Carcinosarcoma of the esophagus: A report of 6 cases associated with zinc finger E-box-binding homeobox 1 expression

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Abstract. Esophageal carcinosarcoma (ECS) has been suggested to result from an epithelial mesenchymal transition (EMT) phenomenon. However, knowledge on its underlying molecular features is limited. The clinical and pathological features, and the prognosis of ECS require further investigation. In the present study, a total of 325 patients with esophageal tumors were observed between January 2004 and December 2014, of which 6 patients were diagnosed pathologically with ECS. The clinicopathological features were compared with those of corresponding cases with the identical pathological T stage (pT) of esophageal squamous cell carcinoma (ESCC). In terms of the clinical T stage (cT), the 6 cases were composed of cT1, cT2, cT3 and cT4 in 1, 1, 3 and 1 case, respectively. Nevertheless, pT was eventually diagnosed as pT1 in all cases. There was a large discrepancy between clinically diagnosed depth of tumor invasion prior to surgery and depth of tumor invasion following surgery. Zinc finger E-box-binding homeobox 1 (ZEB1), an EMT-associated transcription factor, was expressed only in the sarcoma component in all 6 cases of ECS. The ECS cases had a significantly poorer prognosis compared with the 115 pT1 ESCC cases. The present study suggests that the depth of invasion of ECS lesions does not correspond with their respective size, and the EMT of the carcinoma component may affect the prognosis by overexpression of the ZEB1 gene.

Introduction

Esophageal carcinosarcoma (ECS) is a rare type of esophageal cancer that was designated as such by Virchow *et al* (1) due to the presence of carcinomatous and sarcomatous components. The components consist of spindles or polymorphous tumor cells with a mesenchymal character. This tumor often presents with polypoid growth with a stalk, and is characterized by the presence of esophageal squamous cell carcinoma (ESCC) *in situ* surrounding the stalk (2). It has been suggested that the sarcoma component is derived from SCC (3-5).

Epithelial-mesenchymal transition (EMT) has been reported as an important means of tumor invasion and metastasis in numerous types of canonical cancer (6,7). Zinc finger E-box-binding homeobox 1 (ZEB1) is associated with EMT transcription factors (8). Members of the microRNA (miR)-200 family have been reported to serve an important role in dysregulating the epithelial phenotype by targeting ZEB factors, thereby preventing E-cadherin downregulation and resulting in EMT (9). In human cancer, ZEB1 expression has been indicated to be increased when miR-200 expression is decreased due to promoter DNA methylation (10,11). The EMT activator ZEB1, in particular, has also been indicated to confer stemness and resistance to anticancer treatment (9). ZEB1 was specifically recognized in the sarcoma component compared with the carcinoma component in spindle cell carcinoma of the esophagus, among which a number of ECS cases were included (8). To the best of our knowledge, information regarding ZEB1 expression in ECS is limited.

In the present study, the aim was to clarify the molecular features associated with prognosis by comparing the clinicopathological factors and prognosis of ECS and ESCC in pT1.

Materials and methods

Patients and data collection. Esophagectomy was performed in 325 patients with esophageal tumors at Kitasato University Hospital (Sagamihara, Japan) between January 2004 and December 2014, and 5 male and 1 female patient (1.8%), with a mean age of 68 years (range, 60-77 years), were diagnosed with ECS. The present study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of Kitasato University School of Medicine (Sagamihara, Japan). Written consent was obtained from all patients. All tissue samples were collected at the Kitasato University Hospital. The clinical and pathological features

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were analyzed and tumor stage was classified according to the 6th edition of the Union for International Cancer Control Tumor-Node-Metastasis (UICC-TNM 6th edition) staging system of esophageal cancer (12). The median follow-up time was 29 months (range, 16-33 months).

