at 55 kDa. Increase of reaction with the bands was observed with recombinant MAGE-A1 protein in sera from E-8, with recombinant MAGE-A4 protein in sera from E-2, E-4, E-5, E-8 and P-2 and with p53 in sera from E-7, E-8 and P-3 obtained after vaccination.

Specificity of the reaction was further confirmed using transfectants. As shown in Figure 5, sera from E-4 after vaccination and from E-5 before and after vaccination reacted to MAGE-A4 in lysate of MAGE-A4-transfected murine fibrosarcoma CMS5a cells. No reaction was observed with lysate of mock-transfected CMS5a cells.

Discussion

Efficient elicitation of host immune response is a prerequisite for successful immunotherapy using cancer vaccine, and immune monitoring of specific antibody, CD4 and CD8 T cell responses against tumor antigens after vaccination is crucial to evaluate the response. In our study, we investigated antibody response against 13 tumor antigens by ELISA using recombinant proteins to evaluate the immune response more precisely. Nine of ten patients analyzed except E-6 showed an increase or induction of antibody response against NY-ESO-1 and its related LAGE-1 antigen after CHP-NY-ESO-1 vaccination. Eight of these nine patients showed an increase or induction of antibody response to either of these antigens after vaccination. Previously, it was reported that sera from patients vaccinated with recombinant NY-ESO-1 protein and CpG in Montanide sometimes showed nonspecific production of antibody against other recombinant proteins used for control,[11,37] and some of these responses could be attributed to reactivity against bacterial components or His6-tag. To address this possibility, we performed specificity analysis of the antibody response using control recombinant proteins, synthetic peptides and by Western blot that showed heteroclitic responses were not against His6-tag and/or bacterial products included in a preparation of CHP-NY-ESO-1 used for vaccination.

We reported previously that those patients showed NY-ESO-1 specific antibody and CD4 and CD8 T cell responses during vaccination.[14,15] The findings suggest that increase or induction of antibody response against tumor antigens, *e.g.*, MAGE-A3 and MAGE-A4, as well as NY-ESO-1 after CHP-NY-ESO-1 vaccination may be caused by their release from tumor cells damaged by NY-ESO-1-specific immunity. Therefore, antibody response to multiple tumor antigens may suggest an intensity of the overall host immune response against the tumor, and detection of multiple heteroclitic serological responses using a panel of recombinant proteins would be a new tool of immunological monitoring for antitumor responses. A clear correlation between heteroclitic antibody responses and clinical outcomes could not be established in the limited number of patients analyzed in our study (Table 1). However, antibody response as well as CD4 and/or CD8 T cell responses to heteroclitic tumor antigens would be useful for evaluating overall immune response to tumor.

A number of studies have shown the relationship between heteroclitic immune response and clinical response. Germeau *et al.*[19] reported that the frequency of CTL precursor increased tenfold in some patients after vaccination using MAGE antigenic peptides, although they found no significant difference in the levels against immunizing antigens between the tumor-regressor and -progressor patients. They then analyzed CTL precursors against other tumor antigens than that utilized for vaccine and found that the immune responses elicited to those irrelevant antigens after vaccination might contribute to the whole immune response to a given tumor and was correlated to clinical responses. Similarly, Butterfield *et al.*[23,24] reported that peptide-specific T cell response was efficiently induced in most patients by immunization with MART-1/Melan-A peptide pulsed dendritic cells. However, cellular immune responses against not only MART-1/Melan-A but also gp100 and tyrosinase were detected only in a complete clinical responder. These findings suggest a relationship between heteroclitic CTL responses and clinical responses. Furthermore, Disis *et al.* reported induction of both cellular and humoral responses against other intramolecular determinants in patients immunized with HER-2/neu peptide vaccine, and of antibody response to p53 in patients immunized with HER-2/neu peptide vaccine.[17,22] They further studied the effect of HER-2/neu T-helper peptide-based vaccinated patients receiving trastuzumab therapy and observed prolonged immune responses against not only the vaccine antigen but also cryptic antigens.[38] Collectively, the presence of either humoral or cellular immune response to multiple tumor antigens appears to be indicative of the strength of overall response against the tumor and predictive of clinical response. In our study, we used a panel of 13 tumor antigens for the detection of the humoral response. Serological detection of responses to multiple tumor antigens that were shown to be highly immunogenic in cancer patients would be convenient and could be included in routine immune monitoring.

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Minimally Invasive Esophagectomy for Esophageal Cancer: Comparative Analysis of Open and Hand-Assisted Laparoscopic Abdominal Lymphadenectomy with Gastric Conduit Reconstruction

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Background and Objectives: Esophagectomy for esophageal cancer is an invasive procedure. Minimally invasive approaches such as hand-assisted laparoscopic surgery (HALS) might reduce surgical stress and improve postoperative course.

Methods: We retrospectively analyzed 216 consecutive patients who underwent esophagectomy for esophageal cancer through either HALS (109 patients) or open laparotomy (107 patients), through an abdominal approach. The peri- and postoperative outcomes were compared between the two groups.

Results: No significant difference was observed in physical and tumor status between the two groups. The mean operating time (HALS: 452 ± 65, Open: 456 ± 69 min) and mean number of resected lymph nodes (HALS: 19.3 ± 7.1, Open: 20.8 ± 8.3) were similar, while total blood loss was lower in HALS (HALS: 695 ± 369, Open: 1,101 ± 540 ml; $P = 0.0001$). The postoperative course showed marginally lower incidences of pulmonary (HALS: 6.4%, Open: 14.0%; $P = 0.062$) and overall complications (HALS: 23.9%, Open: 35.5%; $P = 0.11$), lower C-reactive protein level at postoperative days 1, 3, and 7, and shorter duration of systemic inflammatory response syndrome (HALS: 2.3 days, Open: 3.5 days; $P = 0.0002$) in HALS than in OPEN. The disease-free survival rates at 2 years were 65% in HALS and 53% in Open.

Conclusions: The findings suggest that HALS is feasible and useful for patients with esophageal cancer.

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KEY WORDS: HALS; esophagectomy; esophageal cancer

INTRODUCTION

Radical esophagectomy currently provides the best cure for resectable esophageal cancer. However, it is a highly invasive surgical procedure associated with high morbidity and mortality. The reported postoperative morbidity rate ranges from 45% to 80%, even in high volume centers [1,2]. Especially, respiratory complications occur at a frequency of 30–75% and are often severe and potentially lethal [3,4]. Therefore, there is a need for alternative techniques that do not only have acceptable oncological outcome but also diminish surgical stress and postoperative complications.

In 1980s, laparoscopic techniques were introduced to the field of upper gastrointestinal surgery and colorectal surgery as less invasive surgery, and subsequent reports indicated that such techniques reduce postoperative pain and complications and hasten recovery [5–7]. In esophageal cancer, esophagectomy via thoracoscopic procedures was introduced in 1992, and various less invasive techniques such as thoracoscopy, laparoscopy, and their combination have since been reported [8–10]. Despite their wide use in clinical practice, the safety and efficacy of these techniques remain controversial [11,12].

Hand-assisted laparoscopic surgery (HALS) has recently gained clinical acceptance as a practical and useful alternative technique to laparoscopic and open surgery [13–16]. HALS is not only less invasive than open surgery but also causes less damage to organs and is easier than laparoscopic surgery based on its manual nature and ability to use retractors. In theory, HALS seems appropriate procedure, similar to the abdominal approach in radical esophagectomy, for patients with thoracic esophageal cancer. To date, no report has evaluated the feasibility and usefulness of HALS compared with open laparotomy.

The aim of the present study was to evaluate the feasibility and usefulness of HALS, and to compare it with the abdominal approach in radical esophagectomy. The study also compared the short-term and mid-term outcomes with those of the open procedure.

PATIENTS AND METHODS

Patients

From January 2005 to January 2010, a total of 310 patients diagnosed histopathologically with esophageal squamous cell carcinoma (ESCC) underwent surgery at Osaka University Hospital. All patients were new cases of ESCC and none had received treatment prior to surgery. All underwent esophageal fiberscopy, esophagography, and enhanced computed tomography (CT) scanning from the neck to the abdomen for tumor staging according to the 6th edition of the TNM classification of the International Union Against Cancer (UICC).

All 216 consecutive patients who met the following inclusion and exclusion criteria were enrolled in this study: (1) subtotal esophagectomy and mediastinal lymph node dissection was performed through right thoracotomy; (2) the primary tumor was located in the thoracic esophagus; (3) the reconstructive organ was the stomach but not the jejunum or colon; (4) the primary tumor or metastatic lymph nodes showed no contiguous spread to adjacent organs such as trachea, aorta, lung in the preoperative evaluation, unless these tumors were

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regarded as completely resectable; (5) no history of previous upper abdominal surgery; (6) no abdominal procedure by totally laparoscopy and/or video-assisted thoracoscopic surgery (VATS); (7) no additional abdominal para-aortic lymph nodes dissection or resection of other organs such as the gallbladder; (8) no pull-up procedure of the gastric conduit through retrosternal or subcutaneous route; and (9) lack of active malignancy in another organ. Abdominal lymphadenectomy and gastric conduit reconstruction was performed by HALS group in 109, while the remaining 106 patients underwent open laparotomy (Open group). In our institution, open laparotomy was performed prior to September 2007 while HALS was employed from October 2007 onwards. Each of the open operations were performed by four upper-GI specialists, while HALS was performed by only two (M.Y. and H.M.). In the first 2 months of the introduction of HALS, only one surgeon (M.Y.) performed HALS as the chief operator with the assistance of the other surgeon (October–November 2007).

