD twice a year from 2003. Furthermore, it is important to learn basic endoscopic skills, including the insertion of a colonoscope and carrying out a polypectomy, in order to progress smoothly. Also, basic skills for endoscopic hemostasis and closure of perforation using hemoclips is necessary during the submucosal dissection process. In future, the standardization of ESD could be facilitated with the development of knives with better control and traction devices.

CONCLUSION

Based on our analysis of the learning curve, approximately 80 procedures should be carried out to acquire ESD skills adequate for consistent, successful removal of large colorectal tumors. However, approximately 40 procedures are sufficient for achieving the target procedure time and perforation rate.

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REPORT

PHASE II STUDY OF CHEMORADIOTHERAPY WITH 5-FLUOROURACIL AND CISPLATIN FOR STAGE II–III ESOPHAGEAL SQUAMOUS CELL CARCINOMA: JCOG TRIAL (JCOG 9906)

KEN KATO, M.D.,* KEI MURO, M.D.,*† KEIKO MINASHI, M.D.,‡ ATSUSHI OHTSU, M.D.,‡ SATOSHI ISHIKURA, M.D.,§ NARIKAZU BOKU, M.D.,¶ HIROYA TAKIUCHI, M.D.,‖ YOSHITO KOMATSU, M.D.,** YOSHINORI MIYATA, M.D.,†† AND HARUHIKO FUKUDA, M.D. § GASTROINTESTINAL ONCOLOGY STUDY GROUP OF THE JAPAN CLINICAL ONCOLOGY GROUP (JCOG).

*Gastrointestinal Oncology Division, National Cancer Center Hospital, Tokyo; †Department of Clinical Oncology, Aichi Cancer Center Hospital, Nagoya, Aichi; ‡Division of Digestive Endoscopy and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Chiba; §Clinical Trials and Practice Support Division, Center for Cancer Control and Information Services, National Cancer Center, Tokyo; ¶Division of Gastrointestinal Oncology, Shizuoka Cancer Center, Sunto-gun, Shizuoka; ‖Cancer Chemotherapy Center, Osaka Medical College Hospital, Takatsuki, Osaka; **Department of Cancer Chemotherapy, Hokkaido University Hospital Cancer Center, Sapporo, Hokkaido; ††Department of Internal Medicine, Saku Central Hospital, Nagano, Japan

Purpose: In this Phase II study, we evaluated the efficacy and toxicity of chemoradiotherapy (CRT) with cisplatin (CDDP) and 5-fluorouracil (5-FU) for Stage II–III esophageal squamous cell carcinoma (ESCC).
Patients and Methods: Patients with clinical Stage II–III (T1N1M0 or T2–3N0–1M0) thoracic ESCC were enrolled between April 2000 and March 2002. Chemotherapy comprised two courses of protracted infusion of 5-FU (400 mg/m^2/day) on Days 1–5 and 8–12, and 2-h infusion of CDDP (40 mg/m^2) on Days 1 and 8; this regimen was repeated every 5 weeks. Concurrent radiotherapy involved 60-Gy irradiation (30 fractions) for 8 weeks with a 2-week break. Responders received two courses of 5-FU (800 mg/m^2/day) on Days 1–5 and CDDP (80 mg/m^2) on Day 1. Final analysis was conducted in March 2007. Survival and late toxicities were monitored for 5 years.
Results: The characteristics of the 76 patients enrolled were as follows: median age, 61 years; male/female, 68/8; performance status 0/1, 59/17 patients; Stage IIA/IIB/III, 26/12/38 patients. Of the 74 eligible patients, 46 (62.2%) achieved complete response. Median survival time was 29 months, with 3- and 5-year survival rates of 44.7% and 36.8%, respectively. Acute toxicities included Grade 3/4 esophagitis (17%), nausea (17%), hyponatremia (16%), and infection without neutropenia (12%). Late toxicities comprised Grade 3/4 esophagitis (13%), pericardial (16%) and pleural (9%) effusion, and radiation pneumonitis (4%), causing 4 deaths.
Conclusions: CRT is effective for Stage II–III ESCC with manageable acute toxicities and can provide a nonsurgical treatment option. However, further improvement is required for reduction in late toxicity. © **2010 Elsevier Inc.**

Esophageal squamous cell carcinoma, Chemoradiotherapy, Long-term toxicity, Salvage surgery.

INTRODUCTION

Esophageal cancer, a highly virulent malignancy, was responsible for 11,182 deaths in Japan in 2005, accounting for 3.4% of the country's total cancer deaths (1), with 35–40% of the patients diagnosed with Stage II–III disease. When this study was planned, the standard treatment for Stage II–III esophageal squamous cell carcinoma (ESCC) in Japan was esophagectomy with three-field lymph node dissection, followed by postoperative chemotherapy; the 5-year survival rate is reported to be 36.8–61% (2–4), with a high morbidity rate.

Chemoradiotherapy (CRT) has proved effective against resectable/unresectable ESCC. The Radiation Therapy Oncology Group (RTOG) trial 85-01 demonstrated the superiority of CRT with cisplatin (CDDP), 5-fluorouracil (5-FU), and concurrent irradiation (50.4 Gy) over radiotherapy alone (64 Gy) in patients with T1–3N0–1M0 esophageal cancer (5), in which the final outcome showed a 5-year survival rate of 26% in the CRT arm compared with 0% in the

Reprint requests to: Ken Kato, M.D., Ph.D., Gastrointestinal Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Tel: (+81) 3-3542-2511; Fax: (+81) 3-3542-3815; E-mail: kenkato@ncc.go.jp

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Conflict of interest: none.

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radiation-alone arm (6). Therefore, CRT is recognized as the standard noninvasive treatment for patients with localized esophageal cancer who opt for nonsurgical treatment.

CRT was introduced in Japan in the early 1990s as a treatment for potentially unresectable locally advanced ESCC. In a Phase II trial, 18 of 54 (33%) patients with clinical T4 and/or M1 lymph node ESCC, who received CDDP/5-FU with concurrent 60-Gy irradiation, achieved complete response (CR) with a 3-year survival rate of 23% (7). Since then, CRT has been clinically indicated for patients with resectable ESCC who refuse surgical resection. In a retrospective analysis, 55 patients with T1–3NanyM0 ESCC, who received CRT with CDDP, 5-FU, and concurrent 60-Gy irradiation, showed a CR of 70% and a 5-year survival rate of 46%, suggesting comparable outcomes with surgery (8). However, the results were retrospective. Thus, we conducted a Phase II study to evaluate the efficacy and toxicity, particularly the long-term outcome, of CRT for Stage II–III ESCC.

PATIENTS AND METHODS

Eligibility

The eligibility criteria were as follows: pathologically confirmed thoracic ESCC; clinical Stage II–III excluding T4 (T1N1M0 or T2–3N1–0M0: International Union Against Cancer [UICC] 1997); Eastern Cooperative Oncology Group (ECOG) performance status (PS), 0 or 1; and age, 20–70 years. Patients who had previously undergone therapy for esophageal cancer or chemotherapy/radiotherapy for other malignancies and who previously had had other active malignancies were excluded. All the patients had to meet the following laboratory criteria within 14 days before registration: leukocytes $\geq$3,000/mm^3; platelet count $\geq$100,000/mm^3; hemoglobin level $\geq$10 g/dL; aspartate aminotransferase (AST)/alanine aminotransferase (ALT) $\leq$2 $\times$ the upper normal limit at the institution; total bilirubin $\leq$1.5 mg/dL; serum creatinine $\leq$1.2 mg/dL; creatinine clearance $\geq$50 mL/min; $PaO_2 \geq$70 mm Hg; and no major electrocardiogram abnormalities. Written informed consent was obtained from all the patients. The study protocol was approved by the JCOG Clinical Trial Review Committee and institutional review boards of the participating institutions.

Chemotherapy

Chemotherapy comprised two courses of protracted infusion of 5-FU (400 mg/m^2/day) on Days 1–5 and 8–12, and 2-h infusion of CDDP (40 mg/m^2) with adequate hydration and antiemetic coverage on Days 1 and 8; this regimen was repeated every 5 weeks. Responders additionally received two courses of 5-FU (800 mg/m^2/day) on Days 1–5 and CDDP (80 mg/m^2) on Day 1 (Fig. 1), repeated every 4 weeks. No further treatment was administered to patients with CR until disease progression. Additional chemotherapy courses were optional for patients with visible disease.