Immunohistochemical staining. Serial tissues sections (4-μm thick) were fixed in 10% formalin at room temperature for 16-24 h and embedded in paraffin. The slices were incubated with 3% H_2O_2 at room temperature for 5 min to deactivate endogenous peroxidase and subsequently washed with PBS. Rabbit anti-human ZEB1 polyclonal antibody (dilution 1:100; cat. no. HPA027524; Atlas Antibodies AB, Stockholm, Sweden), rabbit Snail 1 polyclonal antibody (dilution, 1:500; cat. no. GTX125918; Gene Tex, Los Angeles, USA) and rabbit Twist-related protein 1 (Twist 1) polyclonal antibody (dilution, 1:500; cat. no. ab50581; Abcam, Cambridge, UK) were added, and the slices were incubated at 4˚C overnight. Immune complexes were amplified using a Vectastain Universal Elite ABC kit (dilution, 1:50; cat. no. PK-6200; Vector Laboratories, Inc., Burlingame, CA, USA), at room temperature for 10 min, according to the manufacturer's protocols. These complexes were subsequently detected by incubation at room temperature with the chromogen 3,3'-diaminobenzidine (3%) for 1 min. The invasive front of the carcinoma components and the sarcomatous areas were compared. Using a light microscope (Olympus AX80; Olympus Corporation, Tokyo, Japan) (x20 magnification), positive staining was defined as nuclear immunoreactivity in neoplastic cells. Hematoxylin at 0.1% concentration was used to stain at room temperature for 5 min, then 0.025% eosin was used to stain at room temperature for 3 min. As a control tissue of ZEB1 in the present study, according to the study by Kikuchi *et al* (13), mammary gland tissues were also stained. Triple-negative breast cancer tissues demonstrating the strongest staining were used as a positive control, whereas normal breast tissues that did exhibit staining were used as a negative control (13). The positive and negative controls were primary tumors and the corresponding non-cancerous tissues from 3 patients with triple-negative invasive breast cancer who all underwent a partial resection of the breast in September 1999 at Kitasato University Hospital. Based on previous studies (14-17), gastric adenocarcinoma tissues with confirmed staining were used as a positive control for Twist 1 and Snail 1, while a non-cancerous mucosa sample was used as the negative control. The positive and negative controls were primary tumors and the corresponding non-cancerous tissues from 3 patients with macroscopic type 0-IIc early gastric cancer who underwent laparoscopic gastrectomy in October and November, 2016, and November 2005, respectively, at Kitasato University Hospital.

Statistical analysis. All statistical analyses were performed using JMP® 11.0 software (SAS Institute Inc., Cary, NC, USA). Frequency tables were analyzed using the χ^2 test, while the significance of categorical variables was evaluated with the likelihood ratio. Disease-specific survival was measured from the date of diagnosis to the time of patient mortality due to a specific disease, or censored at the date of the last follow-up evaluation. For evaluation of relapse-free survival, recurrence was defined as development of local recurrence, distant metastasis or patient mortality from ECS (whichever occurred first). Survival functions were estimated by life tables and the Kaplan-Meier method, and compared by log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. The characteristics of the patients with ECS are indicated in Table I. The mean age was 68 years. With the exception of 1 unknown case, all patients had a history of smoking and drinking. The main symptoms reported were difficulty in swallowing, coughing and chest pain in 3, 2 and 1 case, respectively. All cases had an American Society of Anesthesiologists-Physical Status of ≤2 (18). All patients underwent computed tomography, and 5 patients underwent upper gastrointestinal contrast studies. Regarding clinical T stage (cT), cT1, cT2, cT3 and cT4 were indicated in 1, 1, 3 and 1 case, respectively (Fig. 1). The longitudinal length of the tumor was 2.5-10.5 cm (mean, 6.5 cm). Pathological T stage (pT) was eventually diagnosed as pT1 in all cases. According to Japanese Classification of Esophageal Cancer 11th Edition (2), the final pathological morphological type was pedunculated type, sessile (broad based) type, and pedunculated and slightly elevated type in 4, 1, and 1 cases, respectively. All patients underwent radical surgery with complete tumor resection.

Patient 1 received preoperative chemoradiotherapy (CRT), where radiotherapy was applied at 40 Gy due to suspected tracheal invasion (cT4). The CRT therapy included radiotherapy concurrent with nedaplatin + 5-fluorouracil (FU) chemotherapy due to renal dysfunction. Nedaplatin + 5-FU chemotherapy consisted of two courses of chemotherapy with 5-fluorouracil (800 mg/m^2 on days 1-5) and nedaplatin (90 mg/m^2 on day 1) every 4 weeks. Postoperative adjuvant CRT was performed in patient 2 due to vigorous vascular invasion and lymph node metastasis in the pathological results, and chemotherapy [nedaplatin (50 mg/m^2 on day 1) + 5-FU (400 mg/m^2 on days 1-5)] was additionally used due to renal dysfunction. The pathological depth of invasion was pT1 in all cases, and there was a large discrepancy between clinically diagnosed depth of tumor invasion prior to surgery and depth of tumor invasion following surgery. Lymph node metastasis was indicated in 2 cases. Postoperative complications included hoarseness due to recurrent laryngeal nerve paralysis in 3 cases, and anastomotic leakage [Clavien-Dindo classification (19) grade IIIa], which caused a gastrointestinal tract to lung fistula, in 1 case. Postoperative recurrence was observed in 3 cases despite the superficial depth of invasion in all cases. The 3 patients with recurrence succumbed at 20, 28, and 32 months due to the progression of recurrence in the lymph nodes, liver and lungs/bones, respectively. Patient 2, in particular, presented with multiple lung metastases and fifth rib metastasis at 16 months post-surgery, despite preoperative adjuvant CRT. Despite administration of 8 courses of cisplatin (15 mg/m^2 on day 1) + 5-FU (750 mg/m^2 on days 1-5) and 1 course of cisplatin (90 mg/m^2 on day 1) + irinotecan (75 mg/m^2 on day 1), the patient succumbed at 32 months post-surgery. Patient 4 presented with ECS with pT1 (UICC-TNM 6th edition) lymph node metastasis 0, lymphatic invasion 0, venous invasion 0 and granulocyte

Table I. Characteristic of patients with esophageal carcinoma.

Variables	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Sex	Male	Female	Male	Male	Male	Male
Age, years	61	60	73	77	68	71
Smoking status	Former	Unknown	Current	Current	Current	Former
Chief complaint	Chest pain	Dysphagia	Cough	Cough	Dysphagia	Dysphagia
Location	Mt	Mt	Lt	Ut	Mt	Mt
Clinical T factor	cT4	cT3	cT3	cT3	cT2	cT1
Albumin level, g/dl	4.1	4	3.3	3.3	3.4	3.4
Neutrophil to lymphocyte ratio	3	2.9	1.7	2.4	6.9	6.6
ASA-PS	Class 1	Class 1	Class 2	Class 1	Class 1	Class 2
Tumor length, cm	2.5	10.5	4.5	6.5	8.5	6.6
Macroscopic type	0-Ip	0-Ip	0-Ip	0-Isp	0-Ip+IIa	0-Ip
Lymphatic invasion	Unknown	2	0	0	1	0
Venous invasion	Unknown	2	0	0	1	0
pT factor	pT1	pT1	pT1	pT1	pT1	pT1
pN factor	pN0	pN1	pN0	pN0	pN1	pN0
Immunohistochemical findings	Unknown	Vimentin(+)	Vimentin(+)	G-CSF(+)	Vimentin(+)	Vimentin(+)
Initial treatment	NF + RT + Resection	Resection + NF + RT	Resection	Resection	Resection	Resection
Tumor recurrence	No	Yes	No	Yes	Yes	No
Treatment following recurrence	None	5-FU + cisplatin, irinotecan + cisplatin	None	5-FU +	BSC cisplatin	None
Prognosis	Alive	Succumbed to tumor	Succumbed to AMI	Succumbed to tumor	Succumbed to tumor	Alive

Mt, middle thoracic esophagus; Lt, Lower thoracic esophagus; Ut, Upper thoracic esophagus; ASA-PS, American Society of Anesthesiologists physical status; 5-FU, fluorouracil; NF, nedaplatin + 5-FU; RT, radiationtherapy; AMI, acute myocardial infarction; G-CSF, granulocyte colony-stimulating factor; BSC, best supportive care; pT, pathological depth of tumor invasion; pN, pathological lymph node stage.

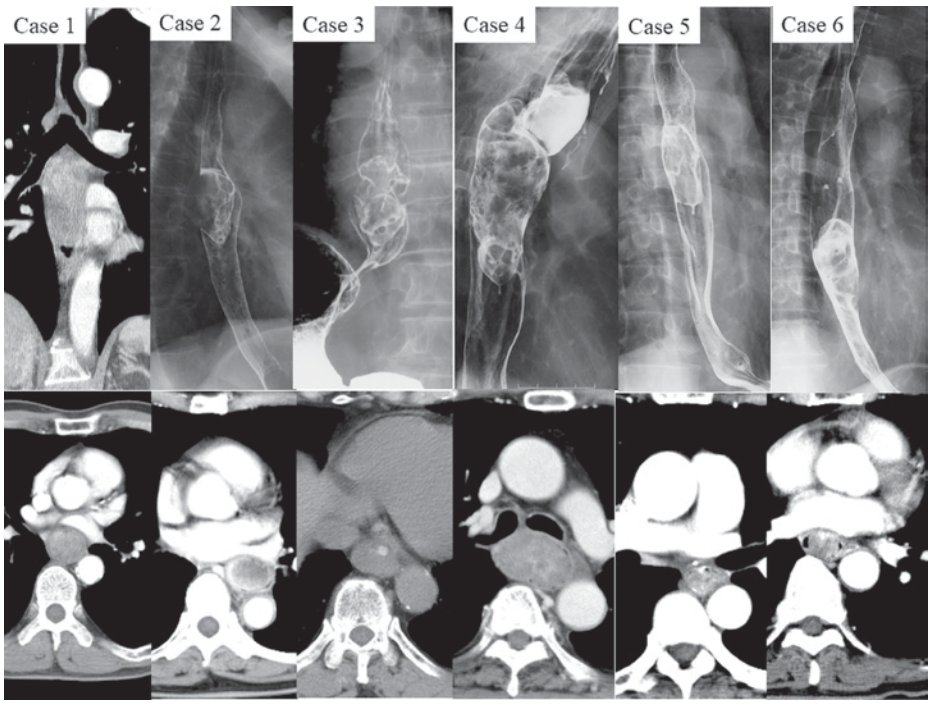

Figure 1. Radiography of the upper gastrointestinal tract and chest computed tomography indicating an intraluminal polypoid lesion in all 6 cases. The mean tumor size was 6.5 cm (major axis, 2.5-10.5 cm).

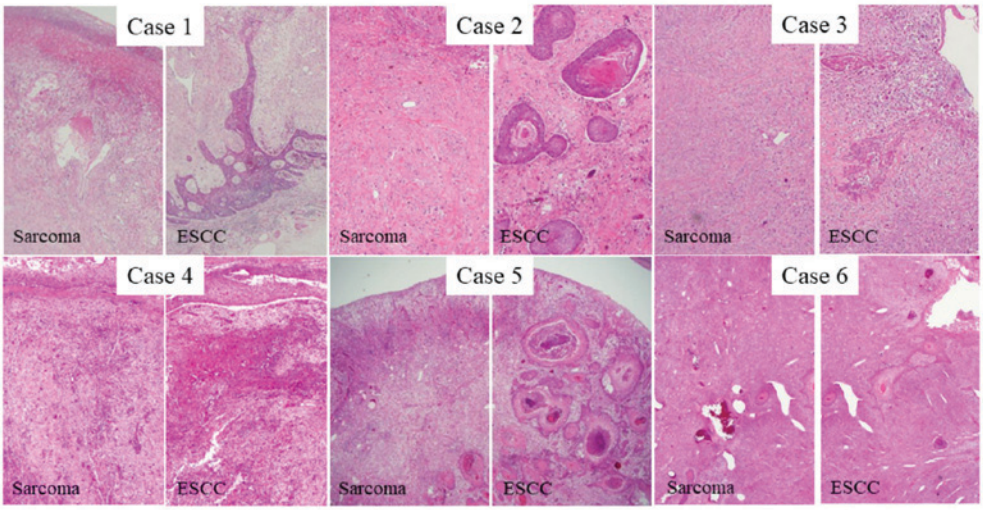

Figure 2. Microscopic appearance of squamous cell carcinoma and spindle-shaped tumor cells of the carcinosarcoma component in all 6 cases (hematoxylin and eosin stain, x20 magnification). ESCC, esophageal squamous cell carcinoma.

colony-stimulation factor-positive tumor cells in the immunohistochemical analysis. This patient experienced recurrence of lymph node metastasis at 6 months post-surgery, and despite administration of 8 courses of cisplatin (60 mg/m^2 on day 1) + 5-FU (800 mg/m^2 on days 1-5), the patient additionally developed brain metastasis and succumbed at 20 months post-surgery. Patient 5 presented with liver metastasis at 23 months post-surgery and additionally developed pleural dissemination, lung metastasis and para-aortic lymph node metastasis at 26 months post-surgery. The patient succumbed 2 months later.

Pathological features. Histopathological findings indicated carcinomatous cells and sarcomatous cells (Fig. 2). The sarcomatous component exhibited spindle-shaped heterotypic cells with varying sizes of enlarged nuclei. ESCC components, scattered in an island shape, were indicated in the lesion. The two components were separate in certain places and intermingled in others. All cases exhibited ZEB1 expression in neoplastic cells distributed uniformly in the sarcomatous component (Fig. 3). Neoplastic cells in the carcinoma components were negative for ZEB1. In 2 cases, metastatic lymph nodes were negative for ZEB1 (Fig. 4). The expression of Twist 1 and Snail 1, which are EMT-associated factors similar to ZEB1, was confirmed. As a result, Snail 1 was weakly expressed in the sarcoma component and the ESCC component (Fig. 5). Twist 1 was strongly expressed in the sarcoma and the ESCC component (Fig. 6). There was no indication that Twist 1 and Snail 1 were expressed specifically in the sarcoma component.

Treatment and clinical outcome. Prognostic analysis of 115 cases of pT1 ESCC with the same depth of tumor invasion as ECS (Table II) indicated a 5-year disease-specific survival rate of 40.0% in ECS and 90.6% in ESCC. ECS had a significantly worse prognosis compared with ESCC in pT1 cases (P=0.0016; Fig. 7A). The 5-year relapse-free survival rate was 22.2% in ECS and 77.1% in ESCC, and ECS had a significantly worse prognosis, compared with ESCC (P=0.0267; Fig. 7B).

Discussion

In the present study, the clinicopathological features of ECS were examined. The results indicated that there was a large discrepancy between the cT factor and the pT factor in ECS. Even in cases with a predicted depth of invasion of cT2 or beyond with large polypoid tumors, the pT factor in all cases indicated that the depth of tumor was superficial invasion only. Regarding the fact that all cases were T1 cases, this was uncommon. According to previous studies, half of reported cases were pT1 (20-22). Due to the high level of patient awareness with regard to health in the area in which the Kitasato University Hospital is situated, the diagnosis of the disease at an early stage is more likely. However, as a number of patients harbored large tumors occupying the lumen, the present study reported an unexpectedly large discrepancy between the pathological depth of tumor invasion and the clinically predicted depth of invasion. It has been reported that >90% of ECS lesions are pedunculated or semipedunculated (23). The polypoid lesions have a predominantly sarcoma component, whereas the superficial ESCC component is mainly present in the stem and base (2). Therefore, sarcomatous metaplasia originally occurs in superficial carcinoma, and only the sarcoma-like component increases rapidly toward the lumen (2).

It has been suggested that in carcinosarcoma, the sarcoma component may derive from EMT (24,25). EMT is involved in a number of developmental milestones, including gastrulation, neural crest formation and heart morphogenesis, which rely on the plastic transition between the epithelium and mesenchyme (6). By contrast, during the progression of epithelial tumors, cancer cells develop increased motility and invasiveness (6). EMT has been established as an important step in the metastatic cascade of epithelial tumors (7). There are numerous molecules that could explain EMT, including ZEB1, Twist 1 and Snail; however, in esophageal carcinosarcoma, ZEB1 has been suggested to serve a critical role in the EMT process (7). In the present study, it is noteworthy that high expression of ZEB1 was recognized only in the sarcoma component. For this

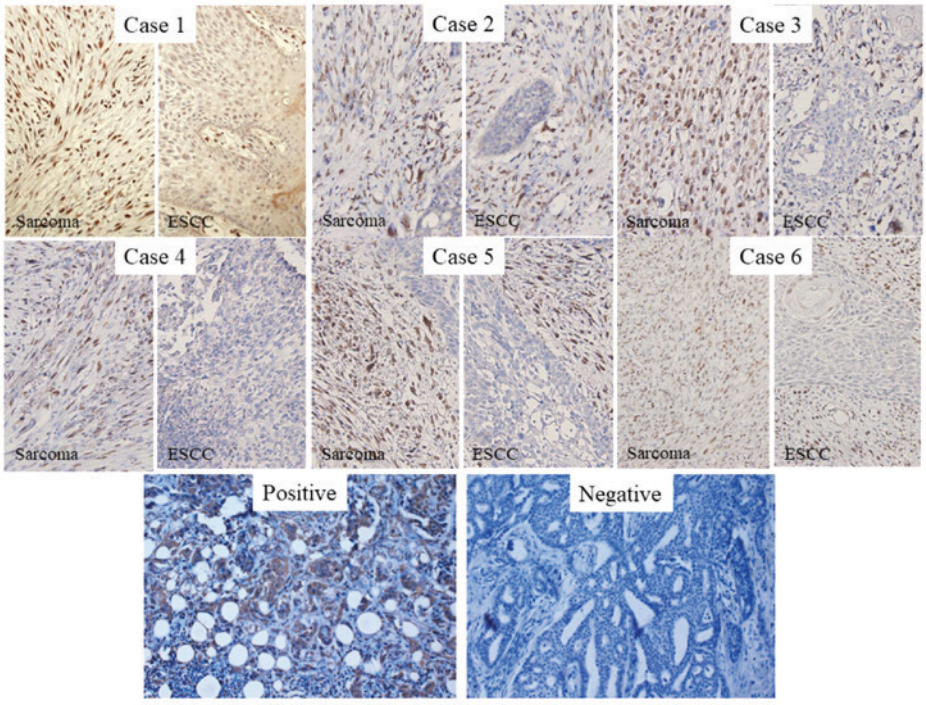

Figure 3. ZEB1 expression (magnification, x100) in neoplastic cells distributes uniformly in the sarcomatous component. All cases indicated the ZEB1 staining pattern. Normal breast tissues and triple-negative breast cancer tissue were defined as negative control and positive control, respectively. ESCC, esophageal squamous cell carcinoma; ZEB1, zinc finger E-box-binding homeobox 1 expression.

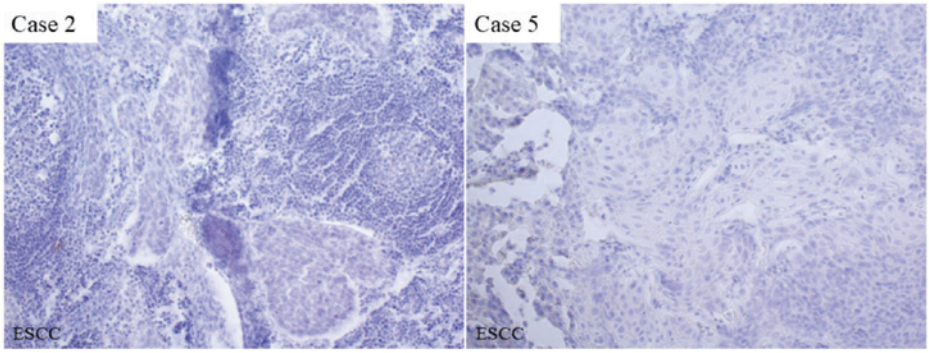

Figure 4. Metastatic lesion of SCC in the lymph node. Cases 2 and 5 were negative for the zinc finger E-box-binding homeobox 1 expression staining pattern. ESCC, esophageal squamous cell carcinoma.

reason, immunohistochemical staining was confirmed in all 6 cases, and as depicted in Fig. 3, the expression of ZEB1 was confirmed to be increased in the ECS component, confirm with the ESCC component. This result suggests that ZEB1 upregulation in ESCC cells may cause EMT and the transformation to carcinosarcoma. In addition, another study reported that phospholipid glutathione peroxidase (GPX4) inhibition, which induces ferroptic cell death in therapy-resistant cancer cells across diverse mesenchymal cell-state contexts, did not exhibit consistent sensitization in Snail 1 and Twist 1, but ZEB1 was correlated with mesenchymal state sensitivity to GPX4 inhibition (26). ZEB1 is a transcriptional factor that can induce EMT through critical mediators, including transforming growth factor β1 (27,28) and miR-200 (29-31), suggesting that the clinical data of the present study may support the underlying molecular mechanism of ZEB1 in EMT.

Zhang *et al* (32) reported that spindle cell carcinoma (SpCC) had a depth of pathological T1/2 (P<0.001) and a good prognosis (P=0.044), compared with typical SCC, with increased tumor depth, compare with SpCC. In the present study, the prognosis of ECS was significantly worse compared with that of ESCC in terms of disease-specific survival rate (P=0.0016). The prognostic relevance between SpCC and ESCC differed between the study by Zhang *et al* (32) and the present study. However, in this previous study, the depth of tumor was significantly shallower in SpCC cases compared with that in typical SCC cases. It was considered that an identical depth of tumor may cancel out the prognostic difference observed. In fact, when the pathological factors of the patient background were stratified into pT1/2 and the prognostic analysis was performed, it was reported that there was no significant difference in the prognosis between SpCC and