Treatment Protocol

The basic strategy for treatment of patients with ESCC had been described previously [17]. In brief, cT4 were indicated for chemoradiotherapy as the initial treatment, and then surgical resection was performed when patients were diagnosed as downstage, release of T4. On the other hand, cN1and/or cM1lym with cT1-3 were indicated for neoadjuvant chemotherapy followed by surgery. Furthermore, cT1-3N0 was indicated for surgery without preoperative treatment between January 2005 and January 2009, and cT2-3N0 was indicated for surgery with neoadjuvant chemotherapy after January 2009. Surgery was performed 4–8 weeks after preoperative chemotherapy.

The preoperative diagnostic workup included physical examination, chest X-ray, lung function assessment by spirometry and arterial blood gases, liver and renal functions by laboratory tests, electrocardiography (ECG), and full assessment for anesthesia.

The postoperative follow-up evaluation was performed every 3–4 months for the first 2 years and every 6 months thereafter by CT scanning and annual endoscopy for 5 years.

Surgery

In October 2008, we introduced VATS combined with 5 cm minithoracotomy, a surgical technique similar to the procedure described by Osugi et al. [18] and have performed it since then in 14 patients. However, these 14 patients were excluded from the present study. The patient was placed on the right-side-up lateral position using left single lung ventilation under general anesthesia, and esophagectomy and mediastinal dissection with extensive lymphadenectomy were performed under a combination of direct visual and thoracoscopically visualized guidance through a 10 cm right thoracotomy through the fourth intercostals space. Subsequently, the patients were repositioned in the supine position, and abdominal lymphadenectomy and gastric conduit reconstruction were performed using either the open technique with an upper-middle abdominal midline laparotomy (fossa epigastrica to umbilicus) or HALS technique with 7 cm upper abdominal midline incision (through which the surgeon's left hand was inserted) and three 5–12 mm long incisions (through which the trocar tubes were inserted; Fig. 1). Following the use of different approaches to the abdominal cavity, the surgical procedures were identical between the two groups:

(1) The greater curve of the stomach was divided taking care in preservation of the gastroepiploic arcade, and the left crus was dissected and incised, allowing hiatal enlargement for lower mediastinal dissection.
(2) The gastrohepatic ligament was divided, and the right crus was dissected.
(3) The left gastric artery was transected at its base on the celiac axis, and the lymph nodes in this region and those around the proximal splenic artery and common hepatic artery were dissected en bloc.
(4) The separated anal side of the esophagus and stomach were exteriorized from the peritoneal cavity. Then the sub-total gastric conduit was constructed. Pyloroplasty was performed manually.
(5) Pull-up of the gastric conduit through the posterior mediastinal route was performed under surgeon's hand guidance, taking care in avoiding torsion, and gently delivering the stomach upward through the hiatus.
(6) Esophagogastric anastomosis was performed by circular end-to-side stapling at the neck.

Based on the location of the primary tumor and the presence or absence of metastases in the lymph nodes chain along the recurrent laryngeal nerve, patients underwent additional cervical lymph node dissection [19–21]. The insertion of a feeding tube in the jejunum was not routinely performed except for patients older than 75 years of age and those with performance status of more than 2.

We applied two energy devices to the surgical procedure involving the abdominal cavity. First, a harmonic scalpel (Ultracision; Ethicon Laboratories, Cincinnati, OH) was used in almost all steps of the dissection in HALS, while hemoclips or ligations were applied to the left gastric and left gastroepiploic vessels. Next, monopolar electrosurgery was used for cutting and coagulation and ligation to obstruct the blood vessels in open laparotomy.

Perioperative Management

In each patient, an epidural catheter was placed into the epidural space between the 5th and 6th thoracic vertebrae, for the injection of an epidural anesthetic postoperatively. Furthermore, each patient received 250 mg of methylprednisolone intravenously perioperatively. All patients were transferred to the intensive care unit (ICU) after surgery and extubated at postoperative day (POD) 1, except when requiring respiratory support. Patients who had difficulty in coughing up sputum and airway secretions underwent bronchoscopy

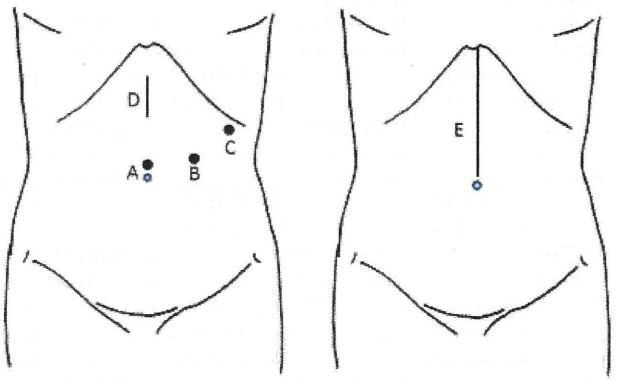

Fig. 1. Diagram of port placement and incision line for abdominal lymph node dissection and gastric mobilization in hand-assisted laparoscopic surgery (HALS) and Open laparotomy. Ports and incisions are placed in this order: Surgeon's right hand (12 mm, **A**), Camera (12 mm, **B**), Grasper (5 mm, **C**), Surgeon's left hand (7 cm, **D**), open laparotomy (**E**).

to clear these secretions from the respiratory tract. Furthermore, a mini-endotracheal tube was inserted to suction excess secretion, remove secretion difficult to discharge and/or aspirate material from the tracheobronchial tree in patients who required more than twice daily the above bronchoscopy. A nasogastric tube was inserted to suction fluids and air from the stomach. These were intermittently suctioned by the nasogastric tube which was connected to the suction device in the first 3 POD. The tube was removed at POD 7 and food intake was allowed if no evidence of leakage was clinically evident at POD 8. Enteral nutrition started at POD 2 in patients with a jejunum feeding tube. There were no differences in the perioperative management in this study.

Postoperative complications were evaluated according to the NCI-CTCAE, version 3. Grade 2 and over adverse events were defined as the appearance of complications. Systemic inflammatory response syndrome (SIRS) was defined by the presence of at least two of the following criteria: (1) heart rate >90 beats/min, (2) respiratory rate >20 breaths/min, (3) body temperature >38 or <36°C, and (4) blood leukocyte count $>12 \times 10^3/mm^3$ or $<4 \times 10^3/mm^3$.

Statistical Analysis

Values were expressed as mean ± standard deviation. Comparison of data of two groups was undertaken using the chi-squared test for categorical data, and the Student *t*-test or Mann–Whitney *U*-test for continuous data. Survival was calculated with the Kaplan–Meier method and differences between groups were evaluated by the log-rank test. Statistical significance for each model was set at $P < 0.05$.

RESULTS

Patients' Demographics

Table I summarizes the characteristics of each group. There was a greater utilization of neoadjuvant chemotherapy in the HALS group ($P = 0.02$), and a greater proportion of cases with advanced disease, albeit insignificantly, in HALS group than Open group ($P = 0.08$). Thus, the differences between the two groups reflect the histopathological changes in the treatment of resectable esophageal cancers in our institution. There was no difference in age, sex, performance status, body mass index (BMI), and preoperative comorbidity between the two groups.

TABLE I. Patient Characteristics and Pathology Demographics of Patients of the Hand-Assisted Laparoscopic Surgery (HALS) and Open Surgery (Open) Groups

	HALS	Open	*P*-value
Number	109	107	
Procedure period	Oct 2007–Jan 2010	Jan 2005–Sep 2007	
Age (years)[a]	64.6 ± 8.5	64.7 ± 8.0	0.99
Sex (M:F)	87:22	95:12	0.1
Performance status (0:1/2)	88:20/1	84:23/0	0.74
BMI (kg/m^2)[a]	21.1 ± 3.1	21.0 ± 2.8	0.62
Location (Ut:Mt:Lt)	23:59:27	18:49:40	0.25
cT (1:2:3:4)	11:24:57:17	17:26:47:17	0.5
cN (0:1)	35:74	43:64	0.22
cStage (1:2:3:4)	7:34:48:20	13:37:44:13	0.08
Neoadjuvant therapy (none:CT:CRT)	24:69:16	39:48:20	0.02
Comorbidity			
Cardiovascular disease	6	4	0.54
Diabetes	10	6	0.31
Hypertension	14	16	0.65
Pulmonary diseases	11	13	0.63
Liver dysfunction	5	9	0.25
Other cancers	5	2	0.25

BMI, body mass index; Ut, upper third of the esophagus; Mt, middle third; Lt, lower third; CT, chemotherapy; CRT, chemoradiotherapy.
[a]Data are mean or number of patients.

Operative Outcome

The operative outcome is summarized for each group in Table II. The mean operating time and time of abdominal manipulation were similar in the two groups. The total and abdominal blood loss was significantly less in the HALS group than in the Open group. The amount of blood transfusion was significantly less in the HALS group than in the Open group (1.2 U vs. 2.1 U; $P = 0.008$). On the other hand, intraoperative fluid balance was similar in the two groups (HALS: 2.2 L, Open: 2.3 L; $P = 0.21$). Three-field lymphadenectomy was performed in 56 and 60 patients in the Open and HALS groups, respectively ($P = 0.69$). None of the HALS procedure required conversion to open surgery.