Administration of both chemotherapy agents was discontinued until toxicity improved to $\leq$Grade 2. The doses were reduced by 25% in the subsequent course after at least one of the following toxicities was observed: leukocytes <1,000/mm^3; platelet count <30,000/mm^3; total bilirubin >2.0 mg/dL; serum creatinine $\geq$2.0 mg/dL; Grade 3/4 stomatitis; or Grade 3/4 esophagitis. Total parenteral nutrition was provided as necessary. Treatment was terminated when disease progression was observed, patients refused to continue, or recovery from toxicity delayed the initiation of the second course by >3 weeks from the planned schedule.

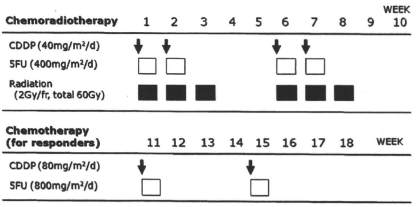

Fig. 1. Protocol scheme.

Radiotherapy

Radiotherapy was delivered using megavoltage ($\geq$6 MV) x-rays; a total dose of 60 Gy was administered in 30 fractions. A 2-week break was provided after 30-Gy irradiation, and radiotherapy was resumed on Day 36 with the second chemotherapy course. The clinical target volume (CTV) for 60-Gy irradiation included the primary tumor plus a 5-cm craniocaudal margin, and the metastatic lymph nodes plus a 1-cm margin. Planning target volume was defined as CTV plus 5- to 20-mm margins for uncertainty. Elective nodal irradiation (40 Gy) of mediastinal and perigastric lymph nodes for all cases, cervical lymph nodes for an upper thoracic primary tumor, and celiac lymph nodes for a lower thoracic primary tumor was also performed. Three-dimensional computed tomography (CT) or X-ray simulation was performed, allowing two-dimensional anterior–posterior opposed fields and bilateral oblique boost. Heterogeneity-uncorrected doses were used.

Assessments

Esophagoscopy and CT were carried out after each course to assess the response. Primary tumor response was evaluated by endoscopy using the modified criteria of the Japanese Society for Esophageal Diseases (9). Complete response of lymph node metastasis was defined as the disappearance of all visible lymph node metastases on the CT or size reduction to $\leq$1 cm for $\geq$3 months after the completion of treatment. Overall CR was declared by an attending physician when CR at both a primary tumor and a lymph node was obtained without the appearance of a new lesion. Complete response was confirmed by reassessment at $\geq$4 weeks after the first assessment. Complete response cases were centrally reviewed, and CR was confirmed by extramural review of the CT scan and images of endoscopy.

Acute toxicities were assessed weekly during CRT and every 2 weeks during additional chemotherapy for 90 days after the completion of CRT. Toxicities were evaluated based on the National Cancer Institute Common Toxicity Criteria (version 2.0). Late toxicity, which first occurred 90 days after CRT initiation, was assessed using the RTOG/European Organization for Research and Treatment of Cancer late radiation morbidity scoring scheme.

Statistical methods

The primary endpoint was overall survival (OS), which was defined as the time from the date of registration to that of death resulting from any cause, and it was censored at the date of the last follow-up for survivors. Progression-free survival (PFS) was defined as the time from the date of registration to that of disease progression or death resulting from any cause, and it was censored at the date of the last visit for patients without progression. Based on the JCOG 9204 trial results (2), in which the 3-year survival rate was 61% for esophagectomy with adjuvant chemotherapy, we initially calculated the sample size expecting a 3-year survival rate of 60%, with a threshold of 45%. With the alpha and beta error levels set at 0.05 and 0.2, respectively, the required number of eligible patients was 68. We finally decided on a sample size of 76, including ineligible patients. The planned accrual and follow-up periods after registration was closed were 1 and 2 years, respectively. For early termination of this study, an interim analysis was planned once 50% of the patients were accrued. A CR point estimate of <60% at the interim analysis would result in early termination of the study.

The JCOG 9204 had enrolled patients based on the pathologic stage after surgery, whereas we enrolled patients based on the clinical stage diagnosed from CT scans. Therefore, this study might include patients with more advanced stages than those in the JCOG 9204. Thus, the protocol was amended to recalculate the sample size from the expected 50% 3-year survival rate and a threshold of 35% in December 2000. The required sample size was 67. The target sample size remained unchanged. The second amendment in February 2007 prolonged the follow-up period to 5 years after the last enrollment to evaluate late toxicity. These amendments were approved by the Data and Safety Monitoring Committee of JCOG.

Secondary endpoints included CR rate, PFS, and acute and late adverse events. Time-to-event distribution was estimated using the Kaplan-Meier method, and confidence intervals (CIs) were calculated using Greenwood's formula. All analyses were performed using SAS Version 9.1.3 software (SAS Institute, Cary, NC, USA) at the JCOG Data Center, with the final analysis conducted in March 2007.

RESULTS

Patient characteristics

Seventy-six patients, whose characteristics are summarized in Table 1, were accrued between April 2000 and March 2002. The median age was 61 years (range, 39–70). Fifty-nine (78%) and 17 (22%) patients showed ECOG PS of 0 and 1, respectively. Fifty-two patients had T3 disease, and 50 had N1 disease. The clinical stages (UICC-TNM) were IIA for 26 patients, IIB for 12 patients, and III for 38.

Table 1. Patient characteristics

Characteristic	Patients (n = 76)	(%)
Male	68	89.4
Female	8	10.6
Age (y)		
Range	39–70	
Median	61	
Performance status		
0	59	77.6
1	17	22.4
Tumor location		
Upper	3	3.9
Middle	44	57.9
Lower	29	38.2
T factor		
T1	8	10.5
T2	16	21.1
T3	52	68.4
N factor		
N0	26	34.2
N1	50	65.8
Stage		
IIA	26	34.2
IIB	12	15.8
III	38	50.0

Response

Two patients were excluded from the efficacy analysis because of inadequate liver function and T4 disease diagnosed after registration (Fig. 2). Of the 74 eligible patients, 46 achieved CR, resulting in a CR rate of 62.2% (95% CI, 50.1–73.2). The confirmed CR rate in 23 patients with T1–2 disease was 78.3% (95% CI, 56.3–92.5), and that in 51 patients with T3 disease was 54.9% (95% CI, 40.3–68.9).

Survival

There were 49 deaths in the final analysis, and all except 5 patients were followed up for >5 years. The median survival time was 2.4 years (Fig. 3); the 3- and 5-year survival rates were 44.7% (90% CI, 35.2–53.8) and 36.8% (95% CI, 26.1-47.5), respectively. The lower limit of 90% CI for the 3-year survival rate exceeded the threshold of 35%, and the

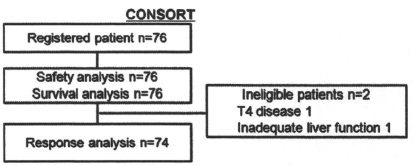

Fig. 2. Consolidated Standards of Reporting Trials diagram.

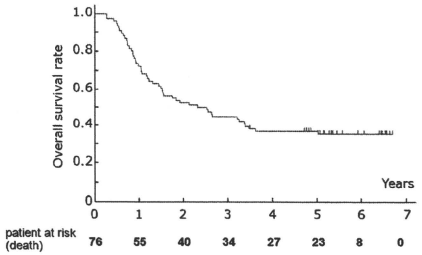

Fig. 3. Overall survival of the 76 patients enrolled in the study.

null hypothesis was rejected ($p = 0.019$). The median PFS was 1 year; the 3- and 5-year PFS rates were 32.9% and 25.6%, respectively (Fig. 4).

Acute toxicity

Data of adverse events for all 76 patients occurring within 90 days after CRT completion are shown in Table 2. Grade 4 leukopenia, neutropenia, anemia, and thrombocytopenia were observed in 1.3%, 1.3%, 2.6%, and 0% of the patients, respectively, whereas Grade 3/4 esophagitis, nausea, infection without neutropenia, and hyponatremia were observed in 17%, 17%, 12%, and 16% of the patients, respectively.

