

Negative influence of programmed death-1-ligands on the survival of esophageal cancer patients treated with chemotherapy

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The programmed death-1/programmed death-1 ligands (PD-1/PD-L) pathway plays an important role in immunological tumor evasion. However, the clinical significance of the PD-L (L1 and L2) expression in esophageal cancer treated with chemotherapy has not been fully investigated. We examined the expression of PD-L of the primary tumors obtained from 180 esophageal cancer patients who underwent radical resection with or without neoadjuvant chemotherapy (NAC) using immunohistochemical staining. The relationship between the expression patterns and clinico-pathological characteristics was examined. In the present study, 53 patients (29.4%) and 88 patients (48.3%) were classified into positive for PD-L1 and PD-L2 expression, respectively. In all the patients examined, overall survival rates of the patients with tumors positive for PD-L1 or PD-L2 were significantly worse than those with tumors negative for PD-L1 or PD-L2 ($P = 0.0010$ and $P = 0.0237$, respectively). However, subgroup analysis showed that these tendencies are only found in the patients treated with NAC, and not in those without NAC. The patients with positive PD-L1 expression had a significantly higher rate of NAC history ($P = 0.0139$), but those with positive PD-L2 expression did not have a significantly high rate of NAC history ($P = 0.6127$). There is no significant relationship between PD-L1 expression and response to chemotherapy ($P = 0.3118$), but patients with positive PD-L2 expression had significantly inferior responses to chemotherapy ($P = 0.0034$). The PD-1/PD-L pathway might be an immunological mechanism associated with the long-term effectiveness of chemotherapy in esophageal cancer patients. Further investigation into the roles of PD-1 pathway in chemotherapy could lead to the development of better treatment options for this disease.

Esophageal cancer is the eighth most common cancer in the world and is the sixth leading cause of cancer death.⁽¹⁾ It is one of the most aggressive and lethal malignancies among gastrointestinal cancers because systematic metastases occur in more than 50% of patients at the time of diagnosis. Furthermore, systemic and local recurrences sometimes occur even after curative resection.⁽²⁾ Although prognosis is gradually improving with the invention of multimodal treatment, including chemotherapy, radiotherapy and surgery, the long-term outcome is still poor, and the overall 5-year survival rate is only around 20–30%.^(3–5) Thus, new treatment strategies are needed to improve the prognosis for esophageal cancer patients.

Immunological therapies, such as the blockade of the regulatory mechanism related to programmed death-1 (PD-1), offer promise for the treatment of esophageal cancer. The PD-1 is an immune-checkpoint receptor that provides inhibitory signals in T-cell activation when engaged by its ligands (programmed death-1 ligands [PD-L]); PD1 ligand 1 (PD-L1) and PD1

ligand 2 (PD-L2).⁽⁶⁾ The PD-1/PD-L pathway plays a critical role in regulating the activity of T cells in effector phases against not only normal cells,^(7,8) but also against tumor cells.⁽⁶⁾ Recently, multiple clinical trials have shown that the blockade of this pathway using antibodies specific to PD-1 or PD-L1 is associated with significant antitumor activity in patients with multiple types of cancer,^(9,10) and extensive clinical development of this modality is in progress. Although the expression of PD-L on the tumor cells might serve as a potential predictive correlates for anti-tumor effects of the treatment with the PD-1/ PD-L1 blockade,^(9,11) the relationship between the tumor expression of PD-L and chemotherapeutic effects has not been fully investigated.

In this study, we examined the expression of PD-L1 and PD-L2 in the tumors of surgically treated esophageal cancer patients, including those who received chemotherapy before the surgery as neo-adjuvant chemotherapy (NAC), and retrospectively investigated their relationship with the clinical and pathological characteristics of the patients.

Patients and Methods

Patients. Expression of PD-L1 and L2 was examined using tumor samples obtained from 180 patients who were diagnosed to have esophageal squamous cell carcinoma (ESCC) and treated in the Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University between February 1999 and December 2012. The median age of the patients was 64 years, with a range of 29–84 years. Among the 180 patients, 111 patients classified as Stage II–IV received NAC prior to the radical resection. According to the principles of our department during the period, NAC was given for patients with any T(cT1–4) and lymph node involvement, including regional lymph nodes (N1) and distant lymph nodes (M1 lym) without distant organ metastasis. Thus, the patients with NAC had tumors of significantly more advanced stages consisting of higher cT, cN and cM when compared with the tumors of patients without NAC (Table S1).