Postoperative Outcome

Table III summarizes the postoperative outcome of both groups. Serum C-reactive protein (CRP) levels at POD 1, 3, and 7 were significantly lower in the HALS group than Open group. The duration of SIRS was also significantly shorter in the HALS group than in the Open group (2.3 days vs. 3.5 days; $P = 0.0002$). The durations of ICU and hospital stay were similar in both groups. The volume of discharge from the abdominal drain on POD 2 and POD 3 was significantly lower in the HALS group than Open group. The time until abdominal drain removal was similar in both groups. The volume of discharge from the nasogastric tube on POD 1, 2, and 3 was also significantly lower in the HALS group than in the Open group (data not shown). The average volume of discharge from the chest drain in the 5 days was similar in both groups (HALS: 221 ml, Open: 234 ml; $P = 0.11$). The time to passing the first flatus was significantly shorter in the HALS group than Open group. The duration of analgesia was similar in both groups (data not shown).

The postoperative complications are shown in Table III. Complications occurred in 64 of 216 patients (29.6%), and the rate in the HALS group (23.9%) was lower than the Open group. The rate of pneumonia in the HALS group was lower, albeit statistically

TABLE II. Operative Outcome in the Hand-Assisted Laparoscopic Surgery (HALS) and Open Surgery (Open) Groups

	HALS	Open	*P*-value
Cervical LN dissection			
No	49	51	0.69
Yes	60	56	
Number of resected abdominal LN	19.3 ± 7.1	20.8 ± 8.3	0.14
Total operating time (min)	452 ± 65	456 ± 69	0.67
Abdominal operating time (min)	172 ± 49	172 ± 44	0.98
Total blood loss (ml)	695 ± 369	1101 ± 540	0.0001
Blood loss in abdominal procedure (ml)	233 ± 222	591 ± 400	0.0001
Amount of transfusion (unit)	1.2 ± 2.1	2.1 ± 2.6	0.008
Intraoperative fluid balance (L)	2.2 ± 0.88	2.3 ± 0.77	0.21
Type of resection			
R0	108	105	0.55
R1	1	2	

LN, lymph node.
Data are mean ± SD or number of patients.

TABLE III. Postoperative Outcome of the Hand-Assisted Laparoscopic Surgery (HALS) and Open Surgery (Open) Groups

	HALS (%)	Open (%)	*P*-value
Complications	26 (23.9)	38 (35.5)	0.11
Anastomotic leakage	6 (5.5)	4 (3.7)	0.54
Pneumonia	7 (6.4)	15 (14.0)	0.062
Chylothorax	2 (1.8)	2 (1.9)	0.98
Hemorrhage	1 (0.9)	3 (2.8)	0.30
Vocal cord palsy	17 (15.6)	20 (18.7)	0.55
Arrhythmias	3 (2.8)	6 (5.6)	0.29
Delirium	0 (0)	2 (1.9)	0.15
Complete conduit ischemia	0 (0)	2 (1.9)	0.15
Mini-endotracheal tubing	21 (19.3)	52 (48.6)	<0.0001
Duration of oxygen use (days)	5.2 ± 6.7	7.8 ± 10.2	0.026
Hospital stay (days)	29.9 ± 15	33.0 ± 25	0.28
In-hospital deaths	0 (0)	2 (1.9)	0.24
Duration of SIRS (days)	2.32 ± 1.6	3.50 ± 2.7	0.0002
Postoperative CRP (mg/dl)			
POD 1	6.3 ± 1.9	7.6 ± 2.7	0.0001
POD 3	13.6 ± 6.4	15.5 ± 5.6	0.019
POD 7	5.4 ± 4.8	6.6 ± 4.1	0.046
Time to passing flatus (days)	3.8 ± 1.9	4.8 ± 1.8	0.0001
Discharge from abdominal drain (ml)			
POD 1	148 ± 134	165 ± 206	0.48
POD 2	110 ± 99	172 ± 186	0.0032
POD 3	77.7 ± 126	142 ± 231	0.027
Discharge from chest drain (ml)	221 ± 43	234 ± 69	0.11

SIRS, systematic inflammatory response syndrome; CRP, C-reactive protein; POD, postoperative day.
Data are mean ± SD or number (%) of patients.

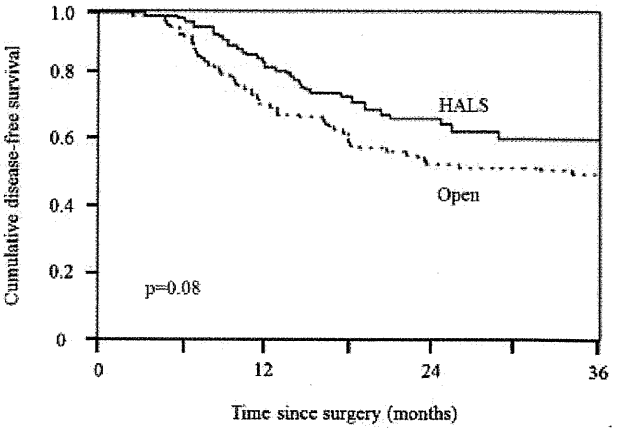

Fig. 2. Kaplan–Meier curves of disease-free survival rates of patients of the HALS group and Open group.

insignificant, than in the Open group (6.4% vs. 14.0%; $P = 0.06$). The proportion of patients who required bronchoscopy was significantly lower in the HALS group (27.5%) than the Open group (60.8%; $P < 0.0001$). The proportion of patients who required insertion of a mini-endotracheal tube was significantly lower in the HALS group (19.3%) than the Open group (48.6%; $P < 0.0001$). The duration of oxygen use during the postoperative period was significantly shorter in the HALS group (5.2 days) than in the Open group (7.8 days; $P = 0.026$). Vocal cord palsy occurred in 37 of 216 patients (17.1%), but was only transient, and the rate of affected patients was similar in both groups.

Outcome of Cancer Treatment

The number of harvested abdominal lymph nodes was not different between the two groups (mean: Open = 20.8, HALS = 19.3; $P = 0.14$). The numbers of patients who developed recurrence of abdominal lymph nodes after surgical dissection were 4 and 2 patients in the Open and HALS group, respectively. The median follow-up period was 35.4 months in the Open group and 20.1 months in the HALS group. The disease-free survival rates of the two groups are shown in Figure 2. The 2-year disease-free survival rate of the Open group was 53.0% compared with 64.9% for the HALS group, but the difference was not statistically significant ($P = 0.08$).

DISCUSSION

The main finding of the present study was the superiority of HALS compared with open laparotomy, based on the following results: (1) HALS resulted in a significantly lower blood loss, (2) HALS did not require longer operating time, (3) low rate of perioperative complications after HALS, (4) significant reduction of postoperative CRP level after HALS, and (5) HALS was associated with early recovery from SIRS. These results indicate that HALS is less invasive than Open laparotomy.

The study showed a lower rate of postoperative complications, especially pulmonary complications, after HALS than open surgery. In addition, it showed a significantly lower need for bronchoscopy and use of a mini-endotracheal tube, and shorter use of oxygen. This could be due to the intact abdominal wall with no associated pain during coughing. This result is clinically meaningful since pulmonary complications were one of the most common reasons of postoperative mortality.

The present study also showed a lower incidence of discharge from the nasogastric tube and abdominal drain and shorter time to the passage of the first flatus after HALS than Open laparotomy. These findings suggest that HALS allows earlier restoration of digestive function, in agreement with previous studies that reported decreased gut dysmotility after endoscopy-assisted laparoscopic surgery and HALS in patients with various colorectal and gastric conditions [22,23].

Admittedly, one must take account of the effects of the ancillary surgical instrumentations, especially energy devices, on the outcome. In the present study, the energy source used in HALS procedure was ultrasonic-activated shears. In comparison, open procedure was performed using monopolar electrosurgery. Some studies reported that the use of the new energy devices such as ultrasonic-activated shears is associated with lower blood loss compared with conventional hemostatic techniques such as monopolar electosurgery [24,25]. Whereas the use of the energy device in HALS could have contributed to the lower blood loss, relative to the open procedure, a larger proportion of the low blood loss in HALS was due to the procedure itself; HALS allowed the surgeon to identify and treat the fine anatomic structures through better visualization using the endoscope, and required the use of only a small abdominal incision.

Another important issue that needs to be discussed is the potential bias introduced by the selection of the surgical operation. In present study, the following aspects of the study design should minimize any such bias. The operative procedure was uniformly performed in all patients because three or all surgeons of four upper-GI specialists participated in each operation as the chief surgeon or assistant surgeon during the entire period of this study. Analysis of the differences among surgeons showed similar operative and postoperative

outcomes among the four surgeons (data not shown). There were also no differences in the operative and postoperative outcomes according to the time these procedures were conducted (early and late periods) between open laparotomy and HALS (data not shown).

