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ORIGINAL ARTICLE

Molecular and clinicopathological analyses of esophageal carcinosarcoma with special reference to morphological change

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Abstract

The molecular pathogenesis of esophageal carcinosarcoma (ECS) has not been fully investigated. This study includes 16 consequent cases of surgically resected ECS. Genetic alterations were independently examined for carcinoma in situ, carcinomatous, and sarcomatous areas. Six cases were analyzed by next-generation sequencing, and the remaining cases were analyzed by Sanger sequencing for *TP53*, *PTEN*, and *INI1*. Sarcomatous components in 3 cases showed histologically heterogenous feature of osteosarcoma. Lymph node metastasis was found in 12 out of 16 cases. Survival analysis revealed 5-year overall survival rate of 59.9%, and the median survival time was 5.37 years. *TP53* was the most frequently mutated gene, being identified in 11 of 16 patients (68.8%), 7 of whom (63.6%) had the same mutations in both carcinomatous and sarcomatous areas. Almost complete concordance was found between p53 immunohistochemistry and *TP53* missense mutations. Five-year overall survival tended to be worse for patients with p53 overexpression, although the data was not significant ($p = 0.186$). Nine of 16 patients (56.3%) showed loss of heterozygosity (LOH) at the *INI1* locus, and this LOH status was consistent with both components. However, interestingly, INI1 expression was preserved in all cases. In addition, copy number variation analysis revealed gene amplification in several tyrosine kinase receptors. Accumulation of mutations in tumor suppressor genes such as *TP53* and *INI1* seemed to occur during ECS development.

Keywords Esophageal carcinosarcoma · Next-generation sequencing · *TP53* · *INI1* · LOH · Tumorigenesis

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Abbreviations

CS Carcinosarcoma
LOH Loss of heterozygosity

Background

Esophageal carcinosarcoma (ECS) is a rare form of esophageal cancer, accounting for 0.2–2.8% of esophageal cancers [1, 2]. This tumor often shows polypoid growth, characterized by the presence of squamous cell carcinoma (SCC) in situ surrounding the stalk. Carcinosarcoma histologically comprises both carcinomatous and sarcomatous components. This tumor is also known as sarcomatoid carcinoma, spindle cell carcinoma, pseudosarcomatous SCC, polypoid carcinoma, metaplastic carcinoma, SCC with a spindle cell component,

and carcinoma with mesenchymal stroma [3–5]. In certain cases, this tumor can contain heterogenous neoplastic bone and cartilage components.

The various names for this tumor reflect the difficulty in the understanding of tumorigenesis. Several theories for the tumorigenesis of carcinosarcoma have been proposed. The collision theory posits that the carcinoma and sarcoma are two independent neoplasms. The combination theory proposes that both components are derived from a single stem cell that undergoes divergent differentiation early in the evolution of the tumor. According to the conversion theory, the sarcomatous element derives from the carcinoma during the evolution of the tumor. Finally, the composition theory suggests that the spindle cell component is a pseudosarcomatous stromal reaction to the presence of the carcinoma [6]. Clinicopathological analyses of ECS have been performed, but the molecular pathogenesis of ECS remains unclear [7–9]. Researches focusing on genetic alteration, especially the comprehensive next-generation sequencing analysis using frozen specimens, have progressed at a remarkable speed. However, such gross analysis is not suitable for the research focusing on the morphological/differentiation process in this kind of heterogenous tumors. The present study was undertaken by the application of the archival pathological tissue specimens using microdissection method to elucidate the genetic alterations involved in the differentiation mechanisms of ECS to explore which of the aforementioned theories may be true.

Materials and methods

Patients and tissue samples

This study included 16 patients diagnosed as ECS out of 1849 (0.87%) esophageal cancer patients surgically treated at Juntendo University Hospital from January 2003 to March 2018. Clinicopathological information was obtained from each patient including patient age, gender, tumor location, tumor size, tumor stage (pT), lymphovascular invasion, lymph node metastasis, cancer stage [10], and survival periods. The definition of the sarcomatous component in carcinosarcoma was based on the histology, specifically spindle or polymorphous tumor cells with a mesenchymal character [3]. The mesenchymal character was confirmed by vimentin immunohistochemistry. Macroscopic features were classified according to the Japanese Esophageal Society criteria as follows: type 0-I, superficial and protruded type, which is subclassified as type 0-Ip consisting of pedunculated lesion with a peduncle or semipeduncle; type 1, protruding type; type 2, ulcerative located type; and type 5, unclassified type [3].This macroscopic classification is also referred to WHO classification [4]. The surgical specimens were fixed in 10% buffered formalin and embedded in paraffin. This study was approved by the Ethical Committee of Juntendo University School of Medicine (2016108).