The chemotherapeutic regimen consisted of the administration of multiple drugs, combining cisplatin and 5-fluorouracil with adriamycin (ACF) or docetaxel (DCF). For the ACF treatment, 70 mg/m² of cisplatin and 35 mg/m² of adriamycin were administered intravenously as bolus infusion on day 1, and 700 mg/m² of 5-fluorouracil was administered intravenously as a continuous infusion on days 1–7. Two courses of ACF chemotherapy were given with a 4-week interval.⁽¹²⁾ For the DCF treatment, 70 mg/m² of cisplatin and 70 mg/m² of docetaxel were administered intravenously as a bolus infusion on day 1, and 700 mg/m² of 5-fluorouracil was administered intravenously as a continuous infusion on days 1–5. Two courses of DCF chemotherapy were given with a 3-week interval.⁽¹³⁾ The tumor samples of all the patients were histologically diagnosed as ESCC, and they are staged according to the criteria of the International Union Against Cancer. The histopathological response was determined according to the criteria of the Japanese Society for Esophageal Diseases.⁽¹⁴⁾ The grades are determined based on the percentage of remaining viable tumor cells within the entire tumor lesion defined as follows: grade 3, no viable residual tumor cells; grade 2, less than two-thirds; grade 1, two-thirds or more; grade 0, all viable (no significant response to chemotherapy). The median follow up for all patients was 24 months, with a range of 1–196 months. Placental tissues obtained from healthy volunteers were also stained along with the tumor samples to be used as positive controls.

This study was approved by the appropriate institutional review board of Osaka University Hospital (approval number: 08226-4) and was conducted in accordance with the Declaration of Helsinki.

Immunohistochemistry. Tissue samples were fixed in 10% phosphate-buffered formalin and embedded in paraffin. A serial section from each specimen was made (4- μ m thick). Some of the sections of each samples were stained with H&E for histological evaluation. Paraffin-embedded tissue sections placed on saline-coated slides (Matsumani Glass, Osaka, Japan) were de-waxed in xylene and ethanol. Heat-induced epitope retrieval was performed by placing slides in citrate buffer (Ph 6.0) using a decloaking chamber (Biocare Medical, Walnut Creek, CA, USA) for approximately 15 min. After the retrieval treatment, endogenous peroxidase was blocked with 0.3% H₂O₂ in PBS for 20 min followed by washing twice in PBS. After neutralization of endogenous peroxidase, the sections on glass slides were pre-incubated with blocking serum and were then incubated overnight with mouse monoclonal

antibodies specific to human PD-L1 (clone 27A2; MBL, Woburn, MA, USA), PD-L2 (Clone 176611, R&D Systems, Minneapolis, MN, USA) or CD8 (clone C8/144b; DAKO, Glostrup, Denmark). After rinsing three times with PBS, the sections were incubated for 20 min with biotinylated secondary antibodies (Vector Laboratories) diluted to 1:250 using a universal blocking reagent, washed three times in PBS, and then incubated for 20 min with avidin biotin complex method reagent (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, USA). The reaction products were rinsed twice with PBS, placed in 0.05 M Tris–HCl buffer (pH 7.5) for 5 min, and then developed in liquid 3,3'-diaminobenzidine (DAKO) for 3 min. After the development, sections were washed twice with distilled water and lightly counterstained with Mayer's hematoxylin. Negative controls of immunohistochemical reactions were made with the staining procedures omitting the primary antibody.

Evaluation of immunostaining. All slides were assessed by two investigators (K.T. and T.K.) independently in a blinded fashion and the scores assigned were determined by consensus afterwards. Every tumor was given a score according to the predominant intensity of the cytoplasmic staining (no staining = 0, weak staining = 1, moderate staining = 2, strong staining = 3) and the extent of stained cells (0% = 0, 1–30% = 1, 30–60% = 2, 60–100% = 3). The intensity of the staining was determined by comparing it to that of the placenta as the positive control, as shown in Figure 1(a). The immunoreactivity (IR) score of individual patients was determined by multiplying the scores for intensity and the extent of the stained cells with a minimum score of 0 and a maximum score of 9 as reported elsewhere.⁽¹⁵⁾ We preliminarily set several cut-off points arbitrarily to select the best value and chose the IR score of 4 for this study; the specimens with a score of 4 or more were classified as positive. The numbers of CD8 T cells were counted in three areas with the most abundant distribution of CD8 cells within the tumor using an ocular grid at $\times 200$ magnification. The average numbers of CD8 T cells of the three areas were used for statistical analysis.