We employed HALS, but not laparoscopic surgery, as minimally invasive esophagectomy. Many studies have reported that laparoscopic surgery has several advantages based on the minimal access approach such as lower operative blood loss, less pain, earlier recovery of bowel activity, and a shorter hospital stay [22,26,27]. However, the laparoscopic procedures are time-consuming and technically demanding and hence have a long learning curve [28]. In comparison, HALS permits direct tactile sensation and allows gentle retraction of large masses of tissues, which enhance exposure, identification of anatomic structures, rapid control of bleeding, and avoidance of tissue injures [14–16]. In particular, HALS was safer and more useful than laparoscopic surgery in the reservation and management of gastric conduit reconstruction and abdominal lymphadenectomy during radical esophagectomy, because it allowed the avoidance of gastric injury commonly seen following grasp with forceps, and assist in pulling up the gastric conduit under surgeon's hand guidance without any torsion. We have experienced only one complete conduit ischemia during laparoscopic surgery but none during HALS. Furthermore, mastering the HALS procedure hardly required a transitional period from open procedure because it is easy to learn and easy to teach as reported previously [29]. The widespread use of laparoscopic surgery, which has been performed in 270 patients with gastric cancer in our institution since 2007, attest to the ease of learning and executing this procedure. Therefore, we consider HALS as a more user-friendly and practical alternative to laparoscopic surgery for gastric conduit reconstruction and abdominal lymphadenectomy in radical esophagectomy.

Our concerns were the oncological results of HALS procedure in esophagectomy. In this study, the number of lymph nodes harvested and the rate of complete pathological resection were not different between the two procedures. Several studies have shown that the total number of surgically removed lymph nodes was independently associated with survival in esophageal cancer [30,31]. In fact, the number of harvested lymph nodes is considered a useful surrogate marker for surgical curability.

Analysis of the survival rate is the most important outcome in assessment of oncological results. In this cohort, there was no difference in the recurrence-free survival between the two groups, and the survival rate was comparable to that reported in other published series [32,33,11]. While our results showed the clear benefits of HALS over conventional open surgery for abdominal lymphadenectomy and gastric conduit reconstruction in radical esophagectomy, further assessment of prognosis and recurrence over a longer period is needed to provide meaningful long-term follow-up data.

CONCLUSIONS

The HALS procedure seems potentially feasible and beneficial for patients with esophageal cancer since it would reduce operative blood loss, the incidence of postoperative pneumonia and systemic inflammation, and allows early restoration of digestive function while retaining equally oncological curative effect compared with the open procedure.

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Survival Factors in Patients with Recurrence After Curative Resection of Esophageal Squamous Cell Carcinomas

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ABSTRACT

Background. Approximately half of patients who undergo curative resection for esophageal cancers develop recurrence postoperatively. The factors affecting survival after such recurrence remain largely unknown.

Methods. To investigate factors affecting survival after recurrence in patients who had undergone curative resection for esophageal cancer, we retrospectively reviewed data for 461 patients who underwent curative esophagectomy with or without preoperative therapy for esophageal squamous cell carcinoma from January 1996 to December 2007. The correlations between several clinicopathological factors and survival after recurrence were examined.

Results. Recurrence occurred in 196 of 461 patients (42.5%), with a median survival time after recurrence of 8.2 months. Multivariate analysis identified advanced tumor stage, preoperative chemoradiotherapy (CRT), number of recurrent tumors, and the presence of recurrence at the local site and liver as associated with shortened survival after recurrence. The analysis also indicated that treatment of the recurrence prolonged survival regardless of the treatment type. Although the pattern of recurrence did not significantly differ according to type of preoperative therapy, patients who underwent preoperative CRT were less often treated with radiotherapy for recurrence. Patients with multiple recurrent tumors less often received radiotherapy or surgery than those with a solitary recurrence. Chemotherapy for recurrence was not associated with either preoperative therapy or the number of recurrences.

Conclusions. Our retrospective study showed that multiple recurrent tumors and preoperative CRT limit the available treatment for recurrence and thereby are associated with poor prognosis. Vigorous treatment for recurrence can extend survival after recurrence in patients who undergo esophagectomy.

Surgical resection remains the primary treatment for thoracic esophageal cancers as it offers the best chance of cure. However, patients who undergo curative tumor resection often develop recurrent disease within a few years after surgery; the 5-year survival rates range from 31 to 55%.[1–4] Surgical series of such patients have documented the pattern and timing of recurrent disease and showed that the recurrence rate after curative esophagectomy ranges from 36% to 56% and the median time to recurrence ranges from 10 to 12 months.[3–10] Significant difficulty is often encountered in treating recurrent disease after esophagectomy, and patient prognosis is generally poor.[5–9] Thus, although recurrent disease after esophagectomy is not uncommon, a recommended treatment strategy remains to be established.

Multimodal treatment combining surgery with other treatments such as preoperative chemotherapy or chemoradiotherapy (CRT) is now used widely to improve resection strategies for esophageal cancers, although the associated survival benefit remains controversial.[11–15] Recent advances in anticancer drug and radiation techniques may particularly benefit patients with recurrent disease after curative resection and open up many new treatment options not previously available. In fact, recurrent disease sometimes responds better to anticancer

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treatment, and patients with recurrence can achieve relatively long-term survival. Thus, the factors affecting this survival after recurrence in patients with thoracic esophageal carcinomas need to be fully explored. Moreover, most patients included in previous studies of recurrent disease after esophagectomy received surgery alone without preoperative treatment, despite preoperative chemotherapy or CRT followed by surgery achieving mainstream status as a curative therapy for advanced esophageal cancer. Whether preoperative treatments affect the pattern of recurrence, treatment for recurrent disease, and survival after recurrence remains to be elucidated.

In the present study, we investigated those factors that affect survival of patients who experienced recurrence after curative resection for esophageal squamous cell carcinoma. Moreover, we also determined the pattern of recurrence according to type of preoperative treatment and examined whether preoperative therapy affects treatment for recurrent disease and survival after recurrence.

MATERIALS AND METHODS

Patient Population

From January 1996 to December 2007, 538 patients with thoracic esophageal cancer underwent surgery at The Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University. Among them, 31 patients underwent surgical resection via a transhiatal approach, and 18 patients underwent incomplete curative resection (R1 or R2). Excluding these 49 patients, 489 patients underwent curative esophagectomy (R0) with systematic lymphadenectomy. The primary tumor was typed histopathologically as squamous cell carcinoma in 461 patients, adenocarcinoma in 15, carcinosarcoma in 4, basaloid in 4, undifferentiated in 3, and melanoma in the remaining 2 patients. This study analyzed the 461 patients with squamous cell carcinoma, of whom 240 patients underwent 2-field lymphadenectomy and the remaining 221 patients underwent 3-field lymphadenectomy. During this period, indication for cervical lymph node dissection was determined, based on intraoperative genetic diagnosis of micrometastasis in recurrent laryngeal nerve chain nodes.[16,17]

Among 461 patients, 120 patients received preoperative chemotherapy followed by surgery and 83 patients received preoperative CRT followed by surgery; the remaining 258 patients received surgery alone. According to the principles of our institute, preoperative CRT followed by surgery was performed for patients showing deeply invading thoracic esophageal cancers (T3–T4) without distant organ metastasis or for those with tumors in the upper third of the thoracic esophagus that had infiltrated into the cervical esophagus. Preoperative chemotherapy followed by surgery was performed for patients with any T (cT1–4) and lymph node involvement, including regional lymph nodes (N1) and distant lymph nodes (M1 lym) without distant organ metastasis.

The study protocol was approved by the Human Ethics Review Committee of Osaka University Graduate School of Medicine.

Preoperative Treatment

The preoperative CRT regimen followed in our hospital comprises simultaneous radiation with administration of 5-fluorouracil (5-FU) and cisplatin as described previously.[18] A single daily fraction of 2 Gy was administered for 4–6 weeks, for a total dose of 40–60 Gy, concurrently with cisplatin and 5-FU. Preoperative chemotherapy in our institution consisted of cisplatin, adriamycin, and 5-FU. Cisplatin was administered at 70 mg/m,^{2} adriamycin at 35 mg/m^2 by rapid intravenous infusion on day 1, and 5-FU at 700 mg/m^2 administered by continuous intravenous infusion on day 1 through day 7. Two courses of chemotherapy were used, separated by a 4-week interval.[19]

Surgical Procedures

Our standard procedures consisted of subtotal esophagectomy with mediastinal lymphadenectomy via right thoracotomy, upper abdominal lymphadenectomy, reconstruction of a gastric tube, and anastomosis in the cervical incision. Two-field lymphadenectomy involved resection of the following lymph nodes based on the International Society for Disease of the Esophagus (ISDE): bilateral recurrent nerve nodes, upper-middle-lower thoracic paraesophageal nodes, tracheobronchial nodes, bifurcation nodes, bilateral main bronchus nodes, posterior mediastinal nodes, supradiaphragmatic nodes, cardiac nodes, celiac artery nodes, left gastric artery nodes, splenic artery nodes, lesser curvature nodes, and common hepatic artery nodes.[10,20] In cases of 3-field lymphadenectomy, deep cervical nodes, supraclavicular nodes, and cervical paraesophageal nodes were additionally removed. Abdominal para-aortic nodes were not usually removed, with rare exceptions. In patients undergoing preoperative chemotherapy or CRT, surgical resection was performed 3–6 weeks after completion of the preoperative therapy.

Adjuvant Therapy

During the period of this study in our institute, postoperative adjuvant therapy was not willingly undertaken. However, selected cases received postoperative adjuvant

therapy based on pathological tumor stage, particularly nodal status, tumor depth, and patient willingness. Of 461 patients, 66 patients (14.3%) received postoperative adjuvant therapy; 3 patients received radiotherapy alone, 7 patients received CRT consisting of simultaneous radiotherapy and administration of 5-FU/cisplatin, and 56 patients received chemotherapy alone (28 cases with 5-FU/cisplatin, 23 cases with intravenous administration of docetaxel or paclitaxel, and 5 cases with oral administration of S-1).

Follow-Up Examination and Definition of Recurrence Pattern

Following hospital discharge, patients were seen every 2 months for the first 2 years, and every 3 months thereafter. Computed tomography of the neck, thorax, and upper abdomen was performed every 4 months for the first 2 years and every 6 months thereafter. Upper gastrointestinal endoscopy was performed annually. When recurrence was suspected by computed tomography scan, more selective investigations such as positron emission tomography (PET), bone scintigraphy, and magnetic resonance imaging were performed to confirm or refute recurrent disease. During follow-up periods, the first site recurrence was noted, and any additional recurrence found within 1 month was considered to have occurred simultaneously. The pattern of recurrence was classified as follows: local recurrence was defined by recurrence at the site of the primary tumors, lymphatic recurrence was defined by recurrence at cervical, mediastinal, and/or abdominal lymph nodes, distant recurrence was defined as recurrence in distant organs such as lung, liver, bone, pleura, or peritoneum. The number of recurrent tumors was defined by adding up the number of recurrent nodules in each recurrent site. All data were collected, entered prospectively into a database, and updated at regular intervals. The median follow-up period of all 461 patients was 55.7 months (range, 22.1–105.8 months). Complete follow-up information until death or December 2009 was available for all patients.

Treatment for Recurrence

During the period of this study, available treatment was recommended for patients showing recurrent disease, providing that their general status permitted such a strategy and that the patient was willing. Surgery, radiation therapy, and different chemotherapy regimens were regarded as different treatments for recurrent disease in this study. Radiotherapy for recurrent disease was defined as that delivered at a dose of more than 30 Gy, excluding radiotherapy against bone metastasis for the purpose of palliative treatment. Radiotherapy delivered at different sites was defined as separate treatments for the purposes of this analysis.

Statistical Analysis

The pattern of recurrence and treatment for recurrence according to preoperative therapy and the number of recurrent tumors were compared using the chi-square test or Mann–Whitney U test. Overall survival was calculated from the date of operation to the event or last known date of follow-up. Actual survival was calculated according to Kaplan–Meyer and statistically evaluated by the log-rank test. The Cox proportional hazards regression model was used to analyze the simultaneous influence of prognostic factors. In all analyses, a P value $< .05$ was accepted as statistically significant. These analyses were carried out using StatView J 5.0 software package (Abacus Concepts, Berkeley, CA).

RESULTS

Pattern of Recurrence

Of 461 patients in this study, recurrence was observed in 196 patients (42.5%); Table 1 details the characteristics of these patients. Among various clinicopathological factors, histology, tumor depth, number of lymph node metastasis, tumor stage, and operative complications were associated with occurrence of recurrent disease. There was no statistically significant difference in incidence of recurrence according to preoperative treatment.

Local recurrence was observed in 36 patients, lymphatic recurrence in 125 (cervical in 35, thoracic in 76, abdominal in 42), and distant metastasis in 113 patients (sites were lung in 44, liver in 43, pleura or peritoneum in 27, bone in 25, skin in 4, brain in 4, adrenal gland in 1, and kidney in 1). Of the 196 cases of recurrent disease, 142 (72.4%) were recognized within the 1st year after resection, and 179 (91.3%) were within 2 years. The median time to recurrence overall was 7.3 months.

Treatment for Recurrent Disease

Of 196 patients, 52 (26.5%) received only treatment for symptomatic relief, while 144 (73.5%) patients received some form of therapy for recurrent disease including 120 (61.2%) of chemotherapy, 59 (30.1%) of radiotherapy, and 18 (9.2%) cases of surgical treatment (Table 2). In total, 235 treatment protocols were performed on the 144 patients; 74 received only 1 treatment, 53 received 2

TABLE 1 Characteristics of patients

	Total	Recurrence		*P* value
		Negative	Positive	
Cases	461	265 (57)	196 (43)	
Age	62.5 ± 8.2	62.7 ± 8.7	62.5 ± 8.9	0.7920
Gender				0.4179
Male	405	230 (57)	175 (43)	
Female	56	35 (63)	21 (37)	
Location				0.7422
Upper third	69	40 (58)	29 (42)	
Middle third	227	134 (59)	93 (41)	
Lower third	165	91 (55)	74 (45)	
Histology				0.0003
Well SCC	116	80 (69)	36 (31)	
Mod SCC	208	124 (60)	84 (40)	
Poorly SCC	137	61 (45)	76 (55)	
Tumor depth				<0.0001
pT0–1	163	126 (77)	37 (27)	
pT2	87	53 (61)	34 (39)	
pT3	192	83 (43)	109 (57)	
pT4	19	3 (16)	16 (84)	
No. of involved LN				<0.0001
<4	338	239 (71)	99 (29)	
≥4	123	26 (21)	97 (79)	
pStage				<0.0001
Stage 0–I	103	92 (89)	11 (11)	
Stage II	162	106 (65)	56 (35)	
Stage III	103	47 (46)	56 (54)	
Stage IV	93	20 (22)	73 (78)	
Preoperative therapy				0.1253
Surgery alone	258	159 (61)	99 (39)	
Chemotherapy	120	62 (52)	58 (48)	
Chemoradiotherapy	83	44 (53)	39 (47)	
Complications				0.0165
Absent	274	170 (62)	104 (38)	
Present	187	95 (51)	92 (49)	
Postoperative therapy				0.0005
Performed	66	25 (38)	41 (62)	
Not performed	395	240 (61)	155 (39)	

Mod moderately, *SCC* squamous cell carcinoma

different treatments, and 17 received 3 or more different treatments.

The most commonly used chemotherapeutic regimen for recurrence in the current study was the combination therapy of cisplatin and 5-FU with or without adriamycin, administered to 42 patients. The second-most commonly used regimen was combination therapy with S-1 and docetaxel, administered to 27 patients. Other less commonly used regimens included combined S-1 and cisplatin, docetaxel alone, paclitaxel alone, or cisplatin alone. Of 59 patients treated with radiotherapy, 17 received radiotherapy alone, while the remaining 42 patients received CRT. There were 18 recurrent diseases removed by surgery at the following sites: 4 cervical lymph node, 3 upper mediastinal lymph node, 3 abdominal lymph node, 2 brain, 2 lung, 2 anastomotic site, 1 liver, and 1 adrenal gland. For 10 patients with recurrence in lymph node, lymph node resection was performed. Among them, 2 patients who had recurrence in upper medial lymph node underwent lymph node resection combined with partial resection of trachea and making entrance of the trachea in anterior chest wall. For patients who had recurrence in brain, lung, and liver, resection of brain metastasis, pulmonary metastasis, and liver metastasis was performed, respectively. For patients with anastomotic recurrence in the neck, partial resection of anastomotic site and reanastomosis was performed. A patient who had recurrence in adrenal gland underwent resection of right adrenal gland.

Recurrence Pattern and Treatment for Recurrence Based on Preoperative Therapy

We next examined whether preoperative therapy affected the pattern and timing of recurrence and treatment for recurrence (Table 2). The patterns of recurrence did not significantly differ according to type of preoperative therapy. Time to recurrence was significantly shorter in patients who underwent preoperative chemotherapy or CRT than in those cases not given preoperative therapy (median time to recurrence; surgery alone, 8.4 months versus preoperative chemotherapy, 6.1 months, preoperative CRT, 5.7 months). Although chemotherapy for recurrence was performed regardless of preoperative therapy, patients who underwent preoperative CRT were less often treated with radiotherapy for recurrence than those who underwent surgery alone (34.3 vs. 15.4%). Similarly, patients who underwent preoperative CRT tended to less often receive surgical resection for recurrence compared with those who underwent either preoperative chemotherapy or surgery alone, although this difference was not statistically significant.

Factors Affecting Survival After Recurrence

The median survival time after recurrence was 8.2 months. Univariate analysis of the factors affecting survival after recurrence identified clinical stage, preoperative therapy, pathological stage, recurrence within 1 year, number of recurrent tumors, and recurrence at a local site, liver, or bone as significantly associated (Table 3). Patients who had 4 or more recurrent tumors showed significantly

TABLE 2 Recurrence pattern and treatment for recurrence according to preoperative therapy

	Preoperative therapy			
	Surgery alone	CT	CRT	Total
Recurrent cases	99	58	39	196
Initial stage				
cStage I	10 (10)	0 (0)	0 (0)	10 (5)
cStage II	38 (38)	9 (16)	6 (15)	53 (27)
cStage III	36 (36)	30 (52)	26 (67)	92 (47)
cStage IV	15 (16)	19 (32)	7 (18)	41 (21)
Pathological stage				
pStage 0–I	8 (8)	1 (2)	3 (8)	12 (6)
pStage II	24 (24)	16 (28)	15 (38)	55 (28)
pStage III	36 (36)	14 (24)	6 (16)	56 (29)
pStage IV	31 (32)	27 (46)	15 (38)	73 (37)
Recurrence within 1 year*				
Present	64 (65)	46 (79)	32 (82)	142 (72)
Absent	35 (35)	12 (21)	7 (18)	54 (28)
First recurrence site				
Local	16 (16)	9 (16)	11 (28)	36 (18)
Lymphatic	62 (63)	42 (72)	21 (54)	125 (64)
Distant	55 (56)	35 (60)	23 (59)	113 (58)
Number of recurrent tumors				
1	43 (43)	15 (26)	16 (41)	74 (38)
2–3	22 (22)	16 (28)	8 (21)	46 (23)
≥4	34 (35)	27 (46)	15 (38)	76 (39)
Treatment used for recurrence				
None	26 (26)	12 (21)	14 (36)	52 (27)
Any treatment	73 (74)	46 (79)	25 (64)	144 (73)
CT	55 (56)	42 (72)	23 (59)	120 (61)
RT	34 (34)**	19 (33)	6 (15)**	59 (30)
Surgery	9 (9)	7 (12)	2 (5)	18 (9)

CT chemotherapy, *CRT* chemoradiation therapy, *RT* radiation therapy
* $P = .0453$; ** $P = .0271$

poor survival after recurrence compared with those with a lower number of recurrent tumors (Fig. 1). Patients given preoperative CRT also showed significantly poorer survival after recurrence compared with surgery-alone patients and those given preoperative chemotherapy (mean survival time; CRT 6.4 months, surgery alone 13.1 months, chemotherapy 10.9 months, Fig. 2). Univariate analysis also showed that chemotherapy, radiation therapy, and surgery for recurrent disease can all improve survival after recurrence (Fig. 3a–d).

