observed; most were cubic epithelial cells, with a few fibrocyte-like cells on the margin of cell clusters (Supplement 1a). These fibrocyte-like cells seemed to disperse from tight clusters and change their shape from cubic to flat and spindle-like (Fig. 2a, b; left). The epithelial marker E-cadherin and the mesenchymal marker vimentin were detected by immunofluorescence staining. Cluster center cells had epithelial-like properties with E-cadherin positive and vimentin negative (Fig. 2a, right). Concurrently, crawling cells had mesenchymal-like properties with E-cadherin negative and vimentin positive (Fig. 2b, right). These observations revealed that the EMT program took place in this cancer cell line.

A highly migratory subclone was selected from the Capan-1 parental cell line by use of the scheme outlined in Fig. 1. Migrating cells were collected after 18 h for each round of selection. In total, the procedure was repeated 9 times until two to threefold repeatable differences in migration and invasion were achieved. For the new migratory variant, Capan1M9, threefold increases in migration ($p < 0.01$, Fig. 2c) and invasion were observed ($p < 0.01$, Fig. 2d) compared with the parental cell line. The selection process took over 10 months to complete and the Capan1M9 cells maintained the significant difference in migration and invasion characteristics compared with the parental cells even after being in culture for over 24 months. The morphology of cultured Capan1M9 cells showed a monolayer growth pattern with flat and loose packed clusters (Supplement 1b). The rate of growth of Capan1M9 cells was significantly faster than that of Capan-1 cells (Fig. 2e).

## Elevated expression of CD133 and EMT-related genes in the Capan1M9 cells

CD133, CD44, CD24, and aldehyde dehydrogenase 1 (ALDH 1) have been proposed as CSC markers for pancreatic cancer in recent years. Therefore, we examined the ratio of $CD133^+$, $CD44^+$, $CD24^+$ or $ALDH1^+$ population by flow-cytometric analysis (Supplement 2a, b). The flow-cytometric analysis showed a high level of CD133 expression in Capan1M9 cells, increasing from 46 to 95%

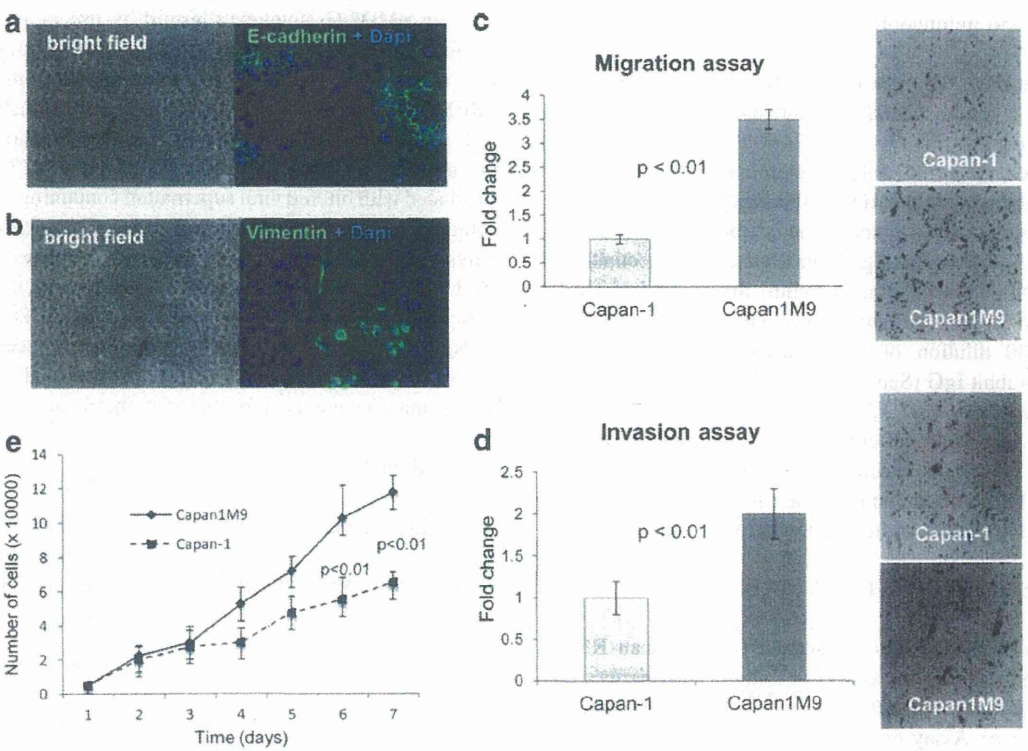


**Fig. 2** Establishment of a highly migratory subclone (Capan1M9) from the parental Capan-1 cells. **a** EMT taking place in crawling cells (*arrow*) surrounding the cluster of cultured Capan-1 cells; bright field ×200 (*left*) and immunofluorescence staining of E-cadherin (*green*) ×200 (*right*). **b** Bright field ×200 (*left*) and immunofluorescence staining of vimentin (*green*) ×200 (*right*). DAPI was subjected to nuclear staining. Migration (**c**) and invasion (**d**) assays between Capan-1 and Capan1M9. **e** The rate of growth of Capan1M9 cells was significantly faster than that of Capan-1 cells (color figure online)