Statistical analysis. Differences between the groups were examined for statistical significance using applicable methods, including Student's *t*-test with Yates' correction, the χ^2 -test, Fisher's exact probability test or the Mann–Whitney *U*-test. Overall survival was calculated from the date of surgery to the event or last known date of follow up. Survival curves were computed using the Kaplan–Meier method, and differences between survival curves were compared using the log-rank test. The differences were considered statistically significant when *P*-values were <0.05. Statistical analysis was performed using JMP ver 9.0 (SAS Institute, Cary, NC, USA).

Results

PD-L1 and PD-L2 expression in tissue samples. PD-L1 and PD-L2 were not detected in normal esophageal epithelium (Fig. 1b). Representative observations for PD-L1 and PD-L2 in the cancer tissues are shown in Figure 1(c) according to the intensity of PD-L staining. The expression of PD-L1 and PD-L2 was detected mainly in the cytoplasm of cancer cells. Figure S1 shows the distribution of the IR score of PD-L1 and PD-L2. According to the cut-off value of 4, 53 patients (29.4%) and 87 patients (48.3%) were considered to be positive for PD-L1 and PD-L2, respectively. A total of 27 patients (15.0%) were considered to be positive for both PD-L1 and

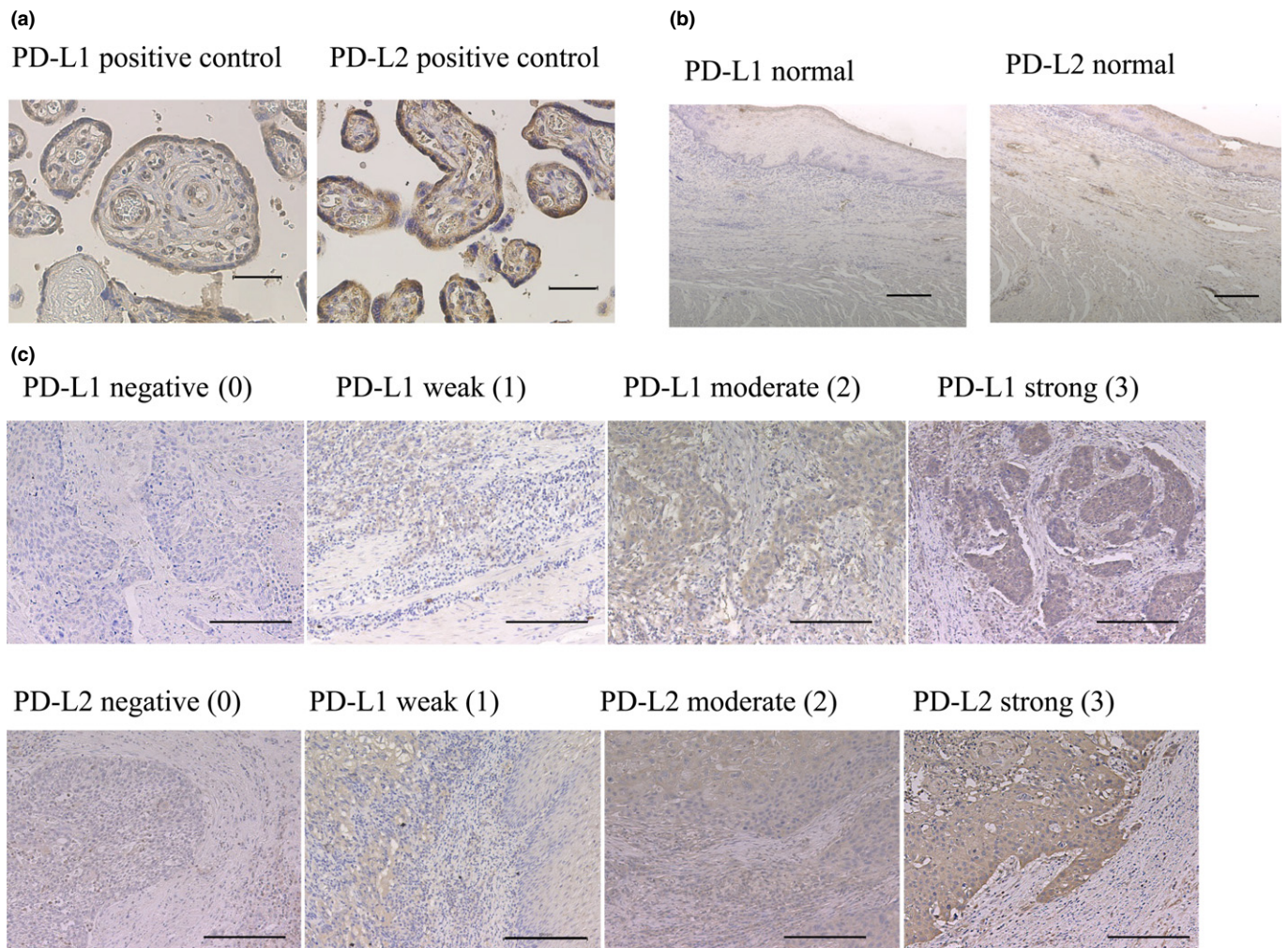


Fig. 1. Representative staining patterns of the samples. (a) Placenta was stained by PD-L1 and PD-L2 and used as positive controls. (b) The PD-L1 and PD-L2 were not detected in normal esophageal epithelium. (c) Representative staining intensities of PD-L1 and PD-L2 in the cancer tissues are shown.

PD-L2 expression. Furthermore, 26 patients (14.4%) showed only PD-L1 expression (PD-L1 positive and PD-L2 negative), and 60 patients (33.3%) showed only PD-L2 expression (PD-L1 negative and PD-L2 positive). Sixty seven patients (37.2%) were considered to be negative for both expressions. There was no significant correlation between PD-L1 and PD-L2 positivity.