Multivariate analysis showed that preoperative CRT, the number of recurrent tumors, and the presence of recurrence at local site and liver were independent factors affecting survival after recurrence, and it identified the number of recurrence tumors as the most important prognostic factor (Table 4, model A). In multivariate analysis, pathological tumor stage was an independent prognostic factor although clinical tumor stage was not. Multivariate analysis also showed that chemotherapy and radiotherapy performed for recurrence were associated with prolonged survival (Table 4, model B). Surgery performed for recurrence showed borderline prognostic significance in multivariate analysis.

Treatment for Recurrence According to Number of Recurrences

Finally, we examined whether the number of recurrent tumors influenced treatment for recurrence. Although chemotherapy for recurrence was performed regardless of the number of recurrent tumors, patients with multiple recurrent tumors significantly less often received radiotherapy or surgery than those with a solitary recurrence (Table 5).

DISCUSSION

In this study, we investigated factors that affect survival of patients who had recurrent disease after curative resection for esophageal squamous cell carcinoma and also examined whether preoperative therapy affects pattern of recurrence, treatment for recurrent disease, and survival after recurrence. Our study found that the number of recurrent tumors, preoperative CRT performed, pathological tumor stage, and the recurrence at local site and liver were identified as independent prognostic factors, and that any type of treatment performed for recurrence can contribute to prolonged survival. Moreover, the success of treatment for recurrence was limited by the number of recurrent tumors and the preoperative therapy.

It is well known that the number of lymph node metastases is the most important prognostic factor in patients who had undergone esophagectomy, and patients with multiple lymph node metastases are recognized as having systemic disease.[21–23] Similarly, patients with multiple recurrent tumors may be recognized as having systemic recurrence at the time of diagnosis. In fact, the proportion of patients who received systemic chemotherapy was not different according to the number of recurrent tumors, whereas the proportion of patients who received radiation therapy or surgical resection as locoregional therapy was significantly lower in patients who had multiple recurrent tumors, especially more than 3, compared with patients with a solitary recurrence.

Previous studies suggested that radiotherapy may be effective therapy for locoregional recurrence. Some previous studies that involved no more than 30 patients reported median survival times from the detection of

TABLE 3 Univariate analysis for survival after recurrence

		HR	95% CI	*P* value
Before recurrence				
Age	≥70	1.25	0.88–1.70	.2275
Gender	Male	1.28	0.81–2.02	.2857
Location	Lower third	1.16	0.87–1.56	.3104
Histology	Poorly SCC	1.22	0.88–1.68	.2309
cStage	Stage III–IV	1.49	1.05–2.13	.0263
Number of LNs	≥4	1.30	0.97–1.72	.0762
pStage	Stage III–IV	1.54	1.13–2.10	.0064
Preoperative therapy				
CRT	Performed	1.83	1.25–2.67	.0018
CT	Performed	0.96	0.70–1.33	.8242
Postoperative complications	Present	1.30	0.98–1.73	.0716
At recurrence				
Recurrence within 1 year	Present	1.84	1.32–2.56	.0004
Recurrence site				
Local	Present	1.63	1.12–2.38	.0099
Lymphatic	Present	0.99	0.74–1.32	.9299
Cervical	Present	1.24	0.86–1.80	.2509
Thoracic	Present	1.12	0.84–1.50	.4401
Abdominal	Present	1.14	0.84–1.52	.4796
Distant	Present	1.58	1.17–2.14	.0031
Lung	Present	1.12	0.80–1.58	.4989
Liver	Present	1.83	1.30–2.58	.0006
Bone	Present	1.92	1.25–2.96	.0029
Dissemination	Present	1.48	0.98–2.23	.0630
No. of recurrent tumor	≥4	2.65	1.92–3.64	<.0001
After recurrence				
CT for recurrence	Performed	0.55	0.40–0.75	.0002
RT for recurrence	Performed	0.41	0.29–0.58	<.0001
Surgery for recurrence	Performed	0.33	0.186–0.60	.0001

SCC squamous cell carcinoma, *CT* chemotherapy, *CRT* chemoradiation therapy, *RT* radiation therapy, *HR* hazard ratio, *95% CI* 95% confidence interval

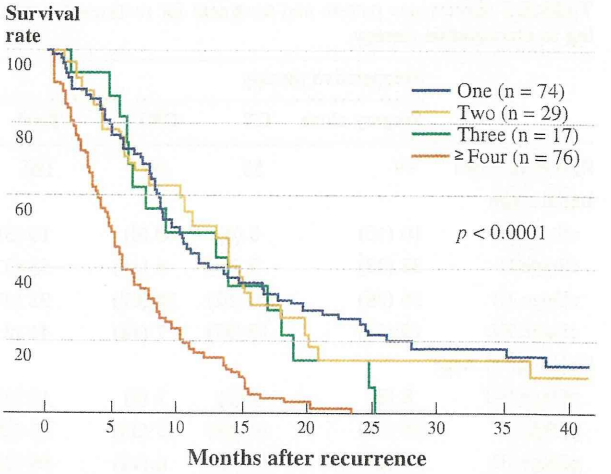

FIG. 1 Overall survival rate in 196 patients with recurrent disease after curative resection of esophageal cancer, according to the number of recurrent tumors. Patients who had more than three recurrent tumors showed significantly poor survival compared with those who had 1–3 recurrent tumors

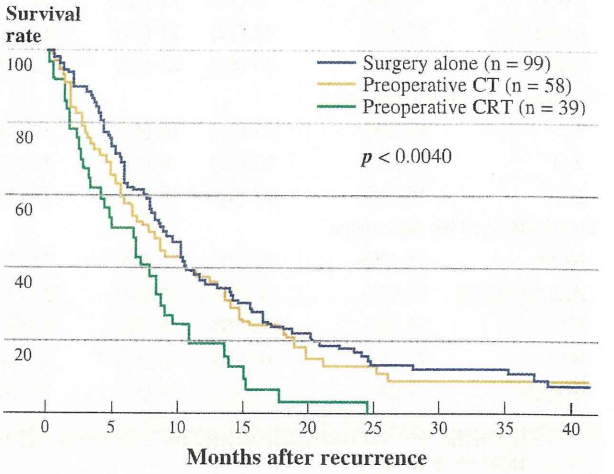

FIG. 2 Overall survival rate in 196 patients with recurrent disease after curative resection of esophageal cancer, according to type of preoperative therapy. Patients who underwent preoperative CRT showed significantly poor survival compared with those who underwent preoperative chemotherapy and surgery alone. *CT* chemotherapy, *CRT* chemoradiation therapy

recurrence of only 7–16 months.[24–27] However, the same studies found that patients with locoregional recurrence without distant organ metastasis and those with small recurrent tumors tended to show longer-term survival.[24,27] In our study, although median survival time after recurrence in 61 patients who received radiotherapy for recurrence was 13.8 months, 16 (26.2%) survived longer than 2 years after recurrence. Thus, it is clear that radiotherapy for recurrence will be effective for some patients. However, the majority of patients who had locoregional recurrence (local and/or lymphatic) after preoperative CRT followed by surgery was excluded from indication for radiation therapy, although proportion of those patients is not negligible. This could contribute partly to current result that patients who received preoperative CRT showed significantly shorter survival after recurrence than those who underwent surgery alone or preoperative chemotherapy.

In general, patients with recurrent disease after esophagectomy showed a poor prognosis, with previously reported survival times after recurrence less than 1 year. Dresner and Griffin reported a median survival time after recurrence of only 2.7 months in patients who developed

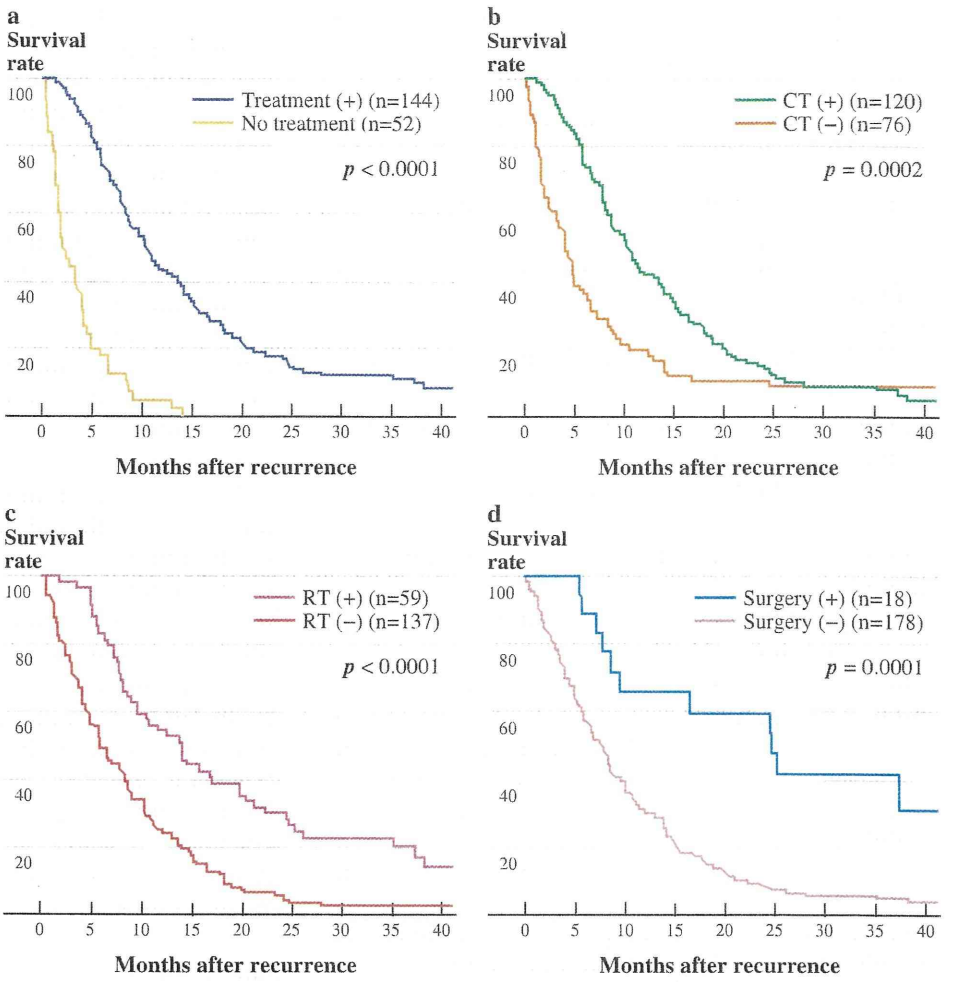

FIG. 3 Overall survival rate in 196 patients with recurrent disease after curative resection of esophageal cancer, according to treatment for recurrence. **a** Treatment versus no treatment. **b** CT versus no CT. **c** RT versus no RT. **d** surgery versus no surgery. All treatments for recurrence including chemotherapy, radiotherapy, and surgery can contribute to prolonged survival after recurrence. *CT* chemotherapy, *RT* radiation therapy

recurrence after curative resection without preoperative therapy, while Mariette et al. reported 7.0 months median survival time in patients with recurrence after surgery alone or neoadjuvant CRT followed by surgery.[3,8] In this study, median survival time after recurrence was 8.2 months. However, 66 of the 196 patients (33.7%) with recurrent disease survived for more than 1 year, and 23 (11.7%) survived for more than 2 years after recurrence. Thus, even patients who develop recurrent disease have a chance of achieving relatively long-term survival after recurrence. Moreover, the current study showed that all available treatments for recurrence including chemotherapy, radiation therapy, and surgery should prolong survival after recurrence. Thus, it is worth treating recurrent disease vigorously using available treatments as far as the patient's condition allows, although patient's condition is determined largely by the number and site of recurrent tumors.

In the present study, the recurrent disease occurred earlier in patients with advanced tumor than those with nonadvanced tumor at the time of operation (data not shown). This finding is consistent with results of previous studies.[8,28] However, the present study also showed that recurrence occurred earlier in patients who received preoperative CRT or preoperative chemotherapy than in those treated by surgery alone, although there is no significant difference in tumor stage at the time of operation (pathological tumor stage) between the former and the latter. One possible explanation for this result may be that postoperative complications is associated with early recurrence in patients who underwent esophagectomy for esophageal cancer.[29] In fact, the incidence of postoperative complication tended to be higher in patients who received preoperative CRT than those treated by surgery alone (49.4 vs. 38.3%). However, recurrence occurred earlier in patients who underwent preoperative chemotherapy than those treated by surgery alone, although there is no difference in incidence of complication between the 2 (39.4 vs. 38.3%). Another reason may be that many of patients who developed recurrent disease after preoperative chemotherapy or preoperative CRT are nonresponders in this study (data not shown). Ineffective preoperative therapy may hasten the occurrence of recurrent disease after

TABLE 4 Results of multivariate analysis for survival after recurrence

	Model A			Model B		
	HR	95% CI	*P* value	HR	95% CI	*P* value
cStage						
III/IV	1.13	0.79–1.60	0.5076	1.13	0.79–1.63	0.4948
Preoperative therapy						
CRT performed	1.64	1.08–2.48	0.0189	1.78	1.17–2.71	0.0068
pStage						
III/IV	1.41	1.01–2.04	0.0498	1.42	0.97–2.09	0.0700
Recurrence within 1 year						
Present	1.33	0.89–1.99	0.1638	1.55	1.00–2.41	0.0498
Number of recurrent tumors						
≥4	2.06	1.40–3.01	<0.0001	1.80	1.21–2.67	0.0035
Recurrence at local site						
Present	1.85	1.23–2.78	0.0032	1.38	0.91–2.09	0.1348
Recurrence in liver						
Present	1.79	1.20–2.68	0.0044	1.68	1.136–2.49	0.0107
Recurrence in bone						
Present	1.06	0.66–1.71	0.8112	1.09	0.68–1.74	0.7208
Treatment for recurrence						
CT performed				0.34	0.23–0.49	<0.0001
RT performed				0.44	0.29–0.67	0.0001
Surgery performed				0.53	0.29–1.05	0.0682

CT chemotherapy, *CRT* chemoradiation therapy, *RT* radiation therapy, *HR* hazard ratio, *95% CI* 95% confidence interval

TABLE 5 Treatment for recurrence according to number of recurrent tumors

	Number of recurrent tumors		
	1	2–3	≥4
n	74	46	76
Treatment used for recurrence			
None*	13 (18)	12 (26)	27 (36)
CT	44 (59)	28 (61)	48 (63)
RT**	35 (47)	16 (35)	8 (11)
Surgery***	14 (19)	3 (7)	1 (1)

CT chemotherapy, *RT* radiation therapy

* $P = 0.0321$; ** $P < 0.0001$; *** $P = 0.0004$

surgery rather than delay it because of suppression of immune function caused by preoperative therapy.

This retrospective study has a limitation by allowing selection bias, in that indication for initial treatment depends not only on patient selection but also on clinical tumor stage. In fact, initial tumor stage in patients who were treated with preoperative chemotherapy or CRT followed by surgery tended to be more advanced than those who underwent surgery alone. However, there was no significant difference in pathological tumor stage after treatment between patients with and without preoperative therapy, when our examination is limited to patients with recurrent disease. As shown in previous studies, whether patients develop recurrent disease after esophagectomy or not depends largely on pathological tumor stage.[3–8,28] Furthermore, in our study, multivariate analysis identified pathological tumor stage but not clinical tumor stage as an independent prognostic factor after recurrence. Therefore, we do not think that comparing the recurrence pattern and survival after recurrence between patients with and without preoperative therapy is invalid in this study.

In conclusion, our study demonstrated that among various clinicopathological factors, pathological tumor stage, preoperative CRT, the number of recurrent tumors, and recurrence at local site and liver are significantly associated with survival of patients who developed recurrence after curative resection of esophageal cancer. Although the choice of treatment option for recurrent disease is limited by the number of recurrent tumors and preoperative CRT performed, our study also revealed that all treatments for recurrence including chemotherapy, radiotherapy, and surgery could contribute to prolonged survival after recurrence. Vigorous treatment for recurrence might therefore extend survival after recurrence in patients who underwent esophagectomy.

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Role of multidrug resistance protein 2 (MRP2) in chemoresistance and clinical outcome in oesophageal squamous cell carcinoma

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BACKGROUND: Although multidrug resistance protein 2 (MRP2) confers chemoresistance in some cancer types, its implication on oesophageal squamous cell carcinoma (ESCC) remains unclear.

METHODS: We evaluated MRP2 expression by immunohistochemistry and RT–PCR using 81 resected specimens from ESCC patients who did or did not receive neo-adjuvant chemotherapy (NACT), including 5-fluorouracil, doxorubicin, and cisplatin (CDDP). Correlation between MRP2 expression and response to chemotherapy was also examined in 42 pre-therapeutic biopsy samples and eight ESCC cell lines.

RESULTS: MRP2-positive immunostaining was more frequently observed in ESCCs with NACT than in those without NACT (27.3 *vs* 5.4%). The MRP2-positive patients showed poorer prognosis than MRP2-negative patients (5-year survival rate, 25.6 *vs* 55.7%). Concordantly, ESCC with NACT showed 2.1-fold higher mRNA expression of MRP2 than those without NACT ($P = 0.0350$). In pre-therapeutic biopsy samples of patients with NACT, non-responders showed 2.9-fold higher mRNA expression of MRP2 than responders ($P = 0.0035$). Among the panel of ESCC cell lines, TE14 showed the highest MRP2 mRNA expression along with the strongest resistance to CDDP. Inhibition of MRP2 expression by small-interfering RNA reduced chemoresistance to CDDP.

CONCLUSION: Our data suggested that MRP2 is one of molecules, which regulate the sensitivity to chemotherapy including CDDP in advanced ESCC patients.

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Keywords: multidrug resistance protein 2; MRP2 expression; prognosis; oesophageal squamous cell carcinoma; chemoresistance; neo-adjuvant chemotherapy

Oesophageal squamous cell carcinoma (ESCC) is the major histological form of oesophageal cancer in East Asian countries. It is one of the most lethal malignancies of the digestive tract and in most cases the initial diagnosis is established only once the malignancy is in the advanced stage (Shimada *et al*, 2003). Multimodal therapies are therefore necessary to prolong the survival of ESCC patients. Chemotherapy has become the standard first-line therapy for advanced ESCC patients, especially neo-adjuvant chemotherapy (NACT) (Tamoto *et al*, 2004). However, the initial response rate for NACT remains at 35–66% (Ajani *et al*, 1992; Iizuka *et al*, 1992; Hilgenberg *et al*, 1988; Ilson *et al*, 1998, 1999; Millar *et al*, 2005) and non-responders risk serious adverse effects without achieving any survival benefit.

The effectiveness of chemotherapy is often limited by drug-resistance factors in the tumours themselves. In fact, some tumours are intrinsically resistant to many kinds of chemotherapeutic agents, whereas other tumours, initially sensitive, often recur or become resistant not only to the initial agents used but also to those used subsequently. These two types of chemoresistance, intrinsic and acquired, are clinically serious problems in many types of cancer including ESCC; however, the molecular mechanisms underlying this resistance are not fully understood. More investigation into the mechanisms of chemoresistance in ESCC is needed with the goal of identifying novel predictive markers that can accurately identify non-responders before the administration of chemotherapy, thus enabling personalised therapies in ESCC patients.

Several members of the ATP-binding cassette (ABC) transporter superfamily have an important role in drug resistance in tumour cell models as well as in the clinic (Lage, 2003). These transporters mediate the ATP-dependent cellular efflux of chemotherapeutic drugs. Of the 48 human ABC transporters, multidrug resistance protein 2 (MRP2; also designated as ABCC2 or cMOAT) is expressed in the hepatocyte canalicular membrane (Kool *et al*, 1997), in which it functions as the major exporter of organic anions from the liver into the bile (Wada *et al*, 1998). Multidrug resistance protein 2 is also expressed in the kidney, gall bladder, small intestine, colon, and lung (Surowiak *et al*, 2006). Interestingly, several cisplatin (CDDP)-resistant human cancer cell lines overexpress MRP2, including ovarian cancer, hepatocellular carcinoma, bladder cancer, and colon cancer (Taniguchi *et al*, 1996; Kool *et al*, 1997; Liedert *et al*, 2003; Materna *et al*, 2005). *In vitro* data also implicated MRP2 in multidrug resistance (MDR) mechanisms during chemotherapy in some cancer cell lines

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(Koike *et al*, 1997; Materna *et al*, 2006; Ma *et al*, 2009). However, few studies have investigated MRP2 expression in ESCC (Gan *et al*, 2010; Tanaka *et al*, 2010), and thus the relationship between MRP2 expression and chemoresistance in ESCC remains unclear. The present study examined the clinical significance of MRP2 expression and its role in intrinsic and acquired resistance to chemotherapy in ESCC patients.

PATIENTS AND METHODS

Patients and treatments

The present study examined samples from 81 patients with histopathologically confirmed primary thoracic oesophageal cancer who underwent surgical resection at our hospital from 1988 to 2007. Table 1 details the patient characteristics. The cohort comprised 9 female and 72 male patients, aged from 42 to 80 years (median 62 years). Sub-total oesophagectomy by right thoracotomy with two or three-field lymphadenectomy was performed in all patients. Curative resection (R0) was achieved in 75 patients (92.6%), whereas the remaining 6 (7.4%) patients underwent a non-curative resection (R1, 2). None of the patients died of post-operative complications. A total of 44 patients (54.3%) with lymph node metastasis at initial diagnosis received NACT comprising two courses of 5-fluorouracil (5-FU), CDDP, and doxorubicin (DXR) (Akita *et al*, 2006; Yano *et al*, 2006; Matsuyama *et al*, 2007; Makino *et al*, 2008, 2010). Only a few patients who showed multiple metastatic lymph nodes in the surgical specimen received a regimen of docetaxel or CDDP plus 5-FU after operation (Ando *et al*, 2003).

Table 1 Correlation between MRP2 expression by immunohistochemistry and various clinico-pathological parameters

	MRP2 expression			
Parameter	**Positive**	**Negative**	**Total**	***P*-value**
Age (years)				
<65	8 (16.3)	41 (83.7)	49	0.7731
⩾65	6 (18.8)	26 (81.2)	32	
Gender				
Male	10 (13.9)	62 (86.1)	72	0.0435
Female	4 (44.4)	5 (55.6)	9	
Histopathology				
Well-, moderately differentiated	10 (16.7)	50 (83.3)	60	0.7500
Poorly differentiated	4 (19.0)	17 (81.0)	21	
Location				
Upper, middle thoracic oesophagus	6 (11.5)	46 (88.5)	52	0.1227
Lower thoracic oesophagus	8 (27.6)	21 (72.4)	29	
Neo-adjuvant chemotherapy				
Yes	12 (27.3)	32 (72.7)	44	0.0161
No	2 (5.4)	35 (94.6)	37	
pT				
T0–2	4 (16.7)	20 (83.3)	24	>0.9999
T3–4	10 (17.5)	47 (82.5)	57	
Number of pN				
<4	6 (11.1)	48 (88.9)	54	0.0594
⩾4	8 (29.6)	19 (70.4)	27	
pStage				
Stages 0–2	4 (12.1)	29 (87.9)	33	0.3800
Stages 3–4	10 (20.8)	38 (79.2)	48	

pT, pN, pStage (pathological classification) according to TNM classification.

After surgery, the patients were surveyed every 3 months by physical examination and measurement of serum tumour markers, every 6 months by CT scan and abdominal ultrasonography, and every year by endoscopy until tumour recurrence was evident. Patients with tumour recurrence received chemotherapy or chemoradiotherapy as long as their systemic condition permitted. The mean overall survival (OS) was 31.6 months and mean disease-free survival was 28.3 months. The mean follow-up period after surgery was 42.9 months.

Immunohistochemical analysis

MRP2 protein accumulation was examined by immunohistochemical (IHC) staining of formalin-fixed and paraffin-embedded ESCC tissue sections (Makino *et al*, 2009). Briefly, after deparaffinization in xylenes and dehydration through graded ethanol solutions; endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 20 min. The tissue sections were then heated at 95°C for 40 min in citrate buffer (0.05 mol l^{-1}, pH 6.0) for antigen retrieval. The sections were then incubated with mouse monoclonal antibody to MRP2 (Clone: M_2III-6, ALEXIS Biochemicals, dilution 1 : 10) for 2 h at room temperature, and antibody binding was visualised using the labeled-streptavidin biotin method. Negative controls for the IHC included omission of the primary antibody. Normal human liver tissue was used as a positive control. MRP2 staining for each ESCC sample was judged 'positive' when more than 10% of the cancer cells in the section were immunoreactive to MRP2, and 'negative' when 10% or less of the cells were positive. All slides were assessed by two observers, independently and then in conference; both were blinded to the clinico-pathological parameters.

Quantitative RT–PCR analysis

Total RNA was extracted from fresh frozen resected tumours or endoscopic biopsy samples from ESCCs patients, and from cancer cell lines using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was generated from 1 μg RNA in a final volume of 20 μl containing oligo-(dT)-15 primer and avian myeloblastosis virus transcriptase, using the Reverse Transcription System (Promega, Madison, WI, USA). Analysis by PCR was performed using a LightCycler, real-time monitoring thermal cycler. Reaction mixture for PCR was prepared containing 2 μl of cDNA template, 3 mmol l^{-1} $MgCl_2$, and 250 nmol l^{-1} of primer pairs, using LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany). The amount of each transcript was normalised against the expression of the housekeeping gene porphobilinogen deaminase (PBGD). Standard curves were constructed with 10-fold serial dilutions of cDNA obtained from non-cancerous oesophageal mucosal cell layers of tissue samples from 10 cases as a standard mixture. The sequences of PCR primers for PBGD, MRP2 were as follows: forward primer 5′-TGTCTGGTAACGGCAATGCGGCTGCAAC-3′, reverse primer 5′-TCAATGTTGCCACCACACTGTCCGTCT-3′ used for amplification of PBGD, forward primer 5′-TAATGGTCCTAGACAACGGG-3′, reverse primer 5′-GGGCCTTCTGCTAGAATTT-3′ for MRP2. The PCR cycling condition was set as follows: an initial denaturing step at 95°C for 10 min and 40 cycles at 95°C for 15 s, 58°C for 10 s, and 72°C for 25 s. The relative amount of cDNA in each sample was measured by interpolation on the standard curve, and then the relative ratio of MRP2/PBGD mRNA expression in log2 scale was calculated for each ESCC sample.

Knockdown analysis using MRP2-siRNAs

Two small-interfering RNA (siRNA-1, -2) of MRP2 (HSS102057, HSS174719) and negative control (NC) (Medium GC duplex of stealth RNAi NC duplexes) were purchased from Invitrogen.