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Clinical significance of soluble forms of immune checkpoint molecules in advanced esophageal cancer

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Abstract
Immune checkpoint molecules are expressed on cancer cells and regulate tumor immunity by binding to ligands on immune cells. Although soluble forms of immune checkpoint molecules have been detected in the blood of patients with some types of tumors, their roles have not been fully elucidated. Soluble PD-L1, PD-1, CD155, LAG3, and CD226 (sPD-L1, sPD-1, sCD155, sLAG3, and sCD226, respectively) were measured in the sera of 47 patients with advanced esophageal cancer and compared with those of 24 control subjects. Pretreatment levels of sPD-1 and sCD155 were significantly higher in the patients with cancer than in the control subjects ($P=0.023$, $P=0.001$). The sPD-1 levels tended to be higher in the patients with lymph node metastasis, a large tumor diameter, and higher levels of serum SCC antigen ($P=0.150$, $P=0.189$, and $P=0.078$, respectively). However, higher levels of sCD155 were associated with a better response to chemotherapy and favorable overall survival ($P=0.111$ and $P=0.068$, respectively). After 2 courses of chemotherapy, the levels of sCD155 and sCD226 were significantly increased ($P<0.001$ and $P=0.002$, respectively). Moreover, the increase in sCD226 during chemotherapy was associated with poor treatment response ($P=0.019$). sPD-1 levels are possibly dependent on the tumor aggressiveness of the esophageal cancer. Furthermore, the pretreatment levels of sCD155 and kinetic change of sCD226 after chemotherapy may be used as biomarkers of the treatment response and prognosis in patients with esophageal cancer.

Keywords Esophageal cancer · Soluble immune checkpoint molecules · Biomarker

Introduction

Esophageal cancer, the sixth leading cause of cancer deaths worldwide, is increasing globally [1]. There are mainly two histological types of esophageal cancer, namely adenocarcinoma and squamous cell carcinoma, the latter of which accounts for 90% of cases worldwide inclusive of Japan. Despite the administration of multidisciplinary treatments, including surgery, chemotherapy, and radiotherapy, esophageal cancer carries a poor prognosis, with the overall 5-year survival following diagnosis being lower than 20% [2]. In order to improve the prognosis of patients with esophageal cancer, biomarkers that can predict the patient's treatment response would be helpful in the choice of optimal treatment strategies. Furthermore, the development of innovative therapies that target novel mechanisms is required.

Immune checkpoint inhibitors represent such innovative cancer therapies, having being rapidly approved for use in the treatment of different malignancies, with hundreds of trials testing their efficacies on various types of tumors. The preliminary results of immune checkpoint inhibitors in esophageal cancer are promising. After the results from two phase II trials in which nivolumab and pembrolizumab (both anti-PD-1 antibodies) were demonstrated to have meaningful clinical activity in heavily pretreated patients with esophageal cancers [3, 4], phase III trials are currently being conducted.

Aside from PD-1 and PD-L1, which are two well-known immune checkpoint molecules that modulate T-cell receptor signals and play a major role in tumor immunity

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escape, various other immune checkpoint molecules have recently been identified. CD155 is overexpressed in various tumors [5–8], modulating host tumor immunity. Its receptors are CD226 and T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT). CD226 is a member of the immunoglobulin superfamily and is expressed on natural killer (NK) cells and T cells. Whereas CD226 is an important receptor for activating NK cell-mediated cytotoxicity, TIGIT has negative effects on both T-cell activation and NK cell activation. The CD4 homolog lymphocyte activation gene 3 (LAG3) is expressed on activated T cells, intrinsically limiting Tcell proliferation, expansion, and viability. LAG3 also contributes to tumor-mediated immune suppression and promotes tumor immunity escape [9].

Recently, the soluble forms of these immune checkpoint molecules have been detected in the blood of patients with some types of tumors [10–14]. However, the roles of these circulating molecules have not been fully elucidated. The aims of this study were to identify the circulating levels of the soluble immune checkpoint molecules sPD-1, sPD-L1, sCD155, sCD226, and sLAG3 in the sera of patients with esophageal cancer, and to evaluate the association between their levels and clinicopathological features and their prognostic significance.