Fifty-three (69.7%) patients completed the 2-course CRT and 2-course additional chemotherapy. Seventy-two (95%) patients received the full dose (60 Gy) of radiation. The treatment protocol was terminated in 23 patients because of disease progression ($n = 10$), toxicity ($n = 11$), patient refusal ($n = 1$), and other reasons ($n = 1$). One early death occurred from esophageal perforation caused by disease progression 21 days after CRT completion. A relationship between early death and the treatment protocol was considered unlikely by the Data and Safety Monitoring Committee.

Late toxicity

Late toxicity data are shown in Table 3. Grade 3–4 late toxicities included pleural (9%) and pericardial (16%) effusion, stenosis, or esophageal fistula (13%), and radiation pneumonitis (4%). Four (5.3%) patients possibly died of treatment-related late toxicity at 3.1, 8.5, 21.3, and 27.8 months after registration. The cause of death were pneumonitis ($n = 2$), pericarditis ($n = 1$), and pleural effusion ($n = 1$). There was no evidence of residual or recurrent disease in these patients. The proportion of any Grade 3/4 late toxicity was 30.1% after 5 years from the initiation of chemoradiation.

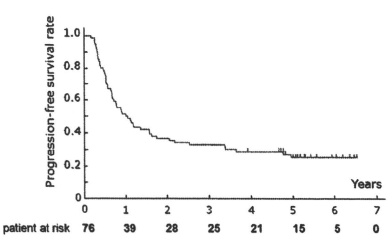

Fig. 4. Progression-free survival rate of the 76 patients enrolled in the study.

Salvage treatment

Twenty-six (34.2%) patients had residual disease or locoregional recurrence without distant metastasis after CRT. Because of inadequate conditions or patient refusal, 7 and 5 patients received chemotherapy and the best supportive care, respectively; the remaining 14 patients received unplanned curative-intent salvage therapy. Eleven patients underwent salvage esophagectomy for residual ($n = 4$) and recurrent ($n = 7$) disease, and the remaining 3 patients underwent endoscopic treatment such as endoscopic mucosal resection (EMR) or argon plasma coagulation. The characteristics of the patients who underwent salvage surgery are described in Table 4.

The median time to salvage surgery after CRT initiation was 13.9 months (range, 4.0–22.7). Six patients underwent esophagectomy with two- or three-field lymph node dissection, 3 patients underwent simple esophagectomy, and 1 underwent only lymphadenectomy; 1 patient could not undergo any resection because of extensive lymph nodes metastasis detected at thoracotomy. Reconstruction was performed using a gastric tube in 7 patients who had R0 resection. There was no operative mortality or hospital death. The median survival time and 3-year survival rate for these 10 patients who received salvage esophagectomy was 16.7 months and 40% (95%C.I: 12.3%–67.0%), respectively.

Of the 3 patients who underwent endoscopic treatment, 1 had mediastinal lymph node metastasis 3 months after argon plasma coagulation, 1 died of surgery-related complication of the pharynx detected 1 year after EMR, and 1 survived for >5 years with no evidence of disease.

DISCUSSION

From the results, CRT for Stage II–III ESCC showed a CR rate of 62.2% (95% CI, 50.1–73.2), a 3-year survival rate of 44.7% (90% CI, 35.2–53.8), and a 5-year survival rate of 36.8% (95% CI, 26.1–47.5). The 3-year survival rate, which is the primary endpoint of this study, met the decision criteria.

Clinically, it is very important to know whether definitive CRT can achieve survival comparable with surgery plus postoperative adjuvant chemotherapy. In this regard, there were several differences in the background between the present study and JCOG 9204 (2) described in Statistical Methods. The study conducted after JCOG 9204, which compared preoperative and postoperative adjuvant chemotherapy comprising the administration of 5-FU and CDDP to Stage II–III esophageal cancer patients (JCOG 9907) (10), could be a reference for this study, because the patients were registered before surgery based on the clinical stage. In the recently

Table 2. Toxicity (n = 76)

Toxicity	NCI-CTC Version 2.0				
	Grade 1	Grade 2	Grade 3	Grade 4	≥Grade 3 (%)
Leukocytes	5	34	32	1	43
Neutrophils	17	31	19	1	26
Hemoglobin	13	35	15	2	22
Platelets	15	13	4	0	5
Dysphagia, esophagitis	29	14	13	0	17
Nausea	25	20	13	–	17
Vomiting	16	6	0	0	0
Diarrhea	10	5	1	0	1.3
Stomatitis/pharyngitis	15	9	6	0	8
Radiation dermatitis	18	4	0	0	0
Febrile neutropenia	–	–	1	0	1.3
Infection without neutropenia	7	8	8	1	12
Hyponatremia	40	–	11	1	16
AST	35	4	3	0	3.9
ALT	43	7	2	1	3.9
Creatinine	15	13	1	0	1.3

Abbreviations: NCI-CTC Version 2.0 = National Cancer Institute Common Toxicity Criteria Version 2.0; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

published results of JCOG 9907, the preoperative chemotherapy arm was highly superior to the postoperative chemotherapy arm in terms of OS. The 5-year survival rate of the postoperative chemotherapy arm in JCOG 9907 did not differ significantly from that in the present study, that is, 38.4% and 36.8%, respectively (10). By contrast, the 5-year survival rate of the preoperative chemotherapy arm in JCOG 9907 was 60.1%, although further follow-up is needed to verify the data. CRT may produce comparable outcomes with surgery plus postoperative adjuvant chemotherapy; however, surgery after preoperative chemotherapy is considered to be superior to CRT. Nevertheless, CRT is one of the treatment options for patients with Stage II and III ESCC because of its apparent advantage of preserving the esophagus, which may provide better quality of life.

Chemoradiotherapy achieves prolonged survival with possibly more late toxicity. Late toxicity after thoracic radiotherapy has been reported in patients with esophageal cancer, lung cancer, and Hodgkin's lymphoma (11–13). Some reports have described that long-term toxicity after CRT results in serious, life-threatening complications. In a previous study, 2 of 78 patients with CR after CRT died of myocardial infarction, and 8 (10.2%) died of pericardial or pleural effusion (14) Late toxicity after CRT against ESCC has not yet been investigated in detail, and early reports of trial outcomes generally seem to underestimate the risk of late toxicity in long-term survivors (15). In the present study, the incidence of ≥Grade 3 late toxicity was similar to that reported in a previous study (14). Most of these events occurred several years after CRT. It is considered that reduction in radiation dose, careful observation, and control of late toxicity may improve post-CRT survival. RTOG 94-05 demonstrated that a higher irradiation dose (64.8 Gy) in CRT was not advantageous with regard to survival and local control, compared with the standard dose (50.4 Gy) (16). One of the reasons was the low tolerability of the high-dose arm because of toxicity. Whereas decreasing the irradiation dose in radiotherapy is essential for reducing late toxicity, the radiation volume is also

Table 3. Late toxicity (n = 76)

Late toxicity	RTOG/EORTC late radiation morbidity scoring scheme					
	Grade 1	Grade 2	Grade 3	Grade 4	≥Grade 3 (%)	≥Grade 4 (%)
Pleural effusion (nonmalignant)	24	5	7	0	9	0
Esophagus-related (dysphagia, stenosis, fistula)	11	4	4	6	13	8
Pericardial effusion	6	5	9	3	16	4
Radiation pneumonitis	33	6	2	1	4	1.3
Skin-related	3	0	0	0	0	0
Spinal cord—related	3	0	0	0	0	0

Abbreviation: RTOG/EORTC: radiation therapy oncology group/european organization for research and treatment of cancer.
four (5.3%) patients possibly died of treatment-related late toxicity: pericarditis (n = 1), pleural effusion (n = 1), and pneumonitis (n = 2).

Table 4. Characteristics and outcomes in patients who underwent salvage surgery

Characteristic	Patients (n = 11)	Characteristic	Patients (n = 11)
Male	11	Residual/Recurrent	4 /7
Female	0		
Age (y)		Surgical curability	
Range	46–70	R0	7
Median	59	R1 + R2	4
Tumor location			
Upper	0	Operative mortality or hospital death	0
Middle	6		
Lower	5	Relapse after surgery	8
Clinical stage*		No relapse	3
IIA	5		
IIB	0		
III	6		

* Clinical stage at the time of registration.

important. In this study, late toxicity might have been caused by the extended volume of irradiation, which corresponds to the dissected area in extended surgery. In the near future, three-dimensional conformal radiotherapy, which was not mandatory in this study, or other methods based on advanced technology such as intensity-modulated radiotherapy and proton therapy, may have potential advantages over conventional two-dimensional radiotherapy in terms of reduced doses for the heart. A clinical trial with these latest radiotherapy techniques is required (17).

Salvage treatment—*e.g.*, salvage surgery (18–20) or salvage EMR (21)—has recently been reported to have therapeutic potential for patients with local failure of CRT. In our study, one-third of the patients did not achieve CR, and 50% of the remaining patients had recurrence after achieving CR. For the latter, salvage treatment should be indicated, if applicable. Mucosal disease can be removed by EMR, and locoregional residual or recurrent disease can be curatively resected by surgery. It has been reported that 6–34% of patients undergo salvage esophagectomy after definitive CRT (22, 23). Although a high rate of hospital deaths (6–33%) is observed compared with that after surgery without preoperative therapy, some patients achieve long-term survival with a 5-year survival rate of 25–35% (24–26). In the present study, 11 (14.5%) patients underwent salvage esophagectomy and 7 had R0 resection. There was no operative mortality or hospital death. The limitations of salvage surgery include patient tolerance, capability of medical staff, and early detection of residual or recurrent disease; however, salvage esophagectomy can achieve long-term survival. Some patients benefit from salvage surgery after definitive CRT; therefore, this procedure is worth further investigation.

Neoadjuvant CRT has recently been recognized as a standard therapy for resectable esophageal cancer in Western countries. According to CALGB 9781, CRT followed by surgery prolonged survival (median survival time, 4.48 vs. 1.79 years) compared with surgery alone in the treatment of esophageal cancer (27). However, most participants in CALGB 9781 had esophageal adenocarcinoma. Meta-analysis has revealed the survival benefit of neoadjuvant CRT in patients with esophageal adenocarcinoma (28). According to FFCD 9102, which included 90% patients with squamous cell carcinoma, surgery after neoadjuvant CRT (40 Gy) and continuation of CRT to 60 Gy without surgery had the same impact on survival and quality of life for responders as induction CRT (29). The results of a randomized trial from Germany, in which 172 ESCC patients randomly received CRT with or without additional surgery, indicated equal efficacy of surgery and CRT. The median survival times were 16.4 months and 14.9 months, respectively, and the 2-year survival rates were 39.9% and 35.4% with and without surgery, respectively (30). This suggests that CRT, which can preserve organ function, is equally effective as surgery for responders. For nonresponders, salvage surgery can be a therapeutic option. Importantly, which types of patients are benefited by salvage surgery or how the surgical procedure is performed after CRT should be prospectively evaluated. We are planning a Phase II trial of CRT for resectable ESCC, followed by salvage surgery for residual or recurrent disease.

CONCLUSION

Chemoradiotherapy is effective for Stage II–III ESCC with manageable acute toxicities and can provide a noninvasive treatment option. However, further improvement is required for reduction in late toxicity.

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RESEARCH Open Access

TNFRSF1B A1466G genotype is predictive of clinical efficacy after treatment with a definitive 5-fluorouracil/cisplatin-based chemoradiotherapy in Japanese patients with esophageal squamous cell carcinoma

Akiko Kuwahara[1,3], Motohiro Yamamori[1,2,3], Megumi Fujita[1,3], Tatsuya Okuno[3], Takao Tamura[3], Kaori Kadoyama[2,3], Noboru Okamura[1,3*], Tsutomu Nakamura[3], Toshiyuki Sakaeda[2,3]

Abstract

Background: Currently definitive 5-fluorouracil (5-FU)/cisplatin (CDDP) -based chemotherapy is recognized as one of the most promising treatments for esophageal cancer. A series of studies performed found genetic polymorphisms and the plasma concentration of 5-FU to be predictive of acute severe toxicities and clinical response. Genetic polymorphisms of *tumor necrosis factor (TNF)* -α and its surface receptors, *TNFRSF1A* and *TNFRSF1B* have been examined in terms of susceptibility to various cancers. In this study, genetic polymorphisms of *TNFRSF1B* gene were evaluated Japanese esophageal squamous cell carcinoma (ESCC) patients treated with the definitive 5-FU/CDDP-based chemoradiotherapy and their predictive values of prognosis or severe acute toxicities were assessed.

Methods: Forty-six patients with ESCC were treated with the definitive 5-FU/CDDP-based chemoradiotherapy, one course of which consisted of the continuous infusion of 5-FU for days 1-5 and 8-12, the infusion of CDDP on days 1 and 8, and the radiation at 2 Gy/day on days 1-5, 8-12, and 15-19, with a second course repeated after 2-week interval. Genetic polymorphisms of a TNF-α receptor *TNFRSF1B* gene were determined by a TaqMan® MGB probe-based polymerase chain reaction.

Results: The genotype of *TNFSR1B* A1466G, but not M196R/T587G or C1493T, was found to be predictive of clinical response, i.e., a complete response or not ($p = 0.040$). Clinical response was predicted by tumor size ($p = 0,002$), lymph node metastasis ($p = 0.007$), distant metastasis ($p = 0.001$) and disease stage ($p < 0.001$), but *TNFRSF1B* A1466G genotype was independent of these factors.

Conclusions: Genetic polymorphism of *TNFRSF1B* A1466G was found to be predictive response in Japanese ESCC patients with a definitive 5-FU/CDDP-based chemoradiotherapy. Further clinical investigation with a large number of patients or experiments in vitro should be performed to assess the predictive value of *TNFRSF1B* A1466G genotype after chemoradiotherapy.

* Correspondence: nokamura@mukogawa-u.ac.jp
[1]School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya 663-8179, Japan

Background

A clinical report published in 1999, the RTOG (Radiation Therapy Oncology Group) 85-01 trial involving 134 patients with T1-3, N0-1 and M0 esophageal cancer, is of great interest in terms of clinical outcome because it demonstrated a 5-year survival rate of 26% [1]. This treatment consists of infusions of 5-fluorouracil (5-FU) and cisplatin (CDDP), and concurrent radiation, without pre- or post-surgical resection. Simultaneously in Japan, a modified version was proposed by Ohtsu and his co-workers for advanced metastatic esophageal cancer [2,3]. Two independent clinical investigations have shown curative potential using this regimen for unresectable esophageal squamous cell carcinoma (ESCC) of T4 or M1a [2,3]. A long-term evaluation of efficacy and toxicity with 139 patients revealed a complete response (CR) rate of 56%, along with a 5-year survival rate of 29% [4,5]. Currently, definitive 5-FU/CDDP-based chemoradiotherapy is recognized as one of the most promising treatments for esophageal cancer [6]. A series of studies performed to find a marker predictive of clinical outcome after treatment with a definitive 5-FU/CDDP-based chemoradiotherapy found a genetic polymorphism, G-1154A, of vascular endothelial growth factor to be a predictor of severe acute leukopenia and cheilitis, and the plasma concentration of 5-FU to be predictive of clinical response [7-9].

Tumor necrosis factor (TNF)-α, a proinflammatory cytokine, plays a key role in the pathogenesis of inflammatory diseases. Its biological effects are elicited by binding to its two cognate cell surface receptors, TNFRSF1A/TNFR1 (p55/60) and TNFRSF1B/TNFR2 (p75/80), both of which are involved in increasing expression of other cytokines and immuno-regulatory molecules through the activation of nuclear factor κB. Through extensive examinations of expression and function, some genetic variations have been shown to explain inter-individual variation. Single nucleotide polymorphisms (SNPs) in the *TNF-α*, *TNFRSF1A* and *TNFRSF1B* genes have been identified, however functional data pertaining to these polymorphisms in scarce. Nonetheless, the putative role of these polymorphisms in disease susceptibility has been examined in genetic association studies of various inflammatory disorders, including Crohn's disease [10-13], ulcerative colitis [10,11,14], systemic lupus erythematosus [15-17] and rheumatoid arthritis [18,19]. More recently, given that cancer progression is preceded by a long period of subclinical inflammation [20-22], the genetic polymorphisms of *TNF-α*, *TNFRSF1A* and *TNFRSF1B* have been examined in terms of susceptibility to various cancers [23-28]. In this study, genetic polymorphisms of the *TNFRSF1B* gene, M196R/T587G, A1466G and C1493T, were evaluated in Japanese ESCC patients treated with a definitive 5-FU/CDDP-based chemoradiotherapy, and their predictive values of prognosis or severe acute toxicities were assessed. To our knowledge, this is the first paper to report that the *TNFRSF1B* genotype is predictive of the clinical efficacy of cancer chemoradiotherapy.

Methods

Patients

Forty-six male ESCC patients were enrolled in this study based on the following criteria: 1) ESCC treated with a definitive 5-FU/CDDP-based chemoradiotherapy at Kobe University Hospital, Japan, from August 2002 to June 2006; 2) clinical stage T1 to T4, N0 or N1, and M0 or M1a according to the International Union Against Cancer tumor-node-metastasis (TNM) classification; 3) age less than 85 years; 4) an Eastern Cooperative Oncology Group performance status of 0 to 2; 5) adequate bone marrow, renal, and hepatic function; 6) no prior chemotherapy; 7) no severe medical complications; and 8) no other active malignancies (except early cancer). The tumors were histologically confirmed to be primary, and no patients with recurrence were included in this study. Written informed consent was obtained from all participants prior to enrollment. This study was conducted with the authorization of the institutional review board and followed the medical research council guidelines of Kobe University.

Protocol

The protocol is presented in Figure 1. A course consisted of the continuous infusion of 5-FU at 400 mg/m^2/day for days 1-5 and 8-12, the infusion of CDDP at 40 mg/m2/day on days 1 and 8, and the radiation at 2 Gy/day on days 1 to 5, 8 to 12, and 15 to 19, with a second course repeated after a 2-week interval [2,3]. If disease progression/recurrence was observed, either salvage surgery, endoscopic treatment, or another regimen of chemotherapy was scheduled.

Genotyping

Genomic DNA was isolated from whole blood with a TaqMan® Sample-to-SNP™ kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's directions. Genetic polymorphisms of *TNFRSF1B*; M196R/T587G, A1466G and C1493T, were determined by a TaqMan® MGB probe-based polymerase chain reaction (PCR) using the StepOne™ real-time PCR system (Applied Biosystems) and pre-manufactured TaqMan® SNP genotyping assays C_8861232_20 (M196R/T587G, rs1061622), C_8861229_10 (A1466G, rs1061624) and C_8861228_20 (C1493T, rs3397) (Applied Biosystems). The PCR was carried out according to the manufacturer's protocol. For each set of reactions,

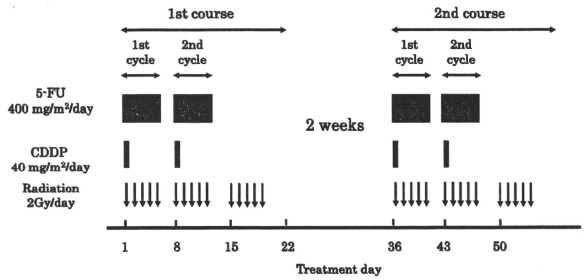

Figure 1 Protocol of a definitive 5-fluorouracil/cisplatin-based chemoradiotherapy. One course of treatment consisted of protracted venous infusions of 5-FU (400 mg/m^2/day, days 1-5 and 8-12) and CDDP (40 mg/m^2/day, days 1 and 8), and radiation (2 Gy/day, days 1-5, 8-12, and 15-19), with a second course (days 36-56) repeated after a 2-week interval.

DNA of cases and controls was taken and a negative control containing H_2O instead of DNA was added to check for contamination.

Clinical response

The clinical response was evaluated according to the method reported previously [2-5]. Briefly, a CR was defined as the complete disappearance of all measurable and assessable disease at the first evaluation, which was performed 1 month after the completion of chemoradiotherapy to determine whether the disease had progressed. The clinical response was evaluated by endoscopy and chest and abdominal computed tomography (CT) scans in each course. A CR at the primary site was evaluated by endoscopic examination when all of the following criteria were satisfied on observation of the entire esophagus: 1) disappearance of the tumor lesion; 2) disappearance of ulceration (slough); and 3) absence of cancer cells in biopsy specimens. If small nodes of 1 cm or less were detected on CT scans, the recovery was defined as an "uncertain CR" after confirmation of no progression for at least 3 months. An "uncertain CR" was included as a CR when calculating the CR rate. When these criteria were not satisfied, a non-CR was assigned. The existence of erosion, a granular protruded lesion, an ulcer scar, and 1.2 w/v% iodine/glycerin-voiding lesions did not prevent an evaluation of CR. The evaluations were performed every month for the first 3 months, and when the criteria for CR were not satisfied at 3 months, the result was changed to non-CR. Follow-up evaluations were performed thereafter every 3 months for 3 years by endoscopy and CT scan. After 3 years, patients were seen every 6 months. During the follow-up period, a routine course of physical examinations and clinical laboratory tests was performed to check the patient's health.

Severe acute toxicities

Definitive 5-FU/CDDP-based chemoradiotherapy is associated with acute toxicities; leucopenia, anemia, thrombocytopenia, nausea/vomiting, diarrhea, mucositis (including stomatitis), esophagitis, and renal dysfunction [2-5]. Here, severe acute leucopenia, stomatitis, and cheilitis were subjected to analysis. Toxicity was evaluated using criteria defined by the Japan Clinical Oncology Group [29]. These criteria were based on the National Cancer Institute Common Toxicity Criteria. Toxicity was assessed on a 2 to 3-day basis during the chemoradiotherapy and subsequent hospitalization period and on every visit after the completion of chemoradiotherapy. Episodes of leucopenia, stomatitis, and cheilitis during the first 2 courses and subsequent 2 weeks (until day 70) were recorded as acute toxicities and those of grade 3 or more as severe acute toxicities.

Survival after the chemoradiotherapy

The survival period was defined as the time from the date of treatment initiation to that of death from any causes or to the last date of confirmation of survival. Survival data were updated on December 31, 2006, and the 2-year survival rate was assessed using the data for 36 patients.

Data analysis and statistics

All values reported are the mean ± standard deviation (SD). The unpaired Student's *t*-test/Welch's test or Mann-Whitney's *U* test was used for two-group comparisons of the concentrations. Fisher's exact test was used for the analysis of contingency tables. The difference of overall

Table 1 Demographic and clinicopathologic characteristics of Japanese patients with esophageal squamous cell carcinoma.

Age, yr	64.6 ± 7.2 (range 48-78)
Height, cm	164.2 ± 6.2 (range 152-180)
Weight, kg	56.7 ± 9.6 (33-79)
Male/Female	46/0
Performance status, 0/1/2/unknown	23/19/3/1
Differentiation, well/moderate/poor/unknown	7/27/6/6
T1/T2/T3/T4	15/6/14/12
N0/N1	21/25
M0/M1a	39/7
Stage I/II/III/IVa	12/10/17/7

The values are the mean ± SD. Noncervical primary tumours with positive supraclavicular lymphnodes were defined as M1a.

survival curves was analyzed by Log-rank test. P values of less than 0.05 (two tailed) were considered to be significant.

Results

Demographic and clinicopathologic characteristics of the 46 ESCC patients are summarized in Table 1. The ratio of T1/T2/T3/T4 was 15/6/14/12, that of N0/N1 was 21/25, and that of M0/M1a was 39/7, resulting in a stage I/II/III/IVa ratio of 12/10/17/7. The CR rate was 47.8% (22/46), and 2-year survival rate was 50.0% (18/36). The clinical response, i.e., CR or non-CR, was predicted by T class ($p = 0.002$), N class ($p = 0.007$), M class ($p = 0.001$) and disease stage ($p < 0.001$). Episodes of severe acute leucopenia, stomatitis and cheilitis occurred in 39.1% (18/46), 13.0% (6/46) and 15.2% (7/46) of cases, respectively and no associations were found with the demographic and clinicopathologic characteristics.

Table 2 indicates the association of the *TNFRSF1B* genetic polymorphisms M196R/T587G, A1466G and C1493T with clinical response in the ESCC patients. *TNFRSF1B* A1466G genotype was predictive of clinical response ($p = 0.040$), whereas M196R/T587G and C1493T were not. No effects of the *TNFRSF1B* genotypes were found for TNM classes and disease stage (data not shown). Figure 2 shows the association of clinical response with overall survival of the patients. The patients with CR survived markedly longer than the non-CR patients ($p < 0.001$, Log-rank test). However, the 2-year survival rate was 25.0%, 60.0% and 50.0% in the patients with the *TNFRSF1B* genotypes AA^{1466}, AG^{1466} and GG^{1466}, and the effect of *TNFRSF1B* A1466G genotype on the overall survival was not significant (Log-rank test). In addition, the effects of *TNFRSF1B* M196R/T587G, A1466G and C1493T genotypes were not found for severe acute leucopenia, stomatitis or cheilitis (data not shown).

Table 2 Effects of *TNFRSF1B* polymorphisms on clinical response in Japanese patients with esophageal squamous cell carcinoma.

		Complete response N = 22	Not complete response N = 24	p
M196R/T587G (rs1061622)	TT	15	21	0.354
	TG	5	2	
	GG	2	1	
	T	35	44	0.135
	G	9	4	
A1466G (rs1061624)	AA	2	10	0.040
	AG	15	10	
	GG	5	4	
	A	19	30	0.094
	G	25	18	
C1493T (rs3397)	CC	9	12	0.787
	CT	9	9	
	TT	4	3	
	C	27	33	0.515
	T	17	15	

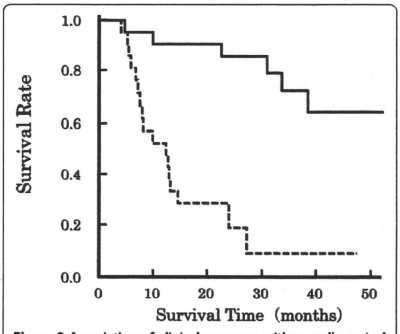

Figure 2 Association of clinical response with overall survival Japanese patients with esophageal squamous cell carcinoma. Line: CR, Dotted line: non-CR. The patients with CR survived extensively longer than the non-CR patients ($p < 0.001$, Log-rank test).

Discussion

The *TNFRSF1B* gene on chromosome 1 at p36 (IBD7) consists of 10 exons and encodes 415 amino acids, whereas the *TNFRSF1A* gene at 12p13 (IBD2) consists of 10 exons and encodes 455 amino acids. TNFRSF1A is

an important factor inducing apoptosis via an intracellular death domain, and TNFRSF1B is thought to be involved in ligand passing, thereby regulating the association of TNF-α with TNFRSF1A. TNFRSF1A is widely expressed, whereas TNFRSF1B is predominantly expressed in cells of the hematopoietic lineage. Several clinical investigations have been conducted to assess the predictive value of the genetic polymorphisms *TNF-α* G-308A, *TNFRSF1A* A36G and G-609T, and *TNFRSF1B* M196R/T587G, A1466G (or A1663G) and C1493T (or C1690T) regarding susceptibility to various inflammatory disorders [10-19], and recently, to cancer [23-28]. As for *TNFRSF1B*, the SNP M196R/T587G has proved predictive of Crohn's disease [13], systemic lupus erythematosus [15-17] and rheumatoid arthritis [18]. *TNFRSF1B* A1466G is not associated with Crohn's disease [13], but the haplotype 1466A-1493T might be important [11]. Recently, *TNFRSF1B* C1493T has been found to be a risk factor of tobacco-related oral carcinoma [28].

In this study, it was demonstrated that the *TNFRSF1B* A1466G genotype was a predictive factor of clinical response to treatment with a definitive 5-FU/CDDP-based chemoradiotherapy in Japanese ESCC patients. The *TNFRSF1B* G-allele at position 1466 is predictive of clinical response, whereas no such association was found for M196R/T587G or C1493T (Table 2). Clinical response was evaluated 1 month after the completion of the chemoradiotherapy, and a CR was defined as the complete disappearance of all measurable and assessable disease. Clinical response was determined by T class (an index of tumor size, $p = 0.002$), N class (lymph node metastasis, $p = 0.007$), M class (distant metastasis, $p = 0.001$) and disease stage ($p < 0.001$), but *TNFRSF1B* A1466G genotype was independent of these factors.

Clinical response was significantly associated with overall survival (Figure 2), however, *TNFRSF1B* A1466G genotype had no effect on the overall survival, presumably because it was not associated with death within 1 year after the completion of chemoradiotherapy. There is no report on the function of this polymorphism but it has been reported that higher expression levels of *TNFRSF1B* gene in colorectal cancer specimens from responding patients were observed compared with those from non-responding patients [30]. Thus, the polymorphism-dependent clinical response might be due to the polymorphism-dependent expression levels, although further studies are needed.

Conclusions

Genetic polymorphisms of the *TNFRSF1B* gene, M196R/T587G, A1466G and C1493T, were evaluated in Japanese ESCC patients treated with a definitive 5-FU/CDDP-based chemoradiotherapy. It was found that A1466G, but not M196R/T587G or C1493T, was a predictive factor of clinical response to chemoradiotherapy. Clinical response was predicted by TNM classes and disease stage, but A1466G genotype was independent of these factors. Further clinical investigation with a large number of patients or experiments in vitro should be performed to assess the predictive value of *TNFRSF1B* A1466G genotype after chemoradiotherapy.

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Author details

[1]School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya 663-8179, Japan. [2]Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan. [3]Kobe University Graduate School of Medicine, Kobe 650-0017, Japan.

Authors' contributions

AK, TT and TS made conception, designed and coordinated the study. MY carried out genotyping study and statistical analysis. MF and NO carried out genotyping study. TO and TT collected samples and evaluated clinical responses. AK, KK, NO, TN and TS prepared the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Development of pancreatic cancers during long-term follow-up of side-branch intraductal papillary mucinous neoplasms

Authors

Y. Sawai[1,2], K. Yamao[2], V. Bhatia[3], T. Chiba[1], N. Mizuno[2], A. Sawaki[2], K. Takahashi[2], M. Tajika[4], Y. Shimizu[5], Y. Yatabe[6], A. Yanagisawa[7]

Institutions

Institutions are listed at the end of article.

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Corresponding author
K. Yamao, MD
Department of Gastroenterology
Aichi Cancer Center Hospital
1-1 Kanakoden, Chikusa-Ku
Nagoya 464-8681
Japan
Fax: +81-52-7642942
kyamao@aichi-ac.jp

Background and study aims: Side-branch intraductal papillary mucinous neoplasms (SB-IPMNs), and associated synchronous and metachronous pancreatic cancers are increasingly detected as imaging modalities become more sensitive. We investigated the natural history of SB-IPMN, and the incidence and characteristics of pancreatic cancers among patients undergoing long-term follow-up.

Patients and methods: We reviewed the clinical, imaging, and pathological features in 103 patients, diagnosed at the Aichi Cancer Center between September 1988 and September 2006 as having SB-IPMN, and conservatively followed up for ≥ 2 years (median 59 months) based on an endoscopic ultrasonography (EUS) database.

Results: 74 (71.8%) patients had nonprogressive lesions. Overall, six patients (5.8%) developed pancreatic cancers during follow-up, with intraductal papillary mucinous (IPM) carcinoma in four, and ductal carcinoma of pancreas that was not IPMN in two patients. Of the six pancreatic cancers, five were diagnosed at a resectable stage. The 5-year and 10-year actuarial rates of development of pancreatic cancer were 2.4% and 20.0%, respectively. Although, at the last follow-up, cyst size, main pancreatic duct (MPD) diameter, mural nodule size, and frequency of metachronous and/or synchronous cancers of other organs were significantly higher in patients who developed IPM carcinoma, resected SB-IPMNs without mural nodules and dilated MPDs had no IPM carcinomas.

Conclusions: The frequency of pancreatic cancers is high on long-term follow-up of SB-IPMN. Although conservative management is appropriate for selected patients, regular and long-term imaging, especially by EUS is essential, even if SB-IPMN remains unchanged for 2 years. Presence of mural nodule and dilated MPD seem to be more appropriate indicators for resection than cyst size alone for SB-IPMNs.

Introduction

Since its first description by Ohhashi et al. in 1982 [1], the reported incidence of intraductal papillary mucinous neoplasms (IPMNs) has been increasing, partly because of growing awareness about them and wider application of sensitive imaging tests. Diagnostic criteria for IPMN have been established, but no consensus exists regarding optimal treatment protocols [2–8]. In 2006, the International Association of Pancreatology (IAP) guidelines for managing IPMN of the pancreas suggested treatment protocols [9], but several controversies still remain.

Most studies detailing the natural history of IPMN have been surgical, and have reported a high prevalence of carcinoma. Malignancy (in situ and invasive) has been identified worldwide in 70% and 25% of resected main pancreatic duct IPMNs, and side-branch IPMNs (SB-IPMNs), respectively [9]. There is an emerging consensus that IPMN is a premalignant condition, with an adenoma-to-carcinoma sequence similar to that of colon carcinoma. Furthermore, it is difficult to preoperatively distinguish between benign and malignant IPMN. For these reasons, early resection of all main duct IPMNs and some SB-IPMNs has been advocated [5,9–12], but the indications for resection of SB-IPMN are still controversial. In addition, synchronous or metachronous development of ductal cancers of the pancreas other than SB-IPMNs has been recently reported in these patients [13–16]. The prognosis for patients with noninvasive IPMN is excellent, whereas that for patients with invasive IPMN or ductal cancer of the pancreas is significantly inferior [17,18]. Hence, we believe that the long-term prognosis of patients with SB-IPMN depends on whether or not pancreatic cancer supervenes in these patients.

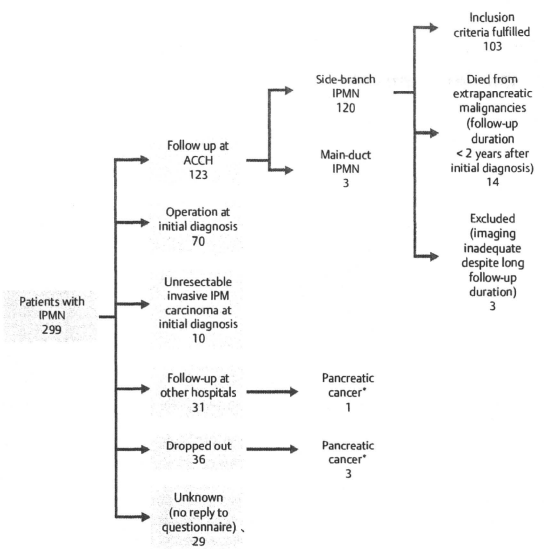

Fig. 1 Inclusion and exclusion of patients with side-branch intraductal papillary mucinous neoplasms (IPMNs) in follow-up study. *These four pancreatic cancers were reported by questionnaire, so we could not tell whether they were ductal carcinoma of the pancreas or intraductal papillary mucinous carcinoma. ACCH, Aichi Cancer Center Hospital.

In the present study, we investigated the frequency of pancreatic cancers found on long-term follow-up among patients with SB-IPMN, the characteristics of these cancers, and the optimal follow-up protocols.

Patients and methods

A review of the database of endoscopic ultrasonography (EUS) procedures performed at Aichi Cancer Center Hospital (ACCH), Nagoya, Japan, between September 1988 and September 2006, revealed a total of 299 patients with IPMN. The inclusion criteria for the present study were: presence of SB-IPMN, a minimal follow-up duration at ACCH of 2 years at September 2008, and availability of findings from serial imaging and of clinical details.

Of the 299 patients, 70 underwent surgery, and another 10 were found to have unresectable IPMC at their initial diagnosis, 31 underwent follow-up at other hospitals, 36 dropped out from follow-up in ACCH or other hospitals, and the final status of 29 was unknown because of failure to reply to the study questionnaire mentioned below (see **Fig. 1**).

Among those who were followed up at other hospitals and those who eventually dropped out, pancreatic cancers (ductal carcinomas of the pancreas, or invasive intraductal papillary mucinous [IPM] carcinomas) developed in 4 of 67 patients. We obtained data from the referring hospitals through a clinical questionnaire that had been approved by the Ethical Committee of the ACCH.

The remaining 123 patients underwent follow up at ACCH. These 123 patients included three patients with main pancreatic duct (MPD) IPMNs (one rejected the option of surgery, and two underwent conservative follow up because of advanced age and poor clinical status), and 120 patients with SB-IPMNs. Of the latter 120 patients, 14 died due to an extrapancreatic malignancy (follow-up duration < 2 years after the initial diagnosis), and imaging was inadequate in three despite a long duration of follow-up; these 17 patients were excluded from the analysis. The remaining 103 patients fulfilled the inclusion criteria and were evaluated in this study (**Fig. 1**).

SB-IPMNs were defined as cystically dilated side-branch lesions with documented ductal communication, and the presence of mucin identified as filling defects in the main duct or side branch by endoscopic retrograde cholangiopancreatography (ERCP). Patients were diagnosed on the basis of the combined results of ERCP, computed tomographic (CT) scans, magnetic resonance cholangiopancreatography (MRCP), EUS, intraductal ultrasonography (IDUS), and peroral pancreatoscopy (POPS).

The follow-up protocol comprised an at least annual review of all new symptoms and signs, and annual imaging studies, primarily EUS. Initially all EUS studies were done using a mechanical radial echo endoscope, but from 1997 curved linear array echo endoscopy was also used for measuring maximal cyst size, MPD diameter, and mural nodule size during follow-up studies. Solid components, wall thickness, and lymphadenopathy were also noted.

Progression of lesions was defined by an increase in the size of the MPD of ≥ 2 mm, of cyst size by ≥ 10 mm, or of mural nodule size by ≥ 1 mm, or the appearance of a pancreatic mass on follow-up imaging. Progression was divided into two categories: higher and lower likelihood of malignancy. Higher likelihood of malignancy was defined as size of mural nodule(s) ≥ 10 mm and/or MPD size of ≥ 10 mm, and/or a rapid increase in size of mural nodule(s) and/or MPD (≥ 5 mm increase since the previous imaging), and/or new appearance of mural nodule(s) in the MPD, and/or the appearance of a pancreatic mass. Progression with lower likelihood of malignancy was defined as an increase in

Age, median (range), years	63 (38 – 84)
Gender, male : female	58 : 45
Follow-up period, median (range), months	59 (24 – 151)
Number of EUS examinations per-patient, median (range), n	5.0 (1* – 14)
Asymptomatic at the initial diagnosis, n (%)	93 (90.3)
Cyst size at the initial diagnosis, median (range), mm	18.0 (4.0 – 50.0)
Presence of mural nodule at initial diagnosis, n (%)	18 (17.5)
Mural nodule size at initial diagnosis, median (range), mm	0.0 (0.0 – 5.0)
Main pancreatic duct diameter at initial diagnosis, median (range), mm	3.0 (1.0 – 7.0)

EUS, endoscopic ultrasonography

* Three patients underwent EUS examination only once; two had further follow-up by ultrasound and computed tomography (CT), and one by endoscopic retrograde cholangiopancreatography (ERCP) and CT.

Table 1 Clinical features in 103 patients with side-branch intraductal papillary mucinous neoplasm (SB-IPMN).

size of the lesions on imaging that did not fulfil the abovementioned criteria.

If there was progression with higher likelihood of malignancy, the patient underwent surgery. In the case of progression with lower likelihood of malignancy, the patient underwent ERCP and CT scan. Those patients in whom cytology detected malignant cells in pancreatic juice underwent surgery, while others were followed up 6 months later. In the case of no progression, we continued annual follow-up.

At our institution, indications for surgery were, as mentioned, progression with higher likelihood of malignancy or cytological detection of malignant cells in pancreatic juice; the presence of significant symptoms (e.g. acute pancreatitis) was another indication. The pathological findings from the resected lesions were reviewed by two pathologists (Y.Y. and A.Y.). Based on the most significant degree of cytoarchitectural atypia, the intraductal components of each tumor were classified as IPMN adenoma, IPMN borderline, IPMN carcinoma in situ (noninvasive IPM carcinoma), or invasive IPM carcinoma, according to the World Health Organization (WHO) classification system [2]. Invasive IPM carcinoma defined according to the WHO system was subclassified into minimally invasive IPM carcinoma and invasive IPM carcinoma, according to the Japan Pancreas Society (JPS) classification [19]. Thus, with the further category of non-IPMN invasive ductal carcinoma of the pancreas, we classified all the tumors into one of six categories.

Statistical analysis

Continuous variables are described using median and range. Patients who developed cancer (noninvasive and invasive) during follow-up were compared with those who remained cancer-free. Intergroup comparisons were done using the χ^2 or Fisher's test for categorical variables, and the Mann-Whitney *U* test for continuous variables. The development rate of pancreatic cancer was estimated using the Kaplan-Meier method. *P* values of < 0.05 were considered significant. Data were statistically analyzed using SPSS software, version 11.0 (SPSS Inc., Chicago, Illinois, USA) and StatView statistical software, version 5.0 (Abacus Concepts Inc, Berkeley, California, USA).

Results

Patient characteristics

The 103 study patients included 58 men (56.3%). The median age of the cohort at the initial diagnosis was 63 years (range 38 – 84). A total of 93 patients (90.3%) were asymptomatic at the initial diagnosis. All patients were followed up for ≥ 2 years, with a median follow-up duration of 59 months (range 24 – 151). Five patients (4.9%) developed symptoms during follow-up. The median number of EUS examinations per-patient during the follow-up period was 5.0 (range 1 – 14). Though three patients underwent EUS examination only once, two of them were followed up using ultrasound and CT, and one using ERCP and CT. At the initial diagnosis, 18 patients (17.5%) had mural nodules.

The median cyst size was 18 mm (range 4.0 – 50.0), median MPD diameter was 3.0 mm (range 1.0 – 7.0), and the median mural nodule was 0.0 mm (range 0.0 – 5.0) in the 103 study patients at the initial diagnosis. The clinical details of the included patients are given in ● **Table 1**.

Progression of lesions

Progression of lesions, as defined earlier, was monitored by serial EUS studies. A total of 10 SB-IPMNs (9.71%) progressed with higher likelihood of malignancy, 19 (18.45%) progressed with lower likelihood of malignancy, and 74 (71.84%) did not progress (● **Fig. 2**).

Requirement for surgery and postoperative pathological findings

We operated on 7 of 10 patients who had progressive lesions with higher likelihood of malignancy, 1 of 19 patients with lower likelihood of malignancy, and 3 of 74 patients with nonprogressive lesions (● **Fig. 2**). Regarding the three patients with higher likelihood of malignancy who did not undergo operation, one was diagnosed as having unresectable invasive IPM carcinoma, one rejected surgery, and one could not have an operation because of respiratory failure. The patient with lower likelihood of malignancy underwent surgery because of recurrent acute pancreatitis. The indications for operation in the three patients with nonprogressive lesions were acute pancreatitis in two patients, and synchronous carcinoid of the papilla in one.

The types of surgery included pancreaticoduodenectomy (n = 6), middle segment pancreatectomy (n = 2), pylorus-preserving pancreaticoduodenectomy (n = 1), distal pancreatectomy (n = 1) and total pancreatectomy (n = 1).

The final pathological diagnoses were: adenomas 5, borderline 1, noninvasive IPM carcinomas 2, minimally invasive IPM carcinoma 1, and non-IPMN invasive ductal carcinomas of pancreas 2. The lesions in all patients with a final diagnosis of malignant disease had shown progression with higher likelihood of malignancy on imaging, while the pathological diagnoses in the four patients who underwent surgery because of pancreatitis (n = 3) and synchronous carcinoid of papilla (n = 1) were adenomas in three cases and borderline IPMN in one case.

Incidence of pancreatic cancer

Pancreatic cancers developed in 6 of 103 patients (5.8%) during the follow-up. These included invasive ductal carcinoma of the pancreas (n = 2), noninvasive IPM carcinoma (n = 2), minimally

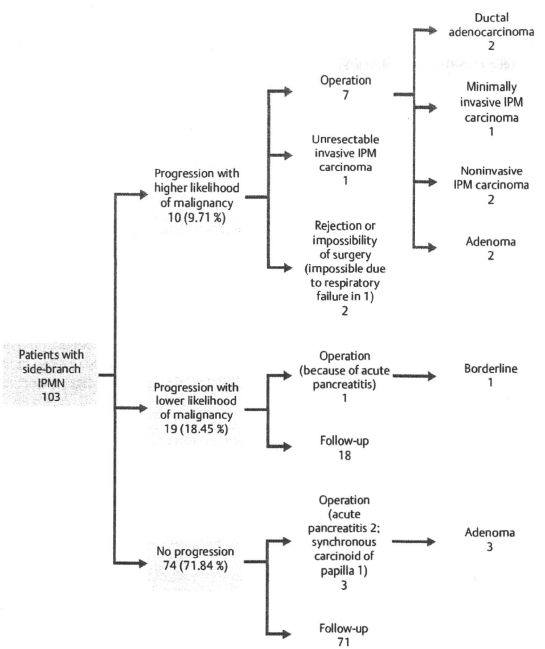

Fig. 2 Follow-up findings in patients with side-branch intraductal papillary mucinous neoplasm (SB-IPMNs).

invasive IPM carcinoma (n = 1), and an unresectable invasive IPM carcinoma (n = 1). The only unresectable carcinoma occurred in a patient 7 years after the initial diagnosis of IPMN. This patient was not regularly examined by EUS. The 5-year and 10-year actuarial rates of pancreatic cancer development were 2.4% and 20.0%, respectively (**Fig. 3**).

Table 2 shows the characteristics of patients who developed pancreatic cancers.

Incidence of extrapancreatic cancers

Overall, 24 cancers of other organs occurred concurrently or subsequently in 18 patients (17.5%). These included five gastric, five colon, three prostate, two breast, two thyroid, two laryngeal, one bladder, one lung, one uterine, and one renal cell carcinoma, and one malignant lymphoma.

Predictors of IPM carcinoma

For evaluating the predictive factors for development of IPM carcinoma, we excluded two patients with ductal adenocarcinoma unrelated to SB-IPMNs, one patient who rejected surgery, and one patient who could not undergo operation because of respiratory failure.

There was no difference in the baseline parameters of gender, age, symptoms, initial MPD diameter, and initial cyst and mural nodule size between patients who developed IPM carcinoma during follow-up and those who did not. However, at the initial diagnosis, the frequency of extrapancreatic cancers was significantly higher in the group who went on to develop IPM carcinoma (**Table 3**).

At the latest diagnosis, cyst size, MPD diameter, and mural nodule diameter were significantly larger in the patient group who had developed IPM carcinoma than in the group who had not, but there was no difference in the frequency of symptoms and the age of patients between the two groups. The frequency of extrapancreatic cancers was also significantly higher in the patients who had developed IPM carcinoma than in those who had not (**Table 4**).

Discussion

The International Association of Pancreatology (IAP) guidelines suggest that management of patients with SB-IPMN should be either follow-up or operation, depending upon the MPD diameter, and the cyst size and mural nodule size.

Although, according to the abovementioned guidelines, cysts of diameter ≥ 30 mm should be surgically resected [9], in practice the cutoff size for operating on cysts remains controversial. Schmidt et al. [20] operated on 103 patients with SB-IPMN and found that there was no difference in the size of cysts between the different pathological types of adenoma, borderline, carcinoma in situ (CIS), and invasive carcinoma. Salvia et al. [21] followed