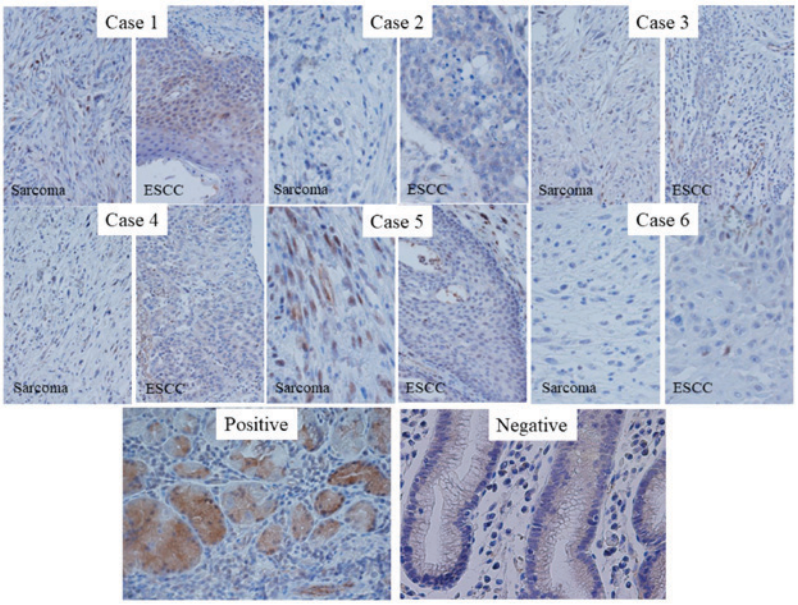

Figure 5. Sarcoma and carcinoma areas are weakly immunohistochemically positive for the expression of Snail 1 (magnification, x100). There was no indication that Snail 1 was expressed specifically in the sarcoma component. All cases indicated the Snail 1 staining pattern (antigen recognized by a rabbit Snail 1 polyclonal antibody). Non-cancerous adjacent mucosa and early gastric cancer tissues were defined as the negative control and positive control, respectively. Snail 1, snail family transcriptional repressor 1; ESCC, esophageal squamous cell carcinoma.

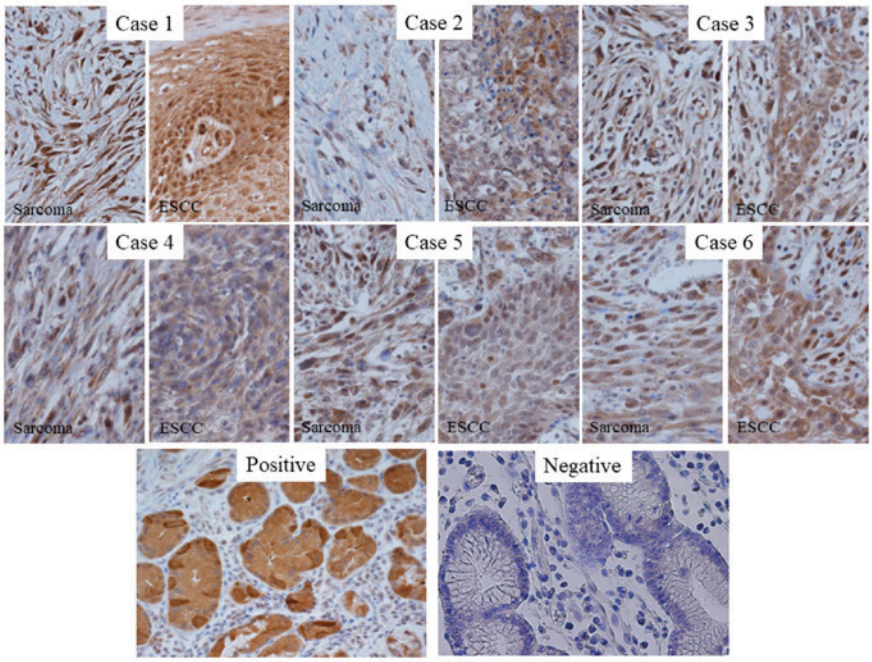

Figure 6. Sarcoma and carcinoma areas are strongly immunohistochemically positive for the expression of Twist 1 (magnification, x100). There was no indication that Twist 1 was expressed specifically in the sarcoma component. All cases indicated the Twist 1 staining pattern (antigen recognized by a rabbit Twist 1 polyclonal antibody). Non-cancerous adjacent mucosa and early gastric cancer tissue were defined as the negative control and positive control, respectively. Twist 1, Twist-related protein 1; ESCC, esophageal squamous cell carcinoma.

typical SCC (32). Therefore, the present study, to the best of our knowledge, included novel findings comparing ECS cases and ESCC cases with pT1.

It has been reported that the expression of ZEB1 in the sarcoma component may be a cause of poor prognosis by participating in treatment resistance, for example, against chemotherapy, and causing distant metastasis (33). In addition, previous studies reported that the inhibition of ZEB1 expression suppressed the tumorigenesis of breast cancer cells (34), that ZEB1 promoted metastasis and loss of cell polarity by repressing the expression of lethal giant larvae homolog 2 in colorectal cancer cells (35), and that ZEB1 was associated with the resistance to chemotherapeutic agents, including gemcitabine, 5-FU and cisplatin in pancreatic cancer cells (36). Recurrent cases were observed in the present study, despite administration of postoperative adjuvant therapy with

Table II. Univariate analysis of esophageal carcinosarcoma and esophageal squamous cell carcinoma with the same depth of tumor invasion.

Characteristics	pT1 carcinosarcoma (n=6)	pT1 squamous cell carcinoma (n=115)	P-value
Sex			0.85
Male	5	99	
Female	1	16	
Age, years			0.65
<65	2	49	
>64	4	66	
Location of tumor			0.99
Cervical esophagus	0	1	
Upper thoracic esophagus	1	20	
Middle thoracic esophagus	4	67	
Lower thoracic esophagus	1	24	
Abdominal esophagus	0	3	
pN factor			0.96
pN1	2	42	
pN0	4	73	
Procedure of esophagectomy			0.61
VATS	1	80	
Thoracotomy	5	35	
Neoadjuvant chemotherapy			0.58
Presence	1	38	
Absence	5	77	
Recurrence			0.03
Presence	3	18	
Absence	3	97	

For all P-values a χ^2 test was performed. VATS, video-assisted thoracic surgery; pT, pathological depth of tumor invasion; pN, pathological lymph node stage.

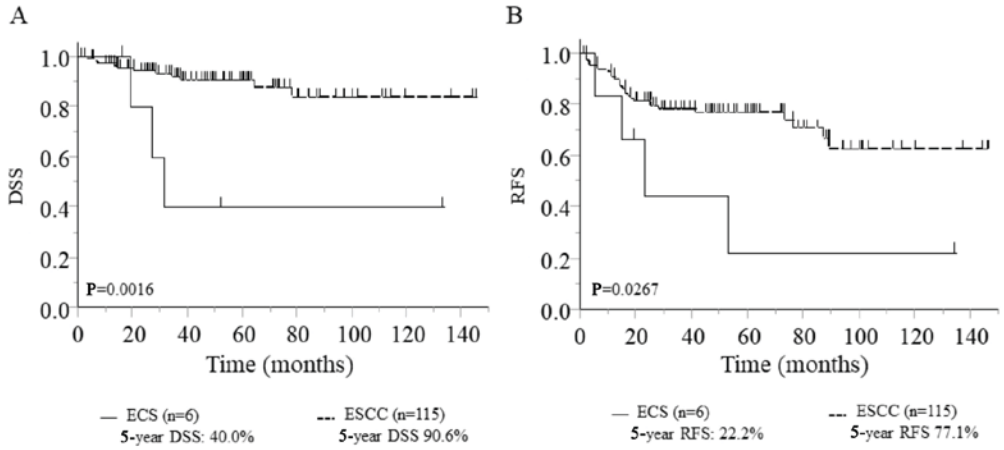

Figure 7. Associations between pathological features and DSS or RFS time. Pathological features consisted of ECS and ESCC with the same depth of tumor invasion. ECS was associated with significantly shorter (A) DSS and (B) RFS times compared with ESCC. DSS, disease-specific survival; RFS, relapse-free survival; ECS, esophageal carcinosarcoma; ESCC, esophageal squamous cell carcinoma.

anticancer drugs. It has been reported that recurrent tumors may be derived from sarcoma (37) with strong expression of ZEB1 being expected. For the treatment of recurrent tumors with expected anticancer drug resistance, no further promising results will be obtained unless molecular targeted treatment is applied. In addition, in a previous study in our

laboratory (Department of Surgery, Kitasato University School of Medicine), it was confirmed that the suppression of ZEB1 expression in triple-negative breast cancer resulted in a loss of resistance to phenylbutyrate in the majority of cases (13).

Based on the aforementioned finding, the present study aimed to indicate the molecular uniqueness of ESC by confirming its specificity, as numerous molecules could be associated with EMT, including ZEB1, Snail 1 and Twist 1. According to the results of the comparative investigation, it was revealed that ZEB1 is highly characteristic of sarcoma components of EMT in ESC. However, a limitation of the present study is that it cannot fully explain the association between ZEB1 and poor prognosis. Despite that ZEB1 indicated an association with ESC, the present study did not examine this association on a molecular level, therefore further investigation is required.

In conclusion, a detailed clinicopathological analysis of ECS was performed in the present study, and its unique clinical and molecular features were identified. The features of ECS indicated in the present study may be of great assistance in further developing a novel therapeutic strategy for the drug-resistant neoplasm.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

HH and KY conducted the conception and design, acquisition of data, analysis of data and drafting the manuscript. MWat performed the acquisition of data, and the drafting and revising of the manuscript. MWas, AE, MK and HMo aided with acquisition of data and assisted with revising the manuscript. YK aided with the statistical analysis and analysis of data. KH and HMi participated in the design and coordination of the study and assisted with revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of Kitasato University School of Medicine (Sagamihara, Japan). Written informed consent was obtained from each patient.

Patient consent for publication

Written informed consent was obtained from each patient for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

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