Materials and methods

Patients and sample collection

The patients enrolled were those with histologically diagnosed advanced esophageal cancer, who had received multidisciplinary treatment at the University Hospital, Kyoto Prefectural University of Medicine (Kyoto, Japan) from April 2015 to March 2017. Other inclusion criteria were as follows: age ≥ 20 years, Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) 0–2, and no prior chemotherapy or radiotherapy. Serum samples were obtained from 47 such patients as well as from 24 healthy (non-patient) volunteers. The sera from the patients were obtained before initiating first-line treatment and at 2 weeks after the completion of 2 courses of chemotherapy. The separated serum samples were immediately stored at − 80 °C until analysis. The disease stage was classified according to The Union for International Cancer Control (UICC)'s *TNM Classification of Malignant Tumours* (7th Edition) descriptions. Written informed consent was obtained from all patients and healthy volunteers. This study was performed with the approval of the Ethics Review Boards of Kyoto Prefectural University of Medicine (Approval No. ERB-C-150-1).

Treatments and response evaluation

The drugs administered during cycle 1 chemotherapy were cisplatin and 5-fluorouracil (5-FU) for the doublet regimen (FP regimen), and cisplatin, 5-FU, and docetaxel for the triplet regimen (DCF regimen). The FP regimen consisted of cisplatin 80 mg/m^2 on day 1 and infused 5-FU 800 mg/m^2 on days 1–5. The DCF regimen consisted of docetaxel 70 mg/m^2 on day 1, cisplatin 70 mg/m^2 on day 1, and infused 5-FU 700 mg/m^2 on days 1–5. The length of 1 chemotherapy cycle of each regimen was 3–4 weeks. Patients with resectable tumors underwent subtotal esophagectomy with regional lymphadenectomy and retrosternal gastric tube reconstruction at 3 or 4 weeks after the last cycle of chemotherapy. For the patients with non-resectable tumors, chemoradiotherapy (60 Gy/30 fractions) with 2 courses of FP regimen or chemotherapy alone was performed. The objective tumor response was assessed by computed tomography scans in accordance with Response Evaluation Criteria in Solid Tumor (RECIST Version 1.1) criteria.

ELISA analysis

sPD-L1, sPD-1, sLAG3, and sCD226 were measured using an enzyme-linked immunosorbent assay (ELISA) kit from Thermo Fisher Scientific (Yokohama, Japan), and sCD155 was measured using an ELISA kit from RayBiotech (Norcross, GA, USA), according to the manufacturers' instructions. The results were expressed as the optical density at 490 nm.

Statistical analysis

Comparisons of clinical data between groups were carried out using the Chi-squared test, the two-tailed Student *t* test, and the Wilcoxon matched test. Overall survival (OS) was measured from the date of diagnosis to the date of last follow-up or death from any cause and estimated using the Kaplan–Meier method. Differences were assessed using the log-rank test at the two-sided significance level of 0.05. The Cox proportional hazards model was used to determine hazard ratios (HRs) and confidence intervals (CIs) at the 95th confidence level. Analyses were performed using JMP software (version 13.0; SAS Institute, Inc., Cary, NC, USA).

Table 1 Patients characteristics

	Cancer patients ($n=47$)
Age years	66 (41–78)
Gender	
Male/female	42/5
Location	
C/U/M/L	2/14/18/13
Stage(UICC)	
II/III/IV	8/24/15
Chemotherapy regimen	
DCF/FP/others	28/17/2
Operation	
Yes/no	26/21
Radiation	
Yes/no	25/22
Treatment response	
CR + PR/SD + PD	29/17

DCF cisplatin, 5-Fluorouracil (5-FU) and docetaxel, *FP* cisplatin and 5-Fluorouracil (5-FU), *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Table 2 Serum soluble marler levels in patients and healthy controls

	n	Median (range)	*P*
PD-L1 (pg/ml)			
Healthy	24	20 (15–35)	0.931
Cancer	47	20 (15–165)	
PD-1 (ng/ml)			
Healthy	24	0.155 (0.11–1.565)	0.023
Cancer	47	0.180 (0.11–0.920)	
CD155 (ng/ml)			
Healthy	24	0.7825 (0.120–15.145)	0.001
Cancer	47	2.9300 (0.335–24.779)	
CD226 (ng/ml)			
Healthy	24	7.5925 (6.575–413.45)	0.257
Cancer	40	9.2275 (6.575–413.45)	
LAG3 (ng/ml)			
Healthy	24	2.9300 (0.335–14.418)	0.066
Cancer	47	3.0325 (0.579–24.779)	

Results

Patient characteristics

The clinical and pathological characteristics of the 47 patients included our study are described in Table 1. The median age was 66 years (range 41–78 years), and all patients were ECOG PS 0–1. Tumor locations were cervical ($n=2$), upper thoracic ($n=14$), middle thoracic ($n=18$), and lower thoracic ($n=13$). Based on UICC TNM classifications, 8, 24, and 15 patients were in clinical stages II, III, and IV, respectively. All 47 patients received chemotherapy, 25 (53.2%) received radiation therapy in combination with chemotherapy, and 26 (55.3%) received surgical resection for esophageal cancer after neoadjuvant chemotherapy. With regard to the treatment response after 2 courses of chemotherapy, 29 patients (63%) showed a complete response (CR) or partial response (PR), 15 had stable disease (SD), 2 had progressive disease (PD), and 1 patient could not be evaluated. There was no difference in age between the patients and healthy control groups, but the proportion of males was significantly higher in the patient group ($P=0.014$).

Serum soluble marker levels at baseline

As shown in Table 2, there was no significant difference between the two study groups in terms of serum sPD-L1 and sCD226 levels. The levels of sLAG3 tended to be higher in the patient group than in the healthy control group ($P=0.0664$), and the levels of sPD-1 and sCD155 were significantly higher in the patient group ($P=0.023$ and $P=0.001$, respectively). Next, we explored the relationship between the clinicopathological characteristics and the elevated sPD-1 and sCD155 levels in the patients (Table 3). Those patients with lymph node metastasis ($P=0.150$), a large tumor diameter ($P=0.189$), and a higher level of serum squamous cell carcinoma-related (SCC) antigen ($P=0.078$) tended to have higher levels of sPD-1. However, the sPD-1 level was found to be unrelated to either the response rate or OS. Furthermore, we did not find any association between the clinicopathological characteristics and the level of sCD155.

To evaluate the clinical significance of these baseline soluble marker levels in chemotherapy or chemoradiotherapy in the patients, we investigated whether the levels of sPD-1 and sCD155 could serve as a predictor of therapeutic effects (i.e., response rate and OS). We evaluated the tumor response after two courses of chemotherapy according to RECIST criteria. Although we did not find any association between the treatment response and the level of sPD-1, the level of sCD155 tended to be higher in patients with a CR or PR to chemotherapy ($P=0.111$; Table 3). Next, we analyzed the data using the Cox proportional hazards model with known risk factors for OS, sCD155, and sPD-1. As shown in Table 4, distant metastasis (HR = 2.931, 95% CI 0.912–9.416, $P=0.052$) and the number of peripheral lymphocytes (HR = 3.003, 95% CI 0.939–11.32, $P=0.061$) were shown to be possibly related to OS in the univariate analysis. In addition to these variables, PS and sCD155 which were possibly associated with OS in the univariate analysis ($P<0.2$) were included in a multivariate analysis model.

Table 3 sPD1and sCD155 in relation to clinicopathological characteristics in esophageall cancer patients

		sPD1		sCD155	
	n	Median	*P*	Median	*P*
Age, years					
≧66	26	0.190 (0.12–0.25)	0.390	2.929 (0.381–24.799)	0.661
<66	21	0.175 (0.11–0.925)		2.974 (0.335–23.674)	
Gender					
Male	42	0.1825 (0.11–0.920)	0.316	2.929 (0.335–24.779)	0.355
Female	5	0.175 (0.12–0.195)		3.137 (0.579–3.740)	
BMI					
≧ 20.95	23	0.190 (0.11–0.92)	0.881	2.974 (0.335–24.779)	0.355
< 20.95	24	0.180 (0.12–0.25)		2.618 (0.381–14.418)	
Tumor size (mm)					
≧ 60	28	0.190 (0.115–0.265)	0.189	2.9300 (0.335–14.418)	0.380
< 60	19	0.160 (0.11–0.92)		3.0325 (0.579–24.779)	
Number of lymph node metastasis					
≧ 3	30	0.190 (0.15–0.92)	0.150	2.618 (0.335–23.674)	0.082
≦ 2	17	0.165 (0.11–0.24)		3.740 (1.236–24.779)	
Distant metastasis					
Yes	15	0.190 (0.115–0.265)	0.793	2.6650 (0.335–23.674)	0.673
No	32	0.1775 (0.11–0.92)		3.0555 (0.579–24.779)	
Stage (UICC)					
II	8	0.1575 (0.11–0.235)	0.561	3.7025 (1.236–20.958)	0.833
III	24	0.1825 (0.12–0.92)		2.951 (0.579–24.770)	
IV	15	0.190 (0.115–0.265)		2.665 (0.335–23.674)	
WBC (/mm^3)					
≧ 7200	24	0.175 (0.11–0.92)	0.907	2.5725 (0.335–23.674)	0.816
< 7200	23	0.190 (0.115–0.25)		3.1370 (0.622–24.779)	
Neutrophil (/mm^3)					
≧ 4629	24	0.175 (0.11–0.92)	0.830	2.433 (0.381–23.674)	0.280
< 4629	23	0.190 (0.115–0.25)		3.406 (0.335–24.779)	
Lymphocyte (/mm^3)					
≧ 1546	24	0.170 (0.11–0.92)	0.342	2.9250 (0.579–24.779)	0.856
< 1546	23	0.1925 (0.12–0.25)		3.055 (0.335–23.674)	
Platelet ($\times 10^4$/mm^3)					
≧ 24.4	24	0.1775 (0.115–0.265)	0.966	2.5705 (0.335–23.674)	0.344
< 24.4	23	0.190 (0.11–0.92)		3.1370 (0.622–24.779)	
Alb (g/dl)					
≧ 3.9	25	0.175 (0.11–0.25)	0.212	3.4060 (0.381–24.779)	0.475
< 3.9	22	0.190 (0.115–0.92)		2.5275 (0.335–23.674)	
CRP (mg/dl)					
≧ 0.27	25	0.175 (0.115–0.265)	0.932	3.431 (0.335–23.674)	0.741
< 0.27	22	0.1875 (0.11–0.92)		2.702 (0.622–24.779)	
SCC (ng/ml)					
≧ 1.6	23	0.190 (0.11–0.265)	0.078	2.4760 (0.622–24.779)	0.883
< 1.6	22	0.1625 (0.115–0.920)		3.0315 (0.335–20.958)	
Treatment response					
CR/PR	29	0.190 (0.12–0.25)	0.600	3.652 (0.335–24.779)	0.111
SD/PD	17	0.175 (0.11–0.92)		2.311 (0.579–9.734)	

BMI Body Mass Index, *WBC* white cell count, *Alb* albumin, *CRP* C-relative protein, *SCC* squamous cell carcinoma-related antigen

Table 4 Prognostic factors of overall survival by uni- and multivariate analyses

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age, years						
< 66	1					
≧ 66	0.743	0.231–2.383	0.607			
Gender						
Female	1					
Male	0.492	0.129–3.211	0.352			
PS						
= 0	1			1		
≧ 1	2.662	0.681–9.092	0.114	1.461	0.313–6.609	0.618
Number of lymph node metastasis						
≦ 2	1					
≧ 3	1.821	0.543–8.200	0.361			
Distant metastasis						
No	1			1		
Yes	2.931	0.912–9.416	0.052	1.727	0.402–7.011	0.451
Neutrophil(/mm^3)						
< 4629	1					
≧ 4629	0.768	0.240–2.461	0.647			
Lymphocyte(/mm^3)						
≧ 1546	1			1		
< 1546	3.003	0.939–11.32	0.061	2.145	0.536–9.254	0.279
Alb(g/dL)						
≧ 3.9	1					
< 3.9	1.421	0.446–5.335	0.565			
CRP(mg/dl)						
< 0.27	1					
≧ 0.27	0.504	0.135–1.606	0.256			
sCD155						
High	1			1		
Low	2.223	0.696–8.369	0.183	3.212	0.921–14.76	0.068
sPD1						
Low	1					
High	1.977	0.619–7.439	0.259			

PS performance status, *Alb* albumin, *CRP* C-relative protein, *HR* hazrd ratio, *CI* confidence interval

After controlling for confounders of the risk factors, sCD155 was found to be a possible independent prognostic factor for increased OS (HR = 3.212, 95% CI 0.921–14.76, $P = 0.068$; Table 4). As shown in Fig. 1, patients with a higher sCD155 level (> 2.952 ng/ml) at baseline showed a trend toward a better OS (Log-rank test: $P = 0.183$).

Changes in soluble marker levels after chemotherapy or chemoradiotherapy

We further evaluated the serum soluble markers after 2 courses of chemotherapy. There was no significant difference in the levels of sPD-1 before and after treatment (data not shown). However, the levels of sCD155 and sCD226 were increased after treatment ($P < 0.001$ and $P = 0.002$, respectively; Fig. 2a). Moreover, whereas there was no relationship between the increase in sCD155 during chemotherapy and the treatment response ($P = 0.479$), the increase in sCD226 during chemotherapy was associated with a poor treatment response ($P = 0.019$; Fig. 2b).

Discussion

It has been found that several soluble immune checkpoint molecules in the serum are elevated in some types of cancer, and although their clinical significance has been reported, little is known about this phenomenon in esophageal cancer.

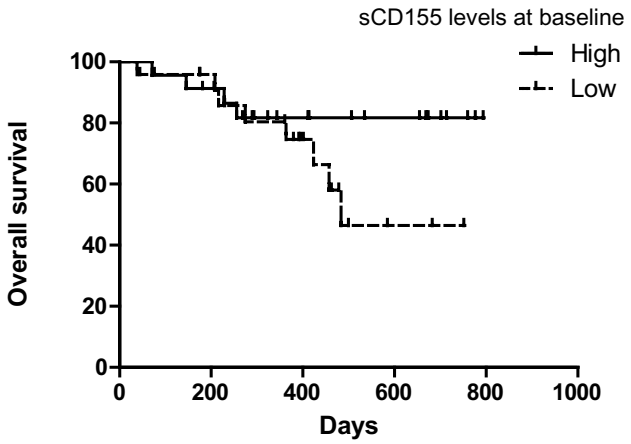

Fig. 1 Kaplan–Meier survival curves according to the serum levels of CD155. The survival curves are in relation to the serum sCD155 levels at baseline. Crosses indicate censored data

Herein, the serum kinetic changes in five soluble immune checkpoint molecules (viz. sPD-1, sPD-L1, sCD155, sCD226, and sLAG3) during chemotherapy were evaluated in patients with advanced esophageal cancer. We found that the levels of sPD-1 and sCD155 were significantly higher in these patients than in the normal healthy subjects ($P=0$ 0.023 and $P=0.001$, respectively). In the patient group, the sPD-1 levels tended to be correlated with the number of lymph node metastases, tumor size, and higher levels of serum SCC. High pretreatment levels of sCD155 had a tendency toward a better CR or PR to chemotherapy and a favorable OS. As for the kinetic changes of these circulating molecules, the levels of sCD155 and sCD226 were significantly increased after chemotherapy ($P<0.001$ and $P=0.002$, respectively). Patients who had a decrease in sCD226 after chemotherapy experienced a favorable treatment response ($P=0.019$). To our best knowledge, this is the first report that has analyzed multiple soluble immune checkpoint molecules in patients with esophageal cancer.

Recently, it was reported that immune checkpoint molecules exist not only on the surface of immune and cancer cells but also as a soluble form in the blood [10–14]. The soluble form of molecules is usually generated by proteolytic cleavage of the membrane-bound form of the costimulatory proteins, or by the translation of alternatively spliced mRNA as in the case of sPD-1 [15, 16]. sPD-L1 is generated by proteolytic cleavage of the membrane-bound

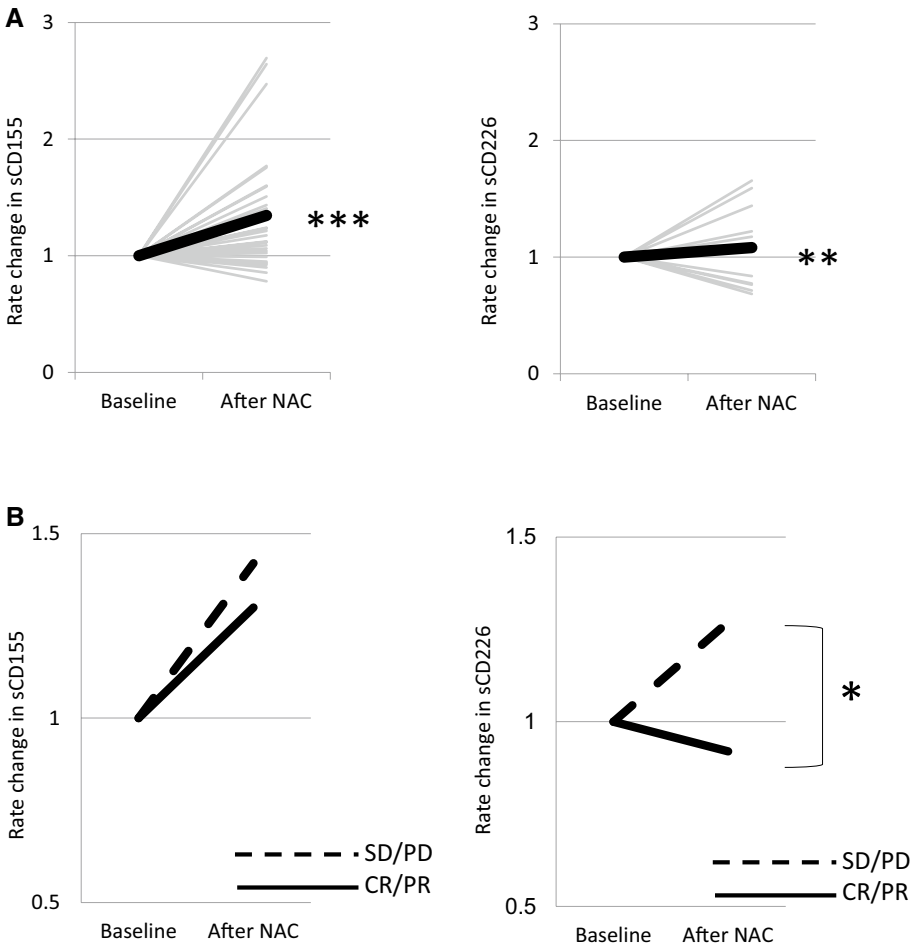

Fig. 2 Changes in serum soluble markers after chemotherapy. **a** The changes in the levels of soluble CD155 and CD226 after 2 courses of chemotherapy were analyzed and expressed relative to their deviation from baseline. The thin lines indicate individual patients. The bold line indicates their average. The average sCD155 and sCD226 levels were significantly increased after treatment. **b** The changes in the levels of soluble CD155 and CD226 after 2 courses of chemotherapy were analyzed according to the treatment response and are expressed relative to their deviation from baseline. Values represent the average. The solid line indicates the CR/PR group, and the broken line indicates the SD/PD group. NAC: neoadjuvant chemotherapy; NAC: neoadjuvant chemotherapy; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease. *$P<0.05$, **$P<0.01$, ***$P<0.001$

form of the costimulatory proteins [17], and its secretion involves the alternative splicing of PD-L1, which lacks the transmembrane domain [18]. Although sPD-L1 has been reported to be elevated in patients with malignancies other than esophageal cancer and to be associated with their OS or treatment response [19–21], we did not find any increase in its levels in our patient cohort compared with the healthy controls or changes in its levels post-treatment. Thus, the clinical significance of sPD-1 is unclear because there are few reports about this soluble immune checkpoint molecule. Although there is no report that sPD-1 is elevated in the serum of patients with cancer, its levels were reported to be positively associated with the risk for hepatocellular carcinoma in males [22]. In this study, we showed that the serum levels of sPD-1 were higher in the patients than in the healthy controls. Since a high sPD-1 level was related to the tumor size and presence of lymph node metastasis, the elevation of sPD-1 may reflect a higher tumor burden and a more aggressive biology of esophageal cancer. Several preclinical studies have suggested that sPD-1 is bioactive and blocks the regulatory properties of membrane-bound PD-L1 and PD-L2, which can lead to restored T-cell function and proliferation, and enhancement of immune-mediated tumor control [23–25]. It has also been reported that the prognosis is good in patients with sPD-1 elevation after treatment for non-small-cell lung cancer [26]. However, in our present study, no association between the pretreatment sPD-1 levels and prognosis was found, and the serum levels of sPD-1 did not change after the treatment. Since sPD-1 could affect T-cell function and proliferation, further studies are needed to clarify the significance of sPD-1 in cancer immunotherapy.

CD155 is a functional ligand for CD226, CD96, and TIGIT on NK and T cells, and can mediate NK and T-cell activation via CD226 and their inhibition via CD96 or TIGIT [27]. Although CD155 is overexpressed in various tumors [5–8], the clinical significance of its expression in tumors remains controversial. In contrast to several reports that showed a positive correlation between high levels of membrane-bound CD155 and poor prognosis in patients with cancer [28–31], one report showed a negative correlation instead [32]. The soluble form of CD155 has been reported to be generated by proteolytic cleavage of the membrane-bound form of the costimulatory proteins [12]. Little is known about the clinical significance of sCD155. Iguchi-Manaka et al. reported that sCD155 levels were significantly higher in patients with cancer ($n=262$), including esophageal cancer ($n=8$), than in healthy subjects and that the level showed positive association with the tumor burden [33]. In our study, the levels of sCD155 were significantly higher in the patients than in the healthy controls, which is in line with the results from Iguchi-Manaka et al. [33]. However, we did not find the association between the sCD155 levels and tumor burden. In addition, our patients with high sCD155 levels showed a trend toward a better treatment response and prognosis. Since CD155 is a complex immune checkpoint molecule involved in both the activation and inactivation of tumor immunity [34], further studies are needed to clarify the biological activity and the clinical significance of sCD155 in esophageal cancer.

CD226, an immunoglobulin supergene family receptor, is expressed mainly in NK cells, T cells, NKT cells, and monocytes, and is involved in cytotoxicity and the cytokine secretion of NK and T cells [35]. sCD226 has been reported to be generated through shedding of its membrane form, and its increased serum levels have been reported in patients with various types of cancer [13, 36]. Unlike these reports, the baseline sCD226 levels were not increased in our patients with esophageal cancer. Moreover, the sCD226 levels increased significantly after chemotherapy, and those patients with a low level of sCD226 after chemotherapy experienced a favorable treatment response. It has been reported that tumor regression after 2 or 3 courses of preoperative chemotherapy for esophageal cancer correlates with overall survival [37, 38], but in order to clarify the significance of sCD226 as a biomarker, it is necessary to evaluate for a longer time with a larger population. Although sCD226 has been reported to have bioactivity, its effects on immune cell function are different between various published studies. Whereas Takahashi et al. had reported that sCD226 can increase the cytotoxicity of NK cells in vitro [36], it has also been reported that it may inhibit NK cytotoxic activity [13]. Since it seems probable that sCD226 plays important roles in the immune system, it may be used as a potential biomarker as well as an immune therapeutic target. Thus, further study is required in the future to verify this.

Several limitations of this study should be acknowledged. Since we did not evaluate the expression of these immune checkpoint molecules on cancer cells or immune cells, we could not show the relationship between the expression levels of the soluble and membrane-bound forms of these molecules. Therefore, the origins of these molecules in the serum are not clear. In addition, this study was performed at a single institute and only examined a relatively small number of patients with esophageal cancer. Our current findings will need to be confirmed in a larger multicenter study. We also believe that further experimental studies are needed to clarify the biological effects of the soluble immune checkpoint molecules in the blood of patients with various forms of cancer.

In conclusion, we have revealed that the serum levels of sPD-1 and sCD155 are significantly elevated in patients with advanced esophageal cancer relative to their levels in healthy subjects. Our results suggest that the sPD-1 levels are possibly dependent on the tumor burden and aggressiveness of the esophageal cancer. In addition, the pretreatment levels of

sCD155 and kinetic change of sCD226 after chemotherapy may be used as biomarkers of the treatment response and prognosis in patients with esophageal cancer. However, these findings are preliminary and will need to be confirmed with further studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate This study was performed with the approval of the Ethics Review Boards of Kyoto Prefectural University of Medicine (Approval No. ERB-C-150-1).

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