NGS

DNA was extracted from the tumoral and non-tumoral tissues of all patients. NGS was performed using the Ion Ampliseq Cancer Hotspot Panel v2 (Thermo Fisher Scientific, Waltham, MA, USA) for six cases that passed the DNA RQ check. Template preparation and chip loading were performed using the Ion Chef system using the Hi-Q View Chef Kit (Thermo Fisher Scientific). Sequencing was performed on Ion PGM (Thermo Fisher Scientific), utilizing the Variant Caller plug-in v.5.2.1.38 with reference to the COSMIC database. NGS amplification was considered successful if an average minimum of ≥ 500 reads was achieved across all target regions and the number of mapped reads was $> 15{,}000$. Mutations with $< 5\%$ allele frequency were excluded. Copy number variation (CNV) analysis was also performed using the Ion Reporter™ software (Thermo Fisher Scientific). CNVs were assessed based on the copy number relative to the non-tumoral samples used.

Immunohistochemistry

Paraffin-embedded tissue blocks containing both carcinomatous and sarcomatous components were selected. Sections 4 μm in thickness were cut and deparaffinized. Immunohistochemistry was performed using the indirect polymer method with the Envision™ system (DAKO Cytomation, Glostrup, Denmark). The monoclonal antibodies used were specific to p53 (PAb1801; Leica, UK; 1:50 dilution), Ki-67 (MIB-1, monoclonal, DAKO, Denmark; 1:200 dilution), and INI1 (25/BAF47, monoclonal, BD Biosciences, USA; 1:200 dilution). Antigen retrievals for p53 and Ki-67 were performed by incubation in Tris-EDTA buffer (pH 9.0) in an autoclave for 10 min at 121 °C. The Ki-67 labeling index was calculated by counting the number of positively stained nuclei from at least 500 cancer cell nuclei. INI1 was judged to have been retained if $> 50\%$ of tumor cells displayed strong nuclear staining.

Sanger sequences

Based on the NGS data, mutations of *TP53*, *PTEN*, and *INI1* were evaluated for the remaining 10 cases not examined by NGS according to the previously described PCR-direct sequencing method [11–13]. DNA was extracted from each carcinoma in situ, carcinomas, and sarcomas by microdissection. Carcinoma in situ (CIS) components disappeared in three cases, probably due to the ulcer formation at the surface during tumor growth (case nos. 2, 7, and 13). DNA could not be extracted from one case (case no. 14) due to the small amount of residual CIS component. CIS and invasive SCC components could not be clearly separated in another case (case no. 10). The same primer pairs listed on the Cancer Hotspot Panel were used for this analysis (Supplementary Table 1). Mutations were presumed if the height of the mutated peak reached 25% of the height of the normal peak.

LOH analysis

Loss of heterozygosity (LOH) analysis of the *INI1* locus was performed by PCR using microsatellite markers on chromosome 22q11.23 (D22S257, D22S301, D22S303, D22S345). In addition, two polymorphic microsatellite markers at the *TP53* locus (17p13.1) were used (Supplementary Table 1). The method was essentially the same as the LOH analysis we previously described [12, 13]. LOH was calculated as the ratio between the short allele-normal (Sn) and the long allele-normal (Ln) and between the short allele-tumor (St) and the long allele-tumor (Lt) using the following formula: (Sn:Ln)/(St:Lt). LOH was scored when one allele (peak) was decreased by > 50% in the tumor sample compared to the same allele in normal tissue, followed by DNA stutter correction when necessary (score, 0.5 or 2.0). A sample was considered non-informative when the control DNA for normal tissue was homozygous for the polymorphic markers (i.e., showing only one peak in the normal control tissue).

Statistical analyses

All p values were two-sided, and p values ≤ 0.05 were considered statistically significant. Survival analysis was performed according to the Kaplan-Meier method. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions and is frequently used in biostatistics.

Results

Clinicopathological characteristics

Typical macroscopic and microscopic features of ECS are shown in Fig. 1. The clinicopathological findings are summarized in Table 1 and Supplementary Table 2. The mean patient age was 66.6 years (range 51–83 years). Sex ratio was 13:3 (male:female). Macroscopically, all cases were protruded lesions and were classified as type 0-I (0-Ip): 8 cases; type 1: 5 cases; type 2: 2 cases; and type 5: 1 case. Most of the cases were accompanied by squamous cell carcinoma in situ except for 3 cases (case nos. 2, 7, and 13). Carcinoma in situ area seemed to have disappeared by ulcer formation in these three cases. The mean tumor size was 54.4 mm (range 26–121 mm). The dominant site of occurrence was in the central esophagus. The depth of invasion was submucosa (SM) in 7 cases, muscularis propria in 3 cases, and adventitia (AD) in 6 cases. Sarcomatous components in 3 cases showed histologically heterogenous feature of osteosarcoma (case nos. 8, 9, and 12). Lymph node metastasis was found in 12 out of 16 cases (75%), and all metastatic lesions were composed of squamous cell carcinoma. Distant metastasis (to the bone, lung, and brain) was noted in 2 cases (case nos. 5 and 6) after surgery. Case 6 received neoadjuvant chemotherapy, and 3 cases received chemotherapy after surgical treatment. Two cases (case nos. 6 and 16) received radiation therapy (total 40 and 50 Gy, respectively) after surgery.

Genetic alterations

Data for genetic alterations are summarized in Table 2. NGS revealed *TP53* as the most frequently mutated gene in ECS (6/6; 100%). The *TP53* mutation patterns were nearly consistent in the two components within the same tumors. The *TP53* mutation was studied in the remaining cases, and combined with the Sanger sequence, *TP53* mutations were found in 11 out of 16 patients (68.8%). Of these 11 patients, 7 (63.6%) possessed the same mutations in both the SCC and sarcomatous areas. Furthermore, a single case harbored the same mutations at two positions in both components and an additional *TP53* mutation was detected only in the carcinomatous area. Mutations in *PTEN* were detected in 3 out of 17 cases, 2 of which were located in the

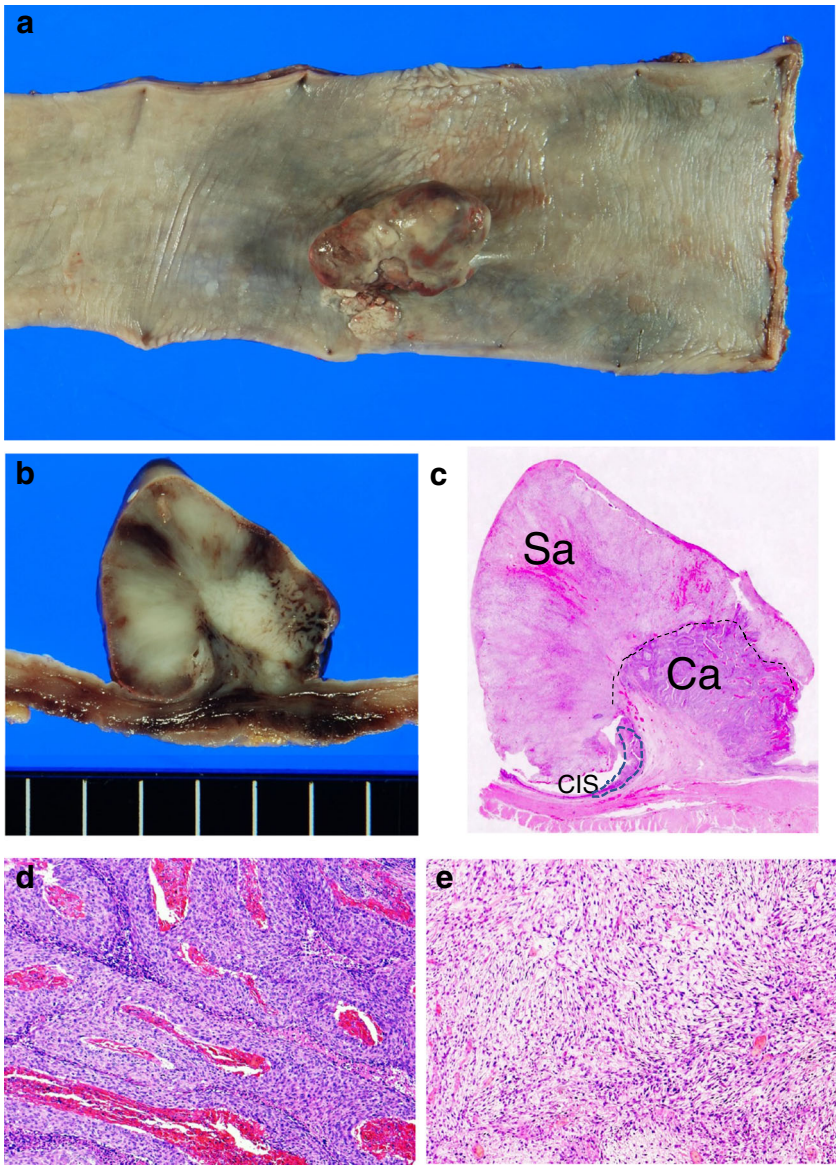

Fig. 1 Macroscopic and microscopic analyses of esophageal carcinosarcoma. A large tumor from esophageal mucosa protrudes into the esophageal lumen (**a**). The tumor seems not to invade deeply despite its large tumor size (**b**) cut surface of tumor, (**c**) Loupe image; CIS carcinoma in situ area, Ca carcinomatous area, Sa sarcomatous area). High-power views of the section show nests of carcinoma cells in the carcinomatous area (**d**) and fascicular proliferation of spindle cells in the sarcomatous area (**e**)

coding regions. These mutations have not been reported in cBioportal. These two mutations were not consistent among the three components. The remaining mutation was the same non-sense mutation detected in both carcinomatous and sarcomatous components, but not in the CIS component. The *INI1* mutation was not detected in any case.