Correlation between PD-L expression and clinicopathological factors. Correlation between PD-L expression and clinicopathological factors was examined. The patients with positive PD-L1 expression had significantly higher chance of having lymph node involvement (pN) ($P = 0.0069$) and history of NAC ($P = 0.0139$). There is no significant relationship between PD-L1 expression and gender, age, tumor depth (pT), distant metastasis (pM) and pathological stage (Table 1). The patients with positive PD-L2 expression had significantly higher chance of having deeper tumor invasion (pT) ($P = 0.0024$), more extensive lymph node involvement (pN) ($P = 0.0005$) and higher pathological stage ($P = 0.0003$). There was no significant relationship between PD-L2 expression and gender, age, distant metastasis (pM) and history of NAC. The patients with positive PD-L1 expression had a significantly higher rate of NAC history ($P = 0.0139$), but those with positive PD-L2 expression did not ($P = 0.6127$).

Expression of PD-L in the tumors and the survival of the patients. In all the patients examined in this study, overall

survival rates of the patients with tumors positive for PD-L1 or PD-L2 were significantly worse than those with tumors negative for PD-L1 or PD-L2 ($P = 0.0010$ and $P = 0.0237$, respectively) (Fig. 2a,b). Multivariate analysis revealed that expression of PD-L1 is a significant predictive factor for overall survival ($P = 0.0114$) in patients with esophageal cancer treated with surgery (Table 2). Next, we analyzed the survival data considering both PD-L1 and PD-L2 expression in each patient. The overall survival rate of patients positive for both PD-L1 and 2 was significantly worse, compared with those of patients positive for PD-L2 alone ($P = 0.0289$) and negative for both ligands ($P < 0.0001$) (Fig. 2c). The evaluation using the other criteria showed similar tendencies (Fig. S2).

We also performed subgroup analysis according to the history of NAC, because NAC-treated patients had more advanced disease at the time of diagnosis when compared with that of the patients not treated with NAC (Table S1). Among the patients without NAC, there is no significant difference in the overall survival rate between patients with positive PD-L1 expression and those with negative PD-L1 expression. Overall survival did not differ between the groups with different PD-L2 expression either ($P = 0.1049$ and $P = 0.5346$, respectively) (Fig. 3a,b). Among the patients treated with NAC, the overall survival rate of patients with tumors positive for PD-L1 was significantly worse than for those with tumors negative