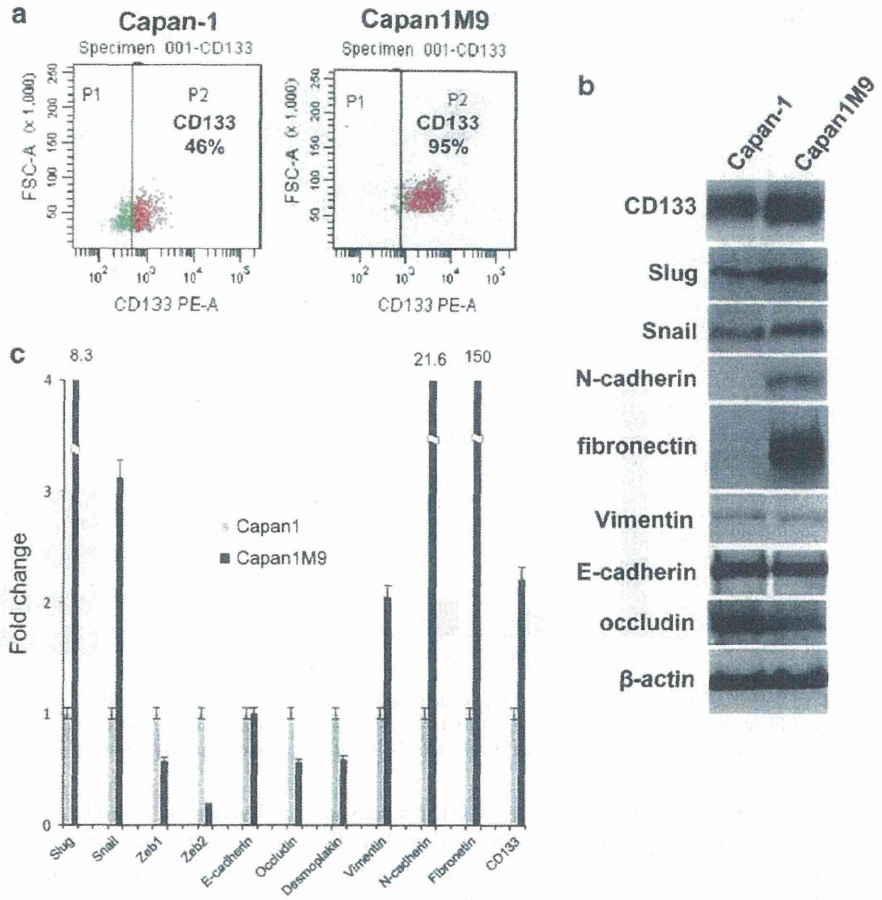


**Fig. 3** Expression of CD133 and EMT-related genes were compared between Capan1M9 and Capan-1 parental cells. **a** Flow-cytometric analysis showed increased CD133 expression percentage. **b** Protein levels of CD133 and EMT-related genes were examined by Western blot. **c** mRNA levels of CD133 and EMT-related genes were detected by real-time RT-PCR

compared with the parental cells (Fig. 3a). To determine whether CD133 is involved in the migration and invasion of Capan1M9 cells, we assessed expression of CD133 in mRNA and protein levels. Immunoblotting showed a higher protein level of CD133 expression in Capan1M9 cells compared with that in the parental cells (Fig. 3b). CD133 mRNA level of Capan1M9 cells also increased more than twofold compared with that of parental cells (Fig. 3c).

Because an EMT program occurred in the migration front of the Capan-1 cells, EMT-associated transcription factors and epithelial and mesenchymal molecules expression levels in the Capan1M9 cells were examined and compared with those in the Capan-1 cells. Snail and Slug mRNA expression levels were found to have increased three and eight times, respectively (Fig. 3c). On the other hand, occludin and desmoplakin expression levels were found to have halved (Fig. 3c). Vimentin, N-cadherin, and fibronectin expression levels were up-regulated by 2, 21, and 150-fold, respectively (Fig. 3c). ZEB1 and ZEB2, EMT-associated transcription factors, were down-regulated slightly in the Capan1M9 cells (Fig. 3c), and Twist could not be detected in either Capan-1 or Capan1M9 cells (data not shown). Protein levels of fibronectin and N-cadherin were significantly greater in the Capan1M9 cells, whereas occludin protein level decreased (Fig. 3b). However, vimentin and E-cadherin protein levels were no different between Capan-1 and Capan1M9 cells (Fig. 3b).

## Decrease of motility as a result of suppressed EMT after endogenous CD133 knockdown

We designed shRNA-GFP for CD133 and expressed it in the Capan1M9 cells (Supplement 3a), and the Capan1M9-GFP cell line was also established as a control. There was no difference in growth rate between these two cell lines (Supplement 3b). Successfully transfected cells with GFP fluorescence distinguished by FCM analysis showed CD133 was effectively knocked down (Fig. 4a). The wound-healing assay was carried out between Capan1M9-GFP-shCD133 and Capan1M9-GFP cells. The Capan1M9-GFP cells had greater motility than the Capan1M9-GFP-shCD133 cells and the wound area in the cell monolayer was completely closed in the Capan1M9-GFP

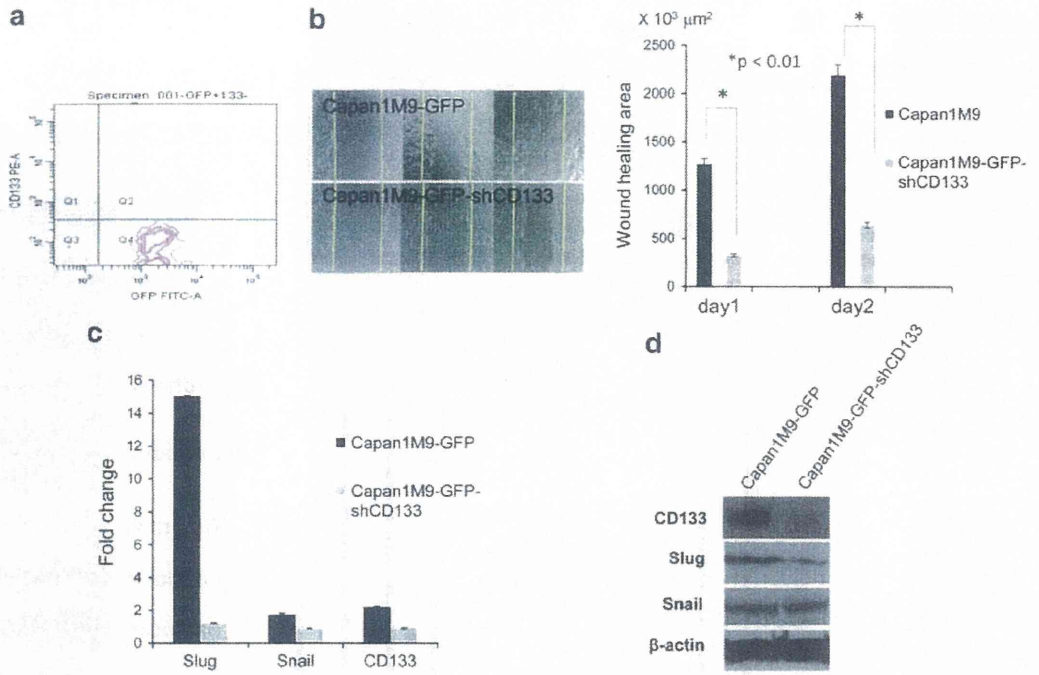


**Fig. 4** Knockdown of CD133 suppressed EMT-related genes and migration. **a** Flow-cytometric analysis after shRNA-CD133 transfection. **b** Wound-healing assay was conducted between Capan1M9-GFP and shCD133-Capan1M9-GFP (*left*), wound-healing area was compared (*right*). CD133, Slug, and Snail expression levels were examined by real-time RT-PCR (**c**) and Western blot (**d**)

after 48 h, In contrast, Capan1M9-GFP-shCD133 achieved only half of the complete wound-healing potential, indicating much lower cell motility (Fig. 4b, left). Capan1M9-GFP-shCD133 cells had significantly lower motility than the Capan1M9-GFP cells by assessment of wound-healing areas (Fig. 4b, right).

mRNA and protein levels of endogenous CD133, Slug, and Snail were examined and compared between Capan1M9-GFP-shCD133 and Capan1M9-GFP cells. CD133-shRNA showed 60% knockdown of CD133 mRNA (Fig. 4c) and effectively suppressed protein level of CD133 expression (Fig. 4d). Slug expression of mRNA level was suppressed by 90% (Fig. 4c) and protein expression also was suppressed (Fig. 4d). However, there was no significant decrease in Snail protein expression (Fig. 4d), although mRNA level of Snail expression was suppressed (Fig. 4c). These results demonstrated that migration ability and Slug expression were suppressed after endogenous CD133 knockdown.

## Discussion

Metastasis, the foremost cause of mortality in cancer patients, accounts for more than 90% all deaths from solid tumor diseases, but the underlying mechanism remains elusive. Although metastasis involves several steps, migration is the first and most important [13, 15]. To evaluate the mechanism of this critical step of metastasis, we established a subclone enriched with high-migratory cells, Capan1M9, from the parental Capan-1. Because we observed the EMT program occurred among Capan-1 cells, EMT program-related genes were examined in Capan1M9 cells to understand whether EMT is important in migration. In Capan1M9 cells, we found that EMT-related transcription factors, Slug and Snail, and mesenchymal molecules, fibronectin and N-cadherin, were up-regulated but the epithelial molecules, occludin and desmoplakin, were suppressed. These results indicated that the EMT program had occurred among the migratory cells. Importantly, Capan1M9 cells strongly express CD133.

EMT converts cells from an epithelial, non-motile morphology to one that is migratory and tends to invade other tissues. This is accompanied by specific changes in gene expression, including up-regulation of the zinc finger transcription factors Slug and Snail, which are potent inducers of EMT [9, 20]. Epithelial-specific proteins mainly include molecules of the cell–cell communication system, for example E-cadherin, desmoplakin, and occludin. These have been shown to be highly valuable tools for identifying and characterizing EMT in vitro [4, 12, 21].

Slug encodes one member of the Snail family of C2H2-type zinc finger transcription factors. Slug induction

triggers the steps of desmosomal disruption, cell spreading, and partial separation at cell–cell borders, which comprise the first and necessary phase of the EMT process [22, 23]. Disruption of the tight junction induces an oncogenic phenotype by down-regulating expression of the tight junction protein, occludin [24–26]. A direct interaction exists between Slug and an E-box within the minimal Raf-1-responsive segment of the occludin promoter. Slug plays a role in mediating Raf-1-induced transcriptional repression of occludin and subsequent EMT [27].

In this study, there was no change in the expression level of E-cadherin between Capan1M9 and the parental cells despite increased levels of Slug expression. Evidence shows that, during EMT, the activity of adherent junctions is highly modified mainly because E-cadherin is replaced or overruled by N-cadherin, a process called "cadherin switching" [28]. Aberrant expression of N-cadherin seems to have a dominant effect in these cell–cell interactions and enhances the motility of tumor cells by destabilizing cell-adhesion complexes even in the presence of E-cadherin [29]. Fibronectin, an extracellular matrix glycoprotein, is of major importance in cell differentiation, growth, and migration. It is involved in processes such as wound healing, embryonic development, and oncogenic transformation [30]. Jia et al. [31] established a highly metastatic human lung adenocarcinoma cell line that had undergone an EMT program because of increased fibronectin expression.

Ultimately, increased expression of Slug, N-cadherin, and fibronectin, and reduced expression of desmoplakin and occludin were observed for the Capan1M9 subclone cells. Concurrently, the potential for cell motility, migration, and invasion was greatly increased. These findings demonstrate that the Capan1M9 cells underwent an EMT program. Furthermore, knockdown of CD133 expression by shRNA led to the down-regulation of Slug and slowed migration. This indicated that the Capan1M9 cells acquire highly migratory and invasive abilities through an EMT program triggered by Slug, which is regulated or facilitated by CD133. These findings demonstrated that CD133 is important in pancreatic cancer migration and invasion as a result of facilitation or regulation of EMT.

CD133 is a glycosylated, approximately 120-kDa, transmembrane protein with five transmembrane domains and two large extracellular loops [32]. The function of CD133 in normal and CSCs is still unknown. It is likely that CD133 interacts with the extracellular matrix or with neighboring cells. This is supported by the cellular localization of CD133 in protrusions of the plasma membrane and by the extracellular environment. Boivin et al. [33] reported that CD133 expressed endogenously or exogenously in medulloblastoma cells is phosphorylated by Src and Fyn, two members of the Src-family tyrosine kinases. Down-regulation of CD133 in melanoma cells increases the gene expression of several Wnt inhibitors, suggesting a link between CD133 and Wnt pathways. Wnt pathways or genes are associated with melanoma stem cell phenotype [17]. Our data confirmed and expanded on the hypothesis that CD133, as a CSC marker, can contribute to migration and invasion. It is speculated that CD133 could either be a regulatory factor triggering the EMT program or work in synergy with other signal pathways to facilitate the EMT program. An interesting finding was that the growth rate between Capan1M9-GFP and Capan1M9-GFP-shRNA-CD133 was not significantly different but Capan1M9 grew faster than Capan-1. Until now, no report has addressed whether CD133 takes part in cell proliferation or apoptosis. This implies that signal pathways other than CD133 may contribute to cell proliferation in these highly migratory cells.

New insights into EMT mechanisms regulated or facilitated by CD133 need further investigation, because the details of the mechanisms regarding how CD133 is involved in Slug regulation are still unclear. Further knowledge of these mechanisms would be of great interest for understanding pancreatic cancer metastasis and potential new therapeutic targets.

In conclusion, this study revealed that CD133 expression in pancreatic cancer cells contributes to migration and invasion by facilitating EMT, especially via Slug. Targeting CD133 could be a promising therapeutic intervention to prevent EMT and to inhibit migration and invasion in the early stage of pancreatic cancer metastasis.

**Acknowledgments** Financial support for this study was provided by Grants-in-Aid for scientific research from the Ministry of Education, Science, Sports, and Culture, Japan.

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Clinical Science

# Endoscopic ultrasonography is useful for monitoring the tumor response of neoadjuvant chemoradiation therapy in esophageal squamous cell carcinoma

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**KEYWORDS:**
Esophageal cancer;
Endoscopic ultrasonography;
Neoadjuvant therapy;
Chemoradiation therapy

**Abstract**

**BACKGROUND:** Recently, neoadjuvant chemoradiation therapy (CRT) has been introduced for treatment of esophageal squamous cell carcinoma (ESCC). This study was performed to investigate the usefulness of endoscopic ultrasonography (EUS) in comparison with EUS findings before and after CRT, and histologic findings.

**METHODS:** There were 33 patients with potentially resectable ESCC who underwent neoadjuvant CRT. Preoperative EUS and histologic findings were compared. EUS criteria were established on the basis of low and high echoic regions. Resected specimens were examined by hematoxylin-eosin, azan, and cytokeratin immunohistochemical staining.

**RESULTS:** Azan and cytokeratin staining clearly delineated fibrous changes and residual tumor. Low echoic regions corresponded to residual tumor and high echoic spots corresponded to fibrosis. All 12 patients classified as grade 1 on EUS diagnosis had histologic grade 1 tumors. Nineteen of 21 cases that presented with high echo were grade 2 or 3. The prognosis according to EUS diagnosis was similar to the histologic effect.

**CONCLUSIONS:** Preoperative EUS findings reflected the histologic effect after neoadjuvant CRT. EUS is a useful tool to assess the effect for CRT and to predict the prognosis in ESCC patients.


Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive diseases in the gastrointestinal tract. Various treatments have been used for advanced ESCC. Extended lymphadenectomy (ie, 3-field lymphadenectomy) has contributed to improvement of the prognosis for some patients with ESCC.[1] Although preoperative radiation therapy or chemotherapy has been attempted, a randomized controlled study indicated that prognosis was not significantly different from surgery alone.[2] Recently, preoperative chemoradiation therapy (CRT) was introduced to improve the prognosis in ESCC. The first randomized report of the

The authors have no conflicts of interest.

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effect of CRT on esophageal cancer by the Radiation Therapy Oncology Group (RTOG 85-01) indicated that the efficacy of CRT for esophageal cancer therapy,[3–5] and the combination of surgery and CRT is thought to be useful.[6–9]

In one randomized trial of preoperative CRT versus surgery alone in patients with locoregional esophageal carcinoma, a significant association was found between survival and histologic response, with responders having longer survival than nonresponders.[10] Thus, the accurate diagnosis of the effect of CRT is useful for the decision-making process regarding additional treatment and prediction of prognosis in ESCC. At present, assessment of the effect of CRT is performed by imaging methods such as barium study, endoscope, computed tomography, magnetic resonance imaging, endoscopic ultrasonography (EUS), and positron emission tomography. However, there have been few studies[11–15] examining the relationship between preoperative EUS findings and histologic effects of CRT. The benefit of the diagnosis by EUS for the effect of neoadjuvant CRT is still controversial. The appreciable discrepancy may be attributable to the endosonographic criteria adopted by the investigators. The purpose of the present study was to establish the endosonographic criteria for the effect of neoadjuvant CRT, and to study the correlation of examination by EUS and histologic response by comparison of the findings of EUS before and after CRT, as well as resected specimens.

## Materials and Methods

### Patients

Single-center experience. A total of 38 patients younger than age 75 years with ESCC received preoperative CRT at Kagoshima University Hospital between 1997 and 2003. In these 38 cases, examination by EUS was possible for 33 cases after CRT. All patients had potentially resectable advanced ESCC with T2 or T3 and N0 or N1, according to the International Union Against Cancer tumor-node-metastasis classification system,[16] and they underwent trans-thoracoabdominal esophagectomy with gastric replacement. These 33 patients were enrolled in the present retrospective study. All patients were followed up after discharge with a radiographic examination every 1 to 3 months, computed tomography every 3 to 6 months, and ultrasonography every 6 months. Informed consent for the treatment of resected specimens without a genetic procedure was acceptable for all patients. The clinicopathologic features of the study group are summarized in Table 1. All the M1 tumors were caused by distant (located on the cervical part or along the celiac artery) lymph node metastases.

**Table 1** Patient characteristics

| | |
|---|---|
| Number | 38 |
| Male/female | 38/0 |
| Age, y | 60.9 ± 8.2 |
| Main location (Ce/Ut/Mt/Lt/Ae) | 1/7/19/10/1 |
| Esophagogram size, mm | 64 ± 16 |
| T factor T(2/3/4) | 8/30/0 |
| T factor T(0/1a/1b) | 34/4/0 |
| pT factor, pT(1/2/3/4) | 2/6/22/8 |
| pN factor, pN(0/1) | 13/25 |
| pM factor, pM(0/1a/1b) | 27/5/6 |
| Pathologic CRT effect, grade 1/2/3 | 14/13/11 |
| Roentgenogram CRT effect, grade PD/NC/PR/CR | 2/9/27/0 |

Ae = abdominal esophagus; Ce = cervical; CR = complete response; Lt = lower thoracic; Mt = middle thoracic; NC = no change; PD = progressive disease; PR = partial response; Ut = upper thoracic.

### CRT

A total radiation dose of 40 Gy was applied; 2 Gy fractions were delivered 5 days per week for 4 weeks to the mediastinum and neck. In the same period, chemotherapy was performed intravenously using 2 anticancer agents: cisplatin (3–5 mg/m$^2$ over 2 h) and 5-fluorouracil (250–300 mg/m$^2$ over 24 h) 5 days a week for 4 weeks. Five to seven weeks after CRT, trans-thoracoabdominal esophagectomy with gastric replacement was performed.

The histologic criteria for the response of CRT were as follows (Japanese Society for Esophageal Diseases).[17] Grade 0, neither necrosis nor cellular or structural changes can be seen throughout the lesion; grade 1, necrosis or disappearance of the tumor is present in no more than two thirds of the whole lesion; grade 2, necrosis or disappearance of the tumor is present in more than two thirds of the whole lesion, but viable tumor cells still remain; and grade 3, the whole lesion falls into necrosis and/or is replaced by fibrosis, with or without granulomatous changes, no viable tumor cells are observed. CRT was judged to be effective in patients whose histologic response was grade 2 or 3. By contrast, in patients whose histologic response was grade 0 or 1, CRT was judged to be ineffective. This response of CRT against ESCC was judged by 2 pathologists without information of EUS diagnosis.

In this study, azan staining to identify fibrous tissues was performed in addition to staining with hematoxylin-eosin to obtain additional information regarding the histologic effects. To identify the residual tumor after CRT, immunohistochemical staining also was performed using the AE1/AE3 monoclonal antibody cocktail (20:1 mixture of AE1 to AE3; Boehringer Mannheim, Mannheim, Germany), which reacts with a broad spectrum of human cytokeratins. Cytokeratin staining was performed according to the method outlined in our previous report.[18]

### EUS examination

Esophagogastroduodenoscopy was performed in all patients before EUS. EUS examination was performed before CRT and within 10 days before surgery using a 7.5-MHz

linear array (PEF-703FA and SSA-270/CE; Toshiba Medical, Tokyo, Japan). We paid attention to low echoic regions and high echoic spots in the primary tumor on EUS examination. Based on the rate of high echoic spots in the primary tumor, the effects of CRT were classified as follows: grade 1 was defined as a wide low echoic region with high echoic spots occupying less than one third of the area; grade 2 was defined as a narrow low echoic region with high echoic spots occupying more than one third of the area; and grade 3 was defined as almost no low echoic lesion with high echoic spots occupying the whole area (Fig. 1).

One or more hardcopy printouts from a transverse plane of the primary lesion were available and the representative largest lesion was analyzed. Two endoscopic specialists who were

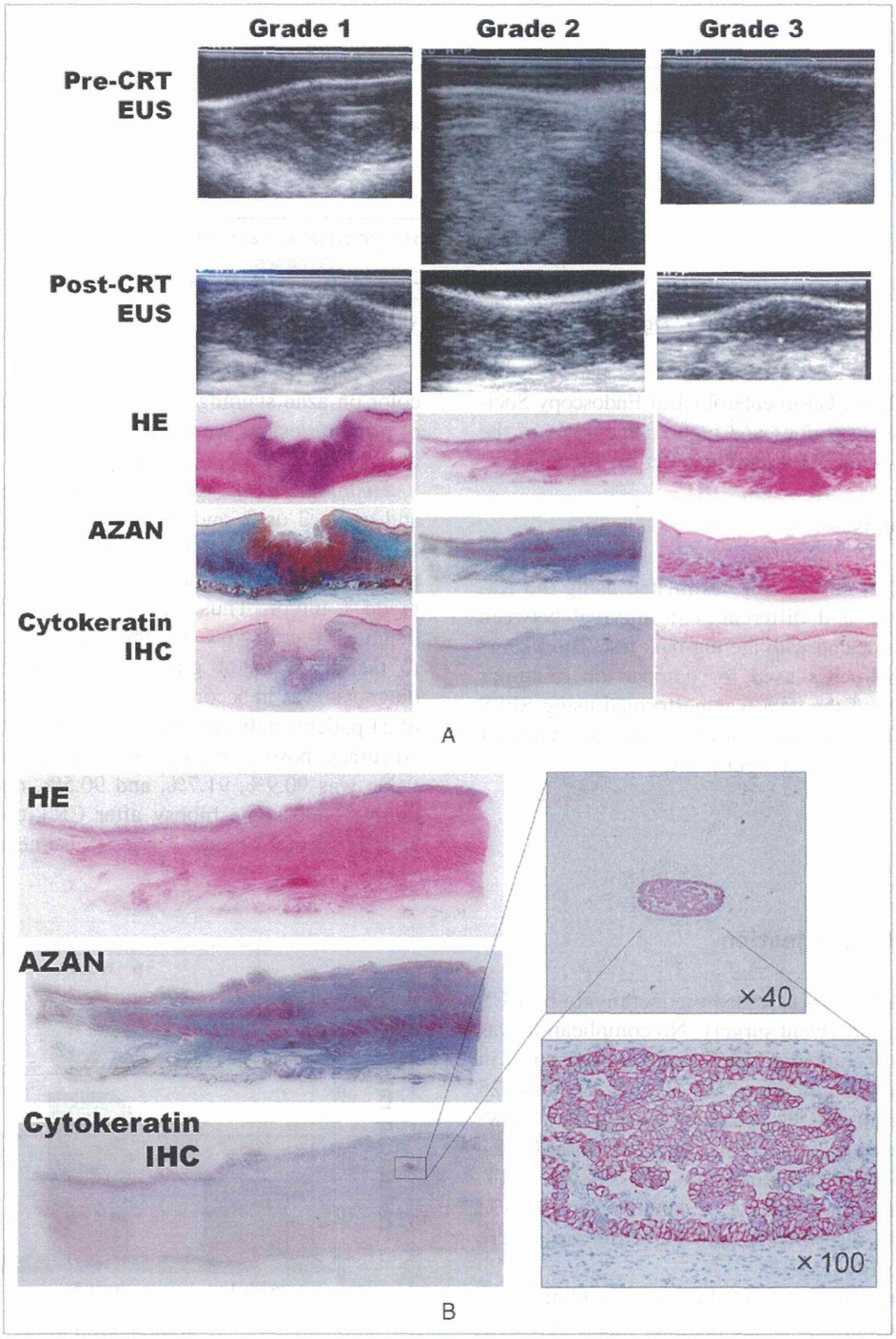


**Figure 1** (A) Classification of tumor response by CRT. HE = hematoxylin-eosin staining; AZAN = azan staining; Cytokeratin IHC = cytokeratin immunohistochemical staining. (B) Small cruster of the alive cancer cells were recognized in grade 2 case. These cells were recognized clearly by immunohistochemical staining using the AE1/AE3 cytokeratin monoclonal antibody cocktail.

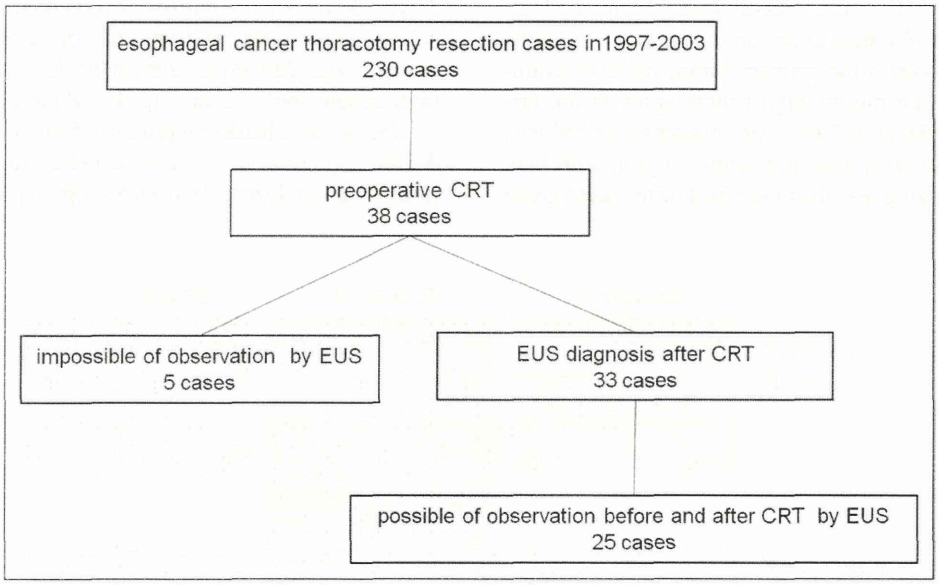


**Figure 2** The breakdown of the present cases.

authorized by the Japan Gastroenterological Endoscopy Society judged low echoic regions and high echoic spots of the tumor under EUS during the examination at the same time.

### Statistical analysis

Actuarial survival curves were estimated using the Kaplan–Meier method, and differences in survival between subgroups were compared with the log-rank test. The Mann–Whitney *U* test also was used for comparison of tumor thickness. Analysis of the data was performed using SPSS version 16.0 (SPSS Japan, Inc, Tokyo, Japan). A *P* value of less than .05 was considered significant.

## Results

### Diagnosis of EUS examination

The primary tumor was assessed preoperatively by EUS in 33 patients who underwent surgery. No complication was recognized in this examination. EUS could be performed in 25 patients both before and after CRT (Fig. 2). Of these 25 patients, the reduction rate was calculated using the following formula: reduction rate = (tumor thickness after CRT - tumor thickness before CRT)/tumor thickness before CRT. The reduction rates for grades 1, 2, and 3 were 12.8%, 27.2%, and 48.1%, respectively, and the differences were significant ($P$ = .014) (Fig. 3).

When comparing the EUS and histologic findings, low echoic areas corresponded to the residual tumor. On both azan and cytokeratin staining, the residual tumor was delineated by red color (Fig. 1B). By contrast, high echoic spots corresponded to fibrous changes indicated by blue color on azan staining. Thus, low and high echoic patterns of primary lesions were important for diagnosing the effect of CRT by EUS (Fig. 1A and B).

Histologic effect was divided into 2 groups of grade 1, and grade 2 or 3, and the results of EUS were compared according to the histologic effect of CRT. Eleven of 12 patients with grade 1 by EUS diagnosis had a histologic grade 1 tumor. Thus, sensitivity and specificity of EUS diagnosis were 84.6% and 95.0%, respectively, with regard to the diagnosis of grade 1 patients. By contrast, EUS diagnosis was in accordance with the histologic effect in 19 of 21 patients indicated by EUS to have grade 2 or 3 disease. Accuracy, positive predictive value, and negative predictive value was 90.9%, 91.7%, and 90.5%, respectively. Preoperative endoscopic biopsy after CRT could detect cancer cells in 10 of 13 grade 1 cases (false-negative rate, 3 of 13

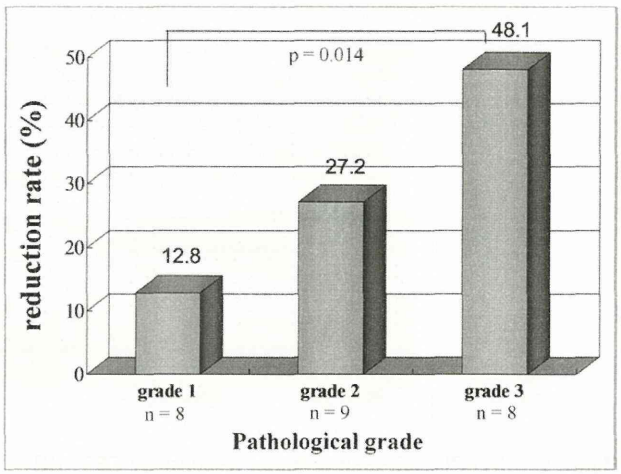


**Figure 3** Reduction rate of the thickness of the primary tumor by CRT.

**Table 2** Comparison of preoperative EUS, pathologic diagnosis, and biopsy under endoscopy after CRT

| | Pathologic diagnosis | |
|---|---|---|
| | Grade 1 | Grade 2 or 3 |
| EUS diagnosis | | |
| Grade 1 | 11 | 1 |
| Grade 2 or 3 | 2 | 19 |
| Biopsy | | |
| Cancer cells positive/negative | 10/3 | 2/18 |

[23.1%]), 2 of 11 grade 2 cases (false-negative rate, 9 of 11 [81.8%]), and 0 of 9 grade 3 cases (false-positive rate, 0 of 9) (Table 2). A few alive cancer cells were recognized by cytokeratin immunohistochemical staining in 2 cases of grade 3 patients who were diagnosed by hematoxylin-eosin staining.

## Prognosis according to CRT effect

No serious complications requiring cessation of CRT were encountered. Follow-up data after surgery were available for all patients with a median follow-up period of 57 months (range, 5–90 mo). The prognoses of patients according to the histologic effect of CRT are shown in Fig. 4. The 5-year survival rates of patients with grades 1 and 2 or grade 3 disease were 22% and 50%, respectively. Patients with grade 1 tumors had a poorer prognosis than those with grade 2 or 3 disease ($P = .02$). When analyzing the relationship between EUS diagnosis for the effect of CRT and prognosis, the 5-year survival rates in patients with grades 1 and 2 or grade 3 were 20% and 50%, respectively (Fig. 5). The prognosis according to EUS diagnosis was similar to the histologic effect.

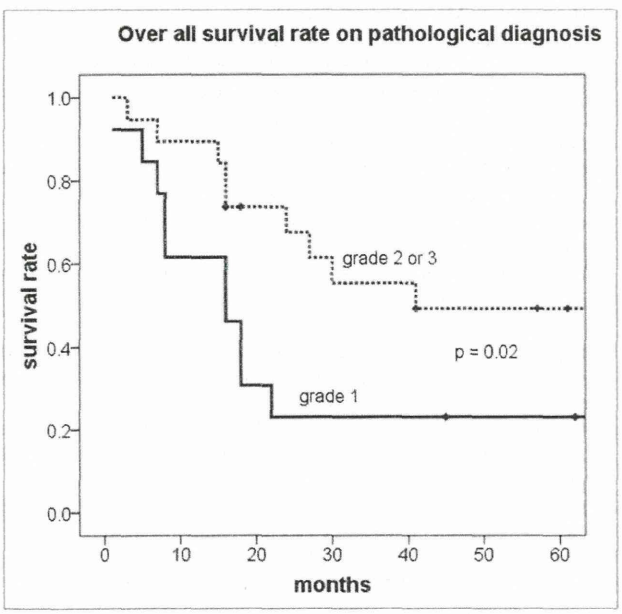


**Figure 4** Survival rate according to histologic effect of CRT.

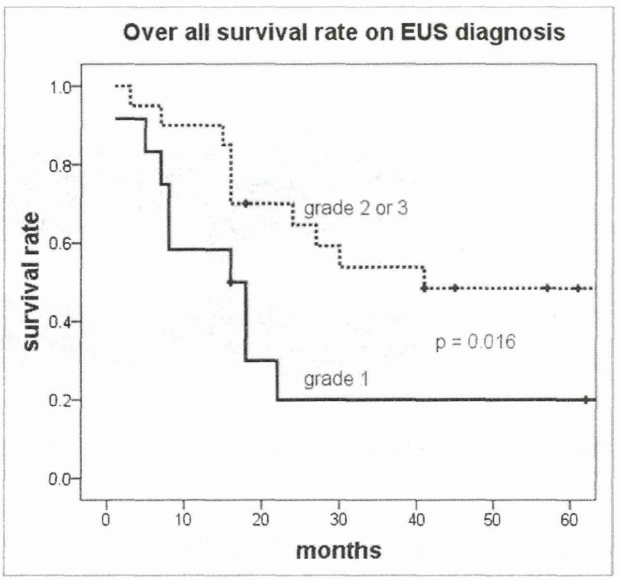


**Figure 5** Survival rate according to the diagnosis of EUS for the effect of CRT.

## Comments

EUS is a useful tool to diagnose the tumor depth and lymph node metastasis in gastrointestinal tract cancer, esophageal cancer,[19–22] gastric cancer, [23–25] and colorectal cancer. [26–29] We also reported that classification of lymph nodes based on EUS findings was useful for planning the treatment strategy and determining the prognosis in patients with ESCC.[19,30–32] EUS provided information regarding not only tumor depth, but also the extent of esophageal infiltration in patients with cardia cancer. In the present study, we examined whether EUS examination is useful for the diagnosis of tumor effect of CRT in ESCC.

In our previous study on lymph node metastasis, the metastatic area (ie, the presence of cancer cells) was shown to correspond to a low echoic area. Similarly, residual tumor after CRT was delineated by a low echoic area by EUS. We noticed high echoic spots in EUS findings after CRT, which corresponded to the areas of fibrosis in the resected specimens. Fibrous changes were clearly recognized by azan staining and residual lesion was identified by cytokeratin staining. We established the criteria of EUS diagnosis for CRT effect based on these findings. In cases in which EUS estimation was possible before and after CRT, the reduction rate of primary tumor also was important.

In the present study, the 5-year survival rate was definitely worse in patients with grade 1 tumors than in those with grade 2 or 3 tumors. Similarly, most studies associated pathologic complete response with improved survival.[6–10,33] These results suggested that assessment of the effect of CRT before surgery

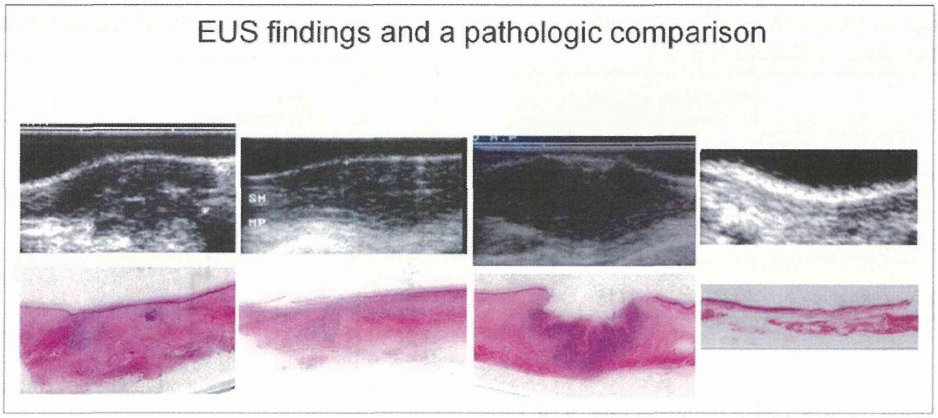


**Figure 6** Comparison of EUS image and the pathology macroimage. The upper row is the EUS image, and the lower row is the resected specimen from the same patients. Low echoic regions and high echo spots accord to vital tumor and fibrotic tissue, respectively.

may provide useful information for further planning of the treatment strategy. Bedenne et al showed that there is no benefit for the addition of surgery after chemoradiation compared with the continuation of additional chemoradiation in the FFCD 9102 trial.[34] However, this randomly assigned study included responders and nonresponders. The patients are available for the choice of the next therapy after CRT more precisely, if they have the information of this first CRT effect. We do not find which is superior in additive CRT and the next esophagectomy according to a responder and a nonresponder in the initial CRT at present. Also, a systematic approach to the endosonographic assessment of lymph node metastasis can improve staging accuracy.[12,35]

Some investigators reported that endoluminal ultrasound could not differentiate between fibrotic tissue and vital tumor residuals.[13–15] Although it was impossible to completely predict histologic response by imaging methods, it is important to determine whether CRT is effective or ineffective because prognosis was significantly different between responders and nonresponders. In fact, in the present study, the survival rates according to the effect of CRT by EUS were quite similar to histologic effect. Willis et al mentioned traced tumor area in transverse cross-section, and they reported that patients with esophageal carcinoma who respond to neoadjuvant treatment as identified by EUS measurement of reduction in tumor size have a significantly better prognosis than nonresponders.[36] We mentioned low echoic regions and high echoic spots, which accord to vital tumor and fibrotic tissue, respectively. Figure 6 shows this correctness.

Radiation (40 Gy) with chemotherapy was performed for neoadjuvant CRT of advanced esophageal cancer in this study, this dose of radiation was acceptable because Apinop et al,[37] Walsh et al,[38] and Reynolds et al[39] chose the same dose of neoadjuvant radiotherapy. However, Lee et al[40] set 45.6 Gy for ESCC, and 50.4 Gy radiation therapy was set by Tepper et al,[41] and 45 to 50.4 Gy radiation for CRT of advanced esophageal cancer might be recommended from now on.

One problem was experience in EUS with a 7.5-MHz linear array. The CRT rigidifies a tumor and may form a firm stenosis. In five cases, the main body of EUS cannot pass the rigid narrow segment. Some patients with rigid stenosis of the esophagus after CRT showed a favorable effect. A linear-array EUS with a small diameter should be used in such patients. In conclusion, we showed the relationship between the EUS findings and histologic effect in patients with ESCC. EUS examination is a useful tool to assess the effects of CRT and to predict prognosis.

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