LOH analysis

LOH at the *TP53* and *INI1* loci was examined because *TP53* was frequently mutated in ECS and since *INI1* was revealed as one of the deleted genes in ECS by CNV analysis of NGS. Five cases were not informative for *TP53* LOH analysis (case nos. 3, 5, 11, 12, and 16). The frequency of LOH of at least one of the microsatellite markers on the *TP53* locus was 63.6% (7/11) for the carcinoma component, 72.7% (8/11) for the sarcomatous component, and 0% (0/7) for the CIS component (Fig. 2a). One case (case no. 1) was positive for the carcinoma component, but negative for the sarcomatous component. Contrastingly, 2 cases (case nos. 8 and 10) were negative for the carcinoma component, but positive only for the sarcomatous component. Among 16 informative cases for LOH analysis at the *INI1* locus, 9 cases (56.3%) showed LOH for at least one of the four microsatellite markers. LOH at the *INI1* locus was positive only in 1 case of the CIS component (case no. 5). This LOH status was consistent in the carcinoma and sarcomatous components in 7 cases. One case (case no. 14) was positive only in the carcinoma component, and 2 cases (case nos. 4 and 10) were positive only in

Table 1 Clinicopathological findings for 16 ECS cases

Age	Mean 66.6 (range 51–83)
Sex	M 13, F 3
Location	CeUt 1, Ut 3, MtUt 2, Mt 8, MtLt 1, Lt 1
Macro	Type 0-I 8, Type 1 5, Type 2 2, Type 5 1
Size (mm)	Mean 54.4 (range 26–121)
T	T1b 7, T2 3, T3 6
ly(+/−)	(11/5)
v(+/−)	(8/8)
N	Positive 12 (all SCC component)
pStage (UICC 8th)	IB 2, IIA 2, IIB 4, IIIA 2, IIIB 4, IVA 2
Survival periods (months)	Mean 52.6 (1.7–187.0)
Ki-67 LI (%)	CIS: mean 30.8, C: mean 50.8, S: mean 44.3

M male, *F* female, *Ce* cervix, *Ut* upper thoracic, *Mt* middle thoracic, *Lt* lower thoracic, *SM* submucosa, *MP* muscularis propria, *AD* adventitia, *CIS* carcinoma in situ area, *C* carcinoma area, *S* sarcomatous area

the sarcomatous component. One case (case no. 5) was positive in all three components, i.e., CIS, carcinomatous, and sarcomatous components (Fig. 2b). LOH in different allele changes was observed in 3 cases (case no. 2 at the AFM238WF2 locus, no. 6 at the D22S303 locus, and no. 14 at the TP53 locus).

IHC

IHC analysis revealed overexpression of p53 in 11 out of 16 cases (68.8%). In all 11 cases, overexpression was observed in both components (Fig. 3a–d). Concordance was found with the p53 IHC staining and *TP53* missense mutations. Five cases showed p53 overexpression without *TP53* mutations. In addition, the carcinomatous component in another case (case no. 3) showed overexpression without *TP53* mutation, while the sarcomatous component harbored the *TP53* missense mutation. The INI1 expression of immunohistochemistry was preserved for the CIS, carcinomatous, and sarcomatous areas in all 16 cases (Fig. 3e, f). Proliferative index assessed by Ki-67 differed in each component within the same tumor in most cases. However, the sarcomatous component did not always show higher index. Rather, it was higher in the carcinoma component (mean 30.8% for CIS and 50.8% for carcinomatous vs. 44.3% for sarcomatous), although this was not statistically significant. The Ki-67 index of CIS seemed to be lower than those of others.

Survival analysis

Survival analysis revealed that the 5-year overall survival rate in the studied cases was 59.9% and the median survival time was 5.37 years (Fig. 4a). The 5-year overall survival tended to be worse for patients with p53 overexpression, although the data was not significant (Fig. 4b; $p = 0.186$).

CNV analysis

Numerous gains and losses were observed in the carcinoma and sarcomatous components, and several differences were also observed between these components (Supplementary Fig. 1). Many genes encoding receptor tyrosine kinase were included among the genes that were amplified.

Discussion

The concept of carcinosarcoma was first proposed by Virchow in 1864 [14]. Although carcinosarcomas of other organs had been reported earlier, the first case of ECS was reported by von Hansemann in 1904 [15]. The clonality of these neoplasms and their corresponding lineages of differentiation have been argued. ECS occurs more commonly in men, typically between the ages of 60 and 70 years. Approximately 60% of the tumors arise in the middle esophagus, nearly one-third in the distal esophagus, and < 10% in the proximal esophagus [8].

The prognosis and malignant potential in conventional SCC (CSCC) can be ascertained by the 5-year survival rate of patients treated by esophagectomy, which was reported to be 54.5% [16]. On the other hand, this study revealed that the 5-year survival rate of 16 ECS patients was 59.9%, being nearly equal to that of conventional esophageal carcinoma. Additionally, another study reported no significant difference in the 5-year survival rate between patients with CSCC and those with ECS [17], although another study demonstrated significantly worse prognosis of ECS than of CSCC in T1 stage tumors [18]. In this study, the proliferative index assessed by Ki-67 was not different between the carcinomatous and sarcomatous areas, while a previous study demonstrated that Ki-67 index was slightly higher in the sarcomatous area than in the carcinomatous area [18]. On the other hand, uterine carcinosarcoma, which usually shows a protruding growth pattern like ECS, has been reported to be more aggressive than high-grade endometrial carcinoma [19–21]. Interestingly, and adding to the controversy, it has been also reported that the carcinomatous component has higher proliferative ability than the sarcomatous area [22]. There are several possible explanations for the similar prognosis of ECS and CSCC

Table 2 Immunohistochemical and molecular features in 16 cases of ECS

Case no.		TP53					INI-1							PTEN	Ki-67 index (%)
		Target sequence/NGS	Markers			IHC	Target sequence/NGS	Markers					IHC	Target sequence/NGS	
			TP53	AFM238WF2	LOH			D22S257	D22S301	D22S303	D22S345	LOH			
1	CIS	(−)	○	○	(−)	+	(−)	○	–	NA	○	(−)	+	(−)	10
	C	(−)	●	●	(+)	+	(−)	●	–	●	●	(+)	+	(−)	42
	S	(−)	○	○	(−)	+	(−)	○	–	●	●	(+)	+	(−)	26
2	C	(−)	●	●†	(+)	+	(−)	●	NA	NA	○	(+)	+	(−)	13
	S	(−)	○	●†	(+)	+	(−)	●	NA	NA	●	(+)	+	g.2264A>T	47
3	CIS	(−)	–	–	–	+	(−)	○	○	NA	○	(−)	+	(−)	2.7
	C	(−)	–	–	–	+	(−)	●	●	–	○	(+)	+	(−)	67
	S	p.A159V	–	–	–	+	(−)	●	●	–	○	(+)	+	(−)	33
4	CIS	(−)	○	○	(−)	–	(−)	○	○	–	–	(−)	+	(−)	17
	C	(−)	○	●	(+)	–	(−)	○	○	–	–	(−)	+	(−)	49
	S	p.E298*	●	●	(+)	–	(−)	●	●	–	–	(+)	+	(−)	65
5	CIS	(−)	–	–	–	+	(−)	–	●	–	●	(+)	+	(−)	48
	C	(−)	–	–	–	+	(−)	–	●	–	○	(+)	+	(−)	43
	S	(−)	–	–	–	+	(−)	–	●	–	●	(+)	+	(−)	46
6	CIS	(−)	○	○	(−)	–	(−)	–	–	○	○	(−)	+	(−)	11
	C	p.E336*	●	○	(+)	–	(−)	–	–	●†	○	(+)	+	(−)	37
	S	p.E336*	●	●	(+)	–	(−)	–	–	●†	○	(+)	+	(−)	29
7	C	p.R273H, p.I251F	–	○	(−)	+	(−)	○	○	○	–	(−)	+	(−)	50
	S	p.R273H, p.I251F	–	○	(−)	+	(−)	○	○	○	–	(−)	+	(−)	27
8	CIS	(−)	–	○	(−)	–	(−)	–	○	○	○	(−)	+	(−)	41
	C	p.E286*	–	○	(−)	–	(−)	–	●	●	●	(+)	+	p.Q171*	54
	S	p.E286*	–	●	(+)	–	(−)	–	●	○	●	(+)	+	p.Q171*	40
9	CIS	p.L130F	○	○	(−)	+	(−)	○	○	○	–	(−)	+	(−)	53
	C	p.L130F	○	●	(+)	+	(−)	○	○	○	–	(−)	+	(−)	54
	S	p.L130F	●	○	(+)	+	(−)	○	○	○	–	(−)	+	(−)	85
10	C/CIS	(−)	–	○	(−)	+	(−)	–	NA	–	○	(−)	+	g.2093C>A	59
	S	(−)	–	●	(+)	+	(−)	–	NA	–	●	(+)	+	(−)	31
11	CIS	c.919_926del	–	–	–	–	(−)	–	NA	–	–	–	+	(−)	26
	C	c.919_926del	–	–	–	–	(−)	–	○	–	–	(−)	+	(−)	35
	S	c.919_926del	–	–	–	–	(−)	–	○	–	–	(−)	+	(−)	49
12	CIS	(−)	–	–	–	+	(−)	–	NA	NA	–	–	+	(−)	28
	C	(−)	–	–	–	+	(−)	–	○	–	–	(−)	+	(−)	62
	S	(−)	–	–	–	+	(−)	–	○	–	–	(−)	+	(−)	19
13	C	p.Y220C, p.C238Y	–	●	(+)	+	(−)	–	○	–	–	(−)	+	(−)	44
	S	p.Y220C	–	●	(+)	+	(−)	–	○	–	–	(−)	+	(−)	16
14	CIS	NA	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	+	(−)	NA
	C	p.E298*	●†	○	(+)	–	(−)	–	●	–	○	(+)	+	(−)	75
	S	(−)	●†	○	(+)	–	(−)	–	○	–	○	(−)	+	(−)	52
15	CIS	(−)	○	○	(−)	+	(−)	○	○	–	○	(−)	+	(−)	48
	C	p.R280G	○	○	(−)	+	(−)	○	○	–	○	(−)	+	(−)	63
	S	p.R280G	○	○	(−)	+	(−)	○	○	–	○	(−)	+	(−)	67
16	CIS	(−)	–	–	–	+	(−)	–	○	○	–	(−)	+	(−)	55
	C	p.C242F, p.R282W, p.P152L	–	–	–	+	(−)	–	○	○	–	(−)	+	(−)	66
	S	p.C242F, p.R282W	–	–	–	+	(−)	–	○	○	–	(−)	+	(−)	80

Nos. 6–9, 15, and 16 were investigated by NGS (carcinoma component and sarcomatous component). Others were investigated by Sanger's sequencing

● LOH, ○ heterozygosity, – not informative, *NA* data not available, † LOH in different allelic change, *CIS* carcinoma in situ, *C* carcinomatous component, *S* sarcomatous component

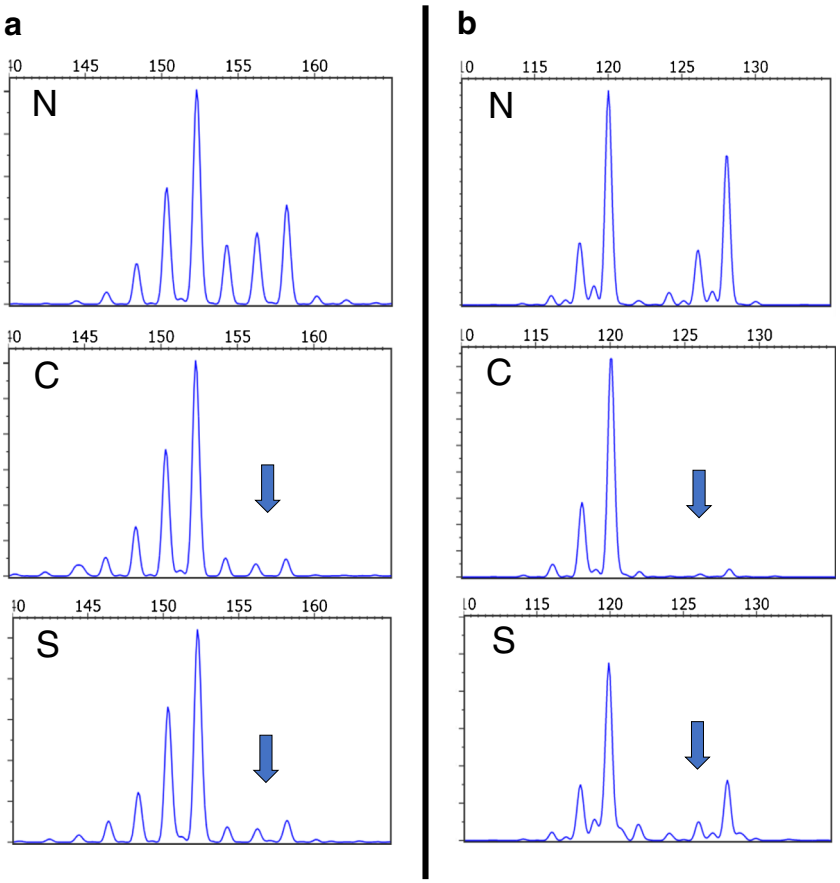

Fig. 2 LOH analysis of the *TP53* (**a**) and *INI1* (**b**) loci. **a** Loss of the long allele (marker: AFM258WF) was observed in both carcinomatous (C) and sarcomatous (S) areas as compared to the corresponding normal tissue (N), indicative of loss of heterozygosity (LOH) of *TP53* locus. **b** LOH is observed in both carcinomatous and sarcomatous areas (marker: D22S257) at the *INI1* locus. N non-tumoral tissue, CIS carcinoma in situ, C carcinomatous area, S sarcomatous area (**a** case no. 2, **b** case no. 13; Both cases did not have *CIS* area.)

compared to uterine CS displaying more aggressive behavior than endometrial carcinoma. First, carcinosarcoma usually shows protruding lesion and shallow lesion. Second, the tumorous lesion is detected as an earlier lesion than in esophageal CSCC. Third, patients often present at an early stage because of the relatively large size and obstructive symptoms like dysphagia [23].

All lymph node metastases were composed of SCC in this study. Although metastasis of a sarcoma-like element was reported in an older case report [23], the sarcomatous component seemed to rarely metastasize to lymph nodes, as shown in sarcomas. Alternatively, as shown in the process of cancer metastasis, epithelial-mesenchymal transition (EMT), which plays a central role in converting both normal and neoplastic epithelial cells into derivatives with a more mesenchymal phenotype [24], might be involved in the metastasis of ECS. Since EMT is a reversible phenomenon, sarcomatous cells need to convert to tumor cells having an epithelial character so that they can metastasize. However, it is not clear whether metastases occurring due to the carcinoma component are more aggressive than those occurring due to the sarcomatous component in ECS or if it is due to EMT.

NGS analysis was performed to elucidate the developmental mechanism of ECS. Tumor suppressor genes including *TP53*, *INI1*, and *PTEN* were frequently altered. LOH was examined at the *INI1* locus, because *INI1* was identified as one of the deleted genes in ECS by CNV analysis of NGS. LOH at the *INI1* locus was frequently observed in ECS, although INI1 expression was preserved. *INI1* is a tumor suppressor gene located on 22q11.23 [25, 26]. Loss of INI1 expression has been reported to be less frequent in sinonasal malignancies including poorly differentiated SCC and undifferentiated carcinoma [27, 28] and SCC of other organs [21]. Frequent loss of INI1 expression was reported to be associated with atypical teratoid/rhabdoid tumors, malignant rhabdoid tumor, and epithelioid sarcoma, while homozygous inactivating genetic alterations were frequent in malignant rhabdoid tumor but rare in epithelioid sarcoma [29]. It is interesting to note that LOH status at the *INI1* locus was almost the same between the carcinomatous and sarcomatous components. LOH at this locus may occur at the early stage during tumorigenesis of ECS. However, the significance of LOH at *INI1* locus in ECS development is not clear, because protein expressions are preserved despite the presence of LOH.

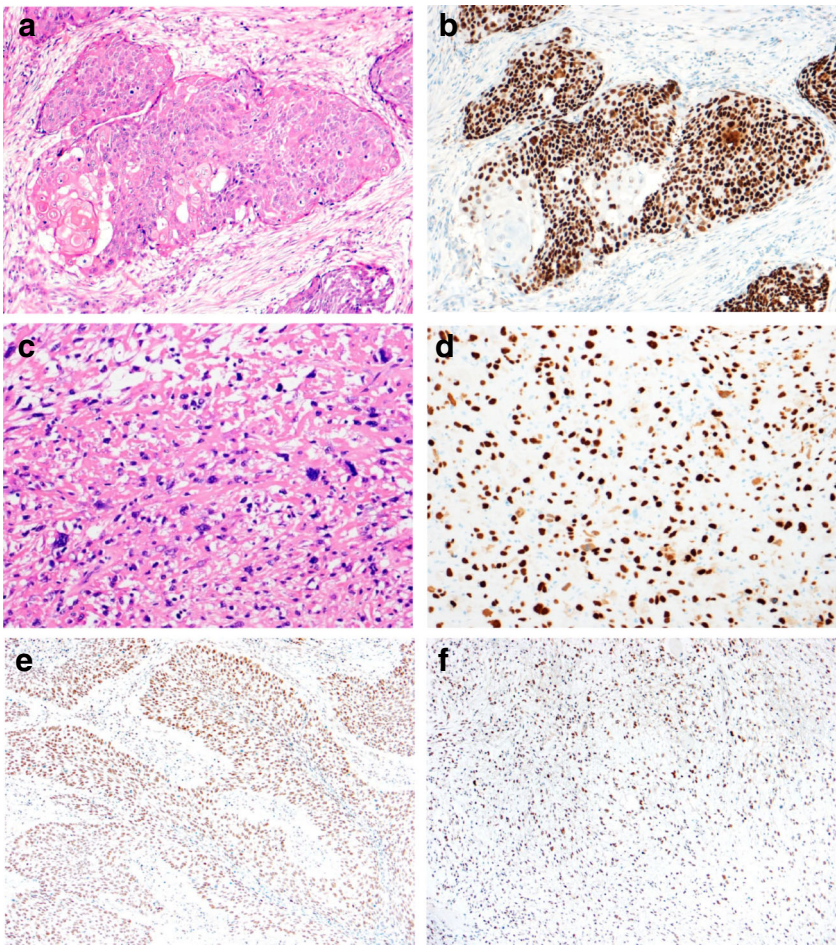

Fig. 3 Immunohistochemistry of p53 and INI1. Diffuse p53 overexpression was observed in both carcinomatous (**a**, **b**) and sarcomatous (**c**, **d**) components. Corresponding histological features are also shown (**a** carcinomatous area; **c** sarcomatous area). Diffuse INI1 staining was preserved in both carcinomatous (**e**) and sarcomatous (**f**) areas

Collectively, these findings suggest that ECSs occur by accumulation of genetic alterations in tumor suppressor genes.

p53 IHC was performed to correlate the *TP53* mutation and overexpression. Each carcinomatous and sarcomatous component showed coincidence in *TP53* mutation status and p53 overexpression in most ECSs. *TP53* is a tumor suppressor gene located at 17p13.1. It is the most frequent mutation in esophageal SCC (86.19% in the Cancer Genome Atlas, 58.2% in the Catalog of Somatic Mutations in Cancer, and 68.7% in cBioportal). Recent studies revealed that carcinosarcomas of esophageal and uterine origins have a high frequency of *TP53* mutations. *TP53* mutation rate is reportedly 61.5% in ECS and 91% in uterine CS [8, 30]. The impact of *TP53* mutation/p53 IHC overexpression on patient survival has been reported in various types of malignancies [31–34]. However, the prognostic impact of the *TP53* mutation/p53 IHC overexpression is unclear in CS. The *KRAS* mutation is a marker of poor prognosis in pulmonary sarcomatoid carcinoma [35]. In the current study, p53 IHC overexpression had a negative prognostic impact in ECS, although it was not statistically significant. These findings suggest that *TP53* mutations are involved in tumor differentiation, rather than in tumor progression. Regarding the *TP53* mutation status and p53 overexpression, each carcinomatous and sarcomatous component within the same tumor showed relative coincidence, although carcinomatous or sarcomatous component-specific mutations were also observed in a few cases. A previous study demonstrated *TP53* mutations in 8 out of 13 cases of ECS, and the same mutations were detected across the both components in 7 of 8 cases [8]. Another case had *TP53* mutation only in the sarcomatous component [8]. Furthermore, it has also been shown that ECS is derived from a single clone originating from a SCC by LOH analysis including *TP53* locus [7]. Based on these findings, *TP53* mutations seemed to occur at an early stage of tumorigenesis in ECS, and the two components could be considered to have the same origin.

NGS also revealed several CNVs including the amplification of genes encoding tyrosine kinase receptors

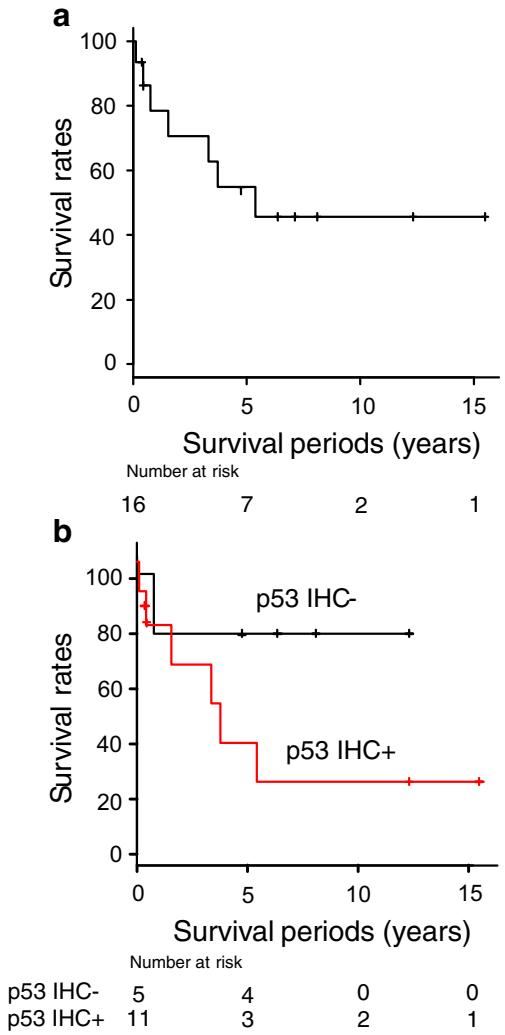

Fig. 4 Survival rate of the studied cases of ECS. **a** Five-year overall survival rate was 59.9%. **b** Survival curve according to p53 IHC status did not reach statistical difference ($p = 0.186$)

in carcinomatous and sarcomatous components. However, these data were not validated by other methods, such as IHC. Combining the clinicopathological characteristics of patients and molecular findings may lead to the development of effective diagnostic and therapeutic targets. Further studies are required to confirm these genes as potential therapeutic targets in ECS.

Finally, it is important for the treatment to diagnose precisely as ECS and to distinguish from other polypoid tumors of the esophagus such as melanoma, basaloid squamous carcinoma. Sometimes, it is quite difficult to distinguish ECS from these tumors by small biopsy specimens and samples obtained from the stalk or "in situ" lesion may lead to the unintentional diagnoses as CSCC. To avoid this kind of unintentional "misdiagnosis," it is recommended to obtain biopsy samples from multiple areas.

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Compliance with ethical standards

This study was approved by the Ethical Committee of Juntendo University School of Medicine (2016108).

Conflict of interest The authors declare that they have no conflict of interest.

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