Table 1. Relationship between expression of PD-L1/PD-L2 and clinico-pathological factors

| | PD-L1 positive N = 53 | PD-L1 negative N = 127 | P-value | PD-L2 positive N = 87 | PD-L2 negative N = 93 | P-value |
|------------------------------|--------------------------|---------------------------|---------|--------------------------|--------------------------|---------|
| Gender (M/F) | 48/5 | 109/18 | 0.4691 | 76/11 | 81/12 | 0.9584 |
| Age | 62.6 ± 10.0 | 65.1 ± 8.4 | 0.1149 | 64.1 ± 9.6 | 64.7 ± 8.4 | 0.9587 |
| pT (1/2/3/4) | 8/10/29/6 | 29/21/65/12 | | 11/12/53/11 | 26/19/41/7 | |
| pT (1–2/3–4) | 18/35 | 50/77 | 0.4952 | 23/64 | 45/48 | 0.0024 |
| pN (0/1) | 8/45 | 45/82 | 0.0069 | 15/72 | 38/55 | 0.0005 |
| pM (0/1) | 35/18 | 97/30 | 0.1528 | 62/26 | 71/22 | 0.3450 |
| p-stage (I/II/III/IV) | 2/14/19/18 | 17/39/41/30 | | 65/18/38/26 | 14/35/22/22 | |
| p-stage (I–II/III–IV) | 16/37 | 56/71 | 0.0826 | 23/64 | 49/44 | 0.0003 |
| Neoadjuvant therapy (yes/no) | 40/13 | 71/56 | 0.0139 | 52/35 | 59/34 | 0.6127 |

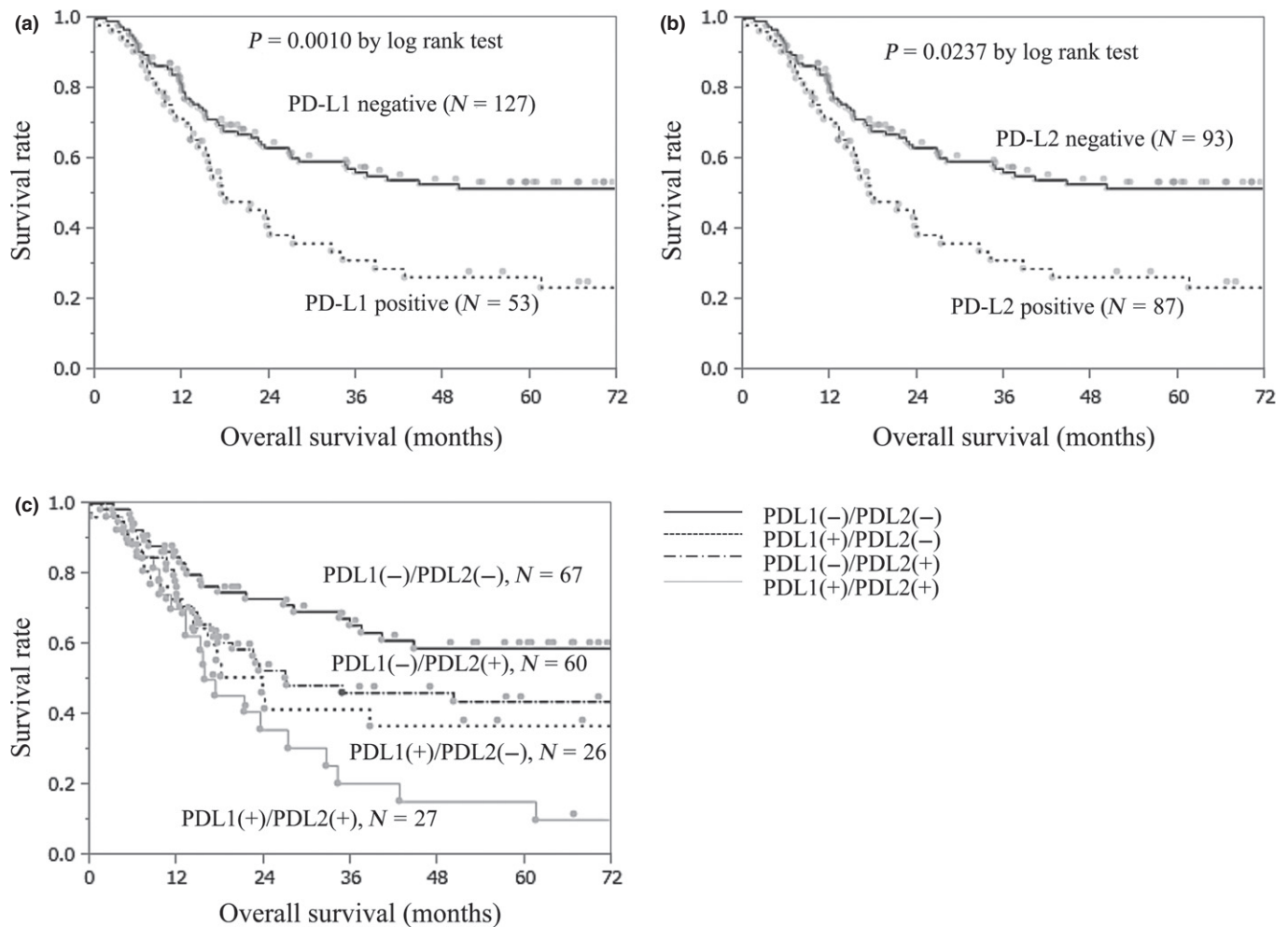


Fig. 2. Overall survival curves for 180 patients with esophageal cancer according to expression level of PD-L1 and PD-L2. Thick line, negative expression group; dotted line, positive expression group. (a) The PD-L1 negative group showed significantly longer overall survival ($P = 0.0010$) than PD-L1 positive group. (b) The PD-L2 negative group showed significantly longer overall survival ($P = 0.0237$) than the PD-L2 positive group. (c) The overall survival of both PD-L1-negative and PD-L2-positive patients was significantly worse than that of both negative patients ($P < 0.0001$).

for PD-L1 ($P = 0.0064$) (Fig. 3c). The same tendency was also observed with regard to PD-L2 expressions ($P = 0.0160$) (Fig. 3d).

Expression of PD-L in the tumors and pathological anti-tumor responses of the patients after neoadjuvant chemotherapy. In the patients who received the neoadjuvant chemotherapy, the relationship between the expression of PD-L1 and PD-L2 and

pathological responses to chemotherapy were examined (Table 2). There was no significant relationship between PD-L1 expression and response to chemotherapy ($P = 0.3118$), but PD-L2 expression correlated inversely with response to chemotherapy ($P = 0.0034$).

Correlation between PD-L expression and CD8+ T cell infiltration. The ligation of the PD-L1 to the PD-1 expressed on the

Table 2. Univariate and multivariate analysis for overall survival

| | 2-year survival rate (%) | Log-rank <i>P</i> -value | Multivariate (cox) <i>P</i> -value | Hazard ratio | 95% CI |
|--------------------------|--------------------------|-----------------------------|---------------------------------------|--------------|---------------|
| Gender | | | | | |
| Male | 56.2 | 0.6578 | | | |
| Female | 59.8 | | | | |
| Age | | | | | |
| >65 | 50.2 | 0.3030 | | | |
| <64 | 62.5 | | | | |
| Tumor depth | | | | | |
| pT1–2 | 75.6 | <0.0001 | 0.0026 | 2.0442 | 1.2756–3.3952 |
| pT3–4 | 45.7 | | | | |
| Lymph node metastasis | | | | | |
| pN0 | 75.6 | <0.0001 | 0.0755 | 1.7150 | 0.9482–3.2997 |
| pN1 | 48.9 | | | | |
| Distant metastasis | | | | | |
| pM0 | 69.3 | <0.0001 | 0.0002 | 2.3334 | 1.5007–3.6164 |
| pM1 | 24.2 | | | | |
| PD-L1 | | | | | |
| Negative | 63.2 | 0.0010 | 0.0114 | 1.7480 | 1.1373–2.6578 |
| Positive | 38.4 | | | | |
| PD-L2 | | | | | |
| Negative | 65.6 | 0.0237 | 0.5155 | 1.1524 | 0.7527–1.7789 |
| Positive | 47.5 | | | | |
| Neoadjuvant chemotherapy | | | | | |
| Performed | 51.9 | 0.3003 | | | |
| Not performed | 63.4 | | | | |

T-cells has been shown to promote the induction of apoptosis of the activated T cells.⁽¹⁴⁾

In contrast, IFN-gamma expressed at the local tumor sites has been reported to be associated with PD-L1 expression on the tumor.⁽¹⁶⁾ To investigate these possibilities in actual cancer patients, we examined the correlation between IR scores for PD-L and the number of tumor-infiltrating CD8+ T cells in the patients with NAC history. Significantly low number of CD8 (+) cells were found only in patients with positive PD-L1 expression in the tumor ($P = 0.0346$) (Fig. 4).

Discussion

To examine the possible roles of PD-L expression in cancer patients, tumor samples surgically resected from 180 esophageal cancer patients, including those treated with NAC, were examined for the expression of these molecules using immunohistochemical staining. Analysis on these patients, not considering NAC history, suggests that positive expression of PD-L of the tumor cells might be associated with the aggressive tumor progression, which significantly affects the survival of esophageal cancer patients. Most of these results are consistent with a previous report on esophageal cancer by Ohigashi *et al.*⁽¹⁷⁾ However, the analysis on the subgroups divided by the experience of NAC revealed unexpected characteristics. The expression patterns of both PD-L1 and PD-L2 on tumors are significantly related to survival only in the patients with NAC ($P = 0.0064$ and 0.0160) but not in the patients without NAC ($P = 0.1049$ and 0.5346). These results might suggest that the tumor expression of the PD-L would have a significant role only in influencing the survival of the chemotherapy for esophageal cancer patients.

Recently, multiple studies have shown the association between the conventional treatment using certain anticancer agents and immune responses against cancer cells.^(18–23) Based on these results, significant numbers of researchers now appear to believe that chemotherapeutic agents can induce anti-tumor immune responses that significantly contribute to the overall anti-tumor effects of the chemotherapeutic agents.⁽²⁴⁾ Our results presented here appear to support this notion and suggest that the PD-1 system might be one of the key players in determining the chemotherapy-related immune responses.

To examine our hypothesis in more detail, we examined the association between the PD-L expression of the tumor and the immediate antitumor response (tumor shrinkage) with chemotherapy. Pathological evaluation on the antitumor responses showed that the patients with strong PD-L2 expression in tumors had significantly higher chance of experiencing inferior antitumor effects (Table S2). However, this correlation was not found for PD-L1 expression. These results might indicate that the tumor expression of PD-L2, but not PD-L1, plays a role in the initial tumor shrinkage induced by the chemotherapeutic effects which might include immunological responses against the tumor. However, we have found that the tumors with positive PD-L1 expression, but not those with positive PD-L2 expression, have significantly lower numbers of CD8+ T-cells infiltrating the tumors. These results might suggest that tumor expression of only PD-L1 is associated with poorer tumor immune responses of the hosts after the chemotherapy. These results might collectively suggest that PD-L1 and PD-L2 have significant impact on survival after chemotherapy but appear to do so through different means. The PD-L2 has been identified as a second PD-1-ligand which inhibits T cell functions,⁽²⁵⁾ but has been shown to have distinct expression

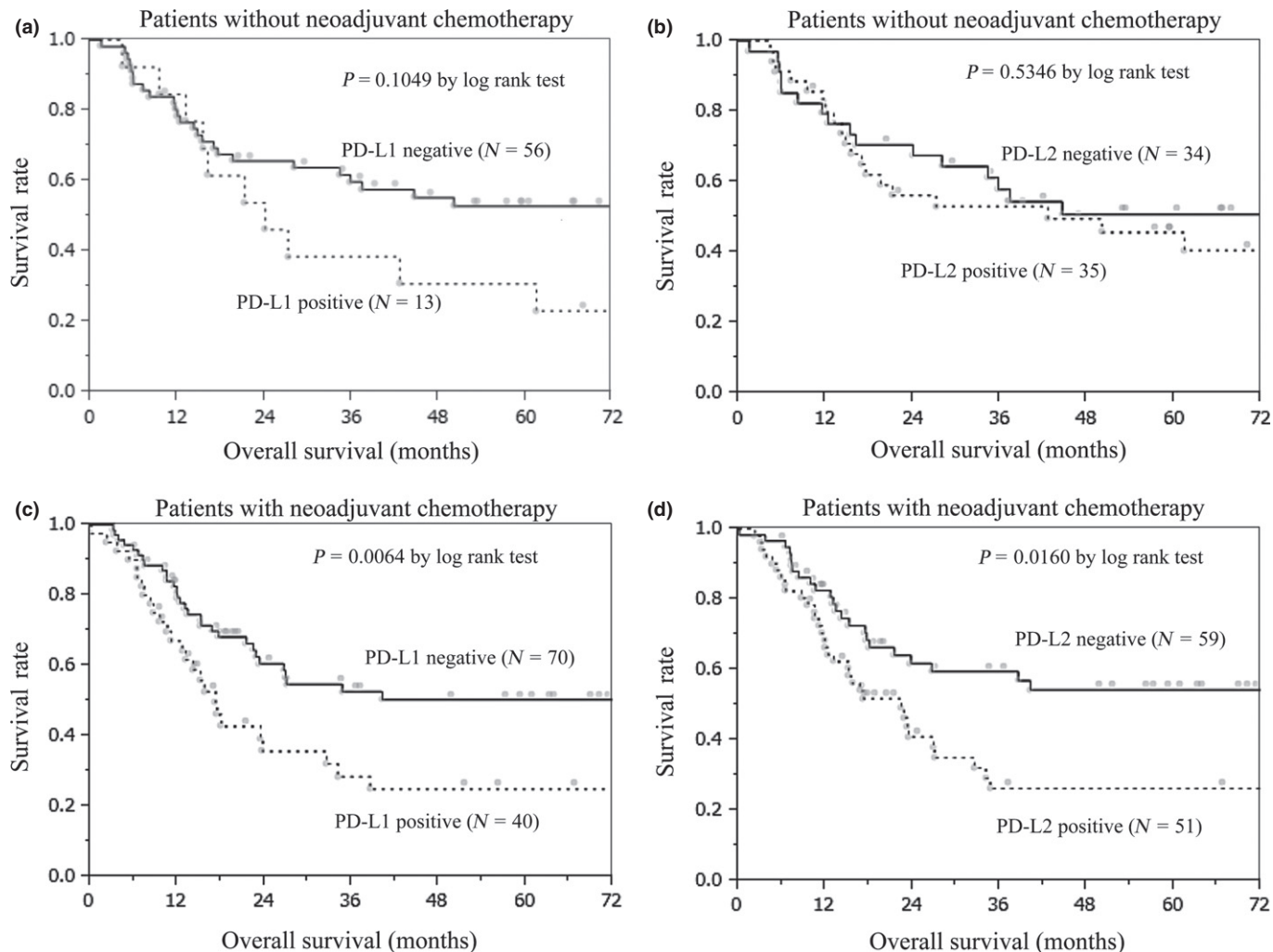


Fig. 3. Overall survival curves of the patients classified according to the history of neoadjuvant chemotherapy and the expression levels of PD-L1 or PD-L2 of the tumors. Thick line, negative expression group; dotted line, positive expression group. (a) Among the patients without receiving neoadjuvant chemotherapy, the overall survival rate was not significantly different between the PD-L1 negative group and the PD-L1 positive group. (b) Among the patients who did not receive neoadjuvant chemotherapy, the overall survival rate was not significantly different between the PD-L2 negative group and the PD-L2 positive group. (c) Among the patients receiving neoadjuvant chemotherapy, the PD-L1 negative group showed significantly longer overall survival ($P = 0.0064$) than the PD-L1 positive group. (d) Among the patients receiving neoadjuvant chemotherapy, the PD-L2 negative group showed significantly longer overall survival ($P = 0.0160$) than the PD-L2 positive group.

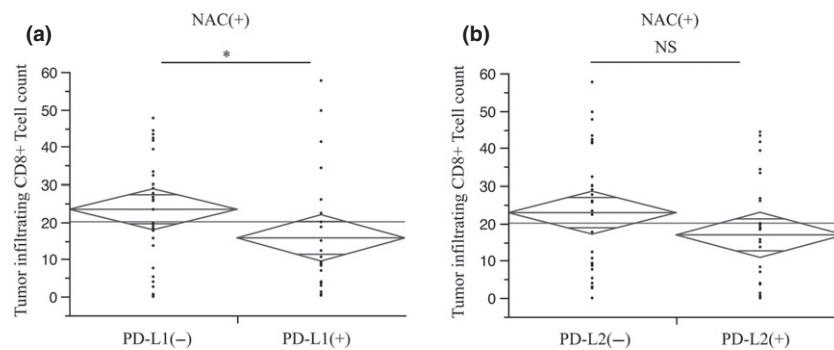


Fig. 4. Correlation between immune-reactivity (IR) scores for PD-L and the numbers of tumor-infiltrating CD8⁺ T cells: (a) PD-L1 ($P = 0.0346$); (b) PD-L2.

patterns and functions when compared with those of PD-L1.⁽²⁶⁾ Our results described here also suggest that PD-L2 functions need to be further investigated and compared with those of PD-L1.

Our results also include other interesting implications related to chemotherapy as well. Because more than half of the

patients examined in this study received chemotherapy before surgery, as NAC, we were able to look into the PD-L expression of the tumors treated with chemotherapy. Surprisingly, the average IR score for PD-L1 in patients treated with NAC was significantly higher than that in patients not treated with NAC. In contrast, the average IR score for PD-L2 in patients treated

with NAC was not significantly different from that in patients not treated with NAC. These results might suggest that expression of only PD-L1, but not PD-L2, in tumor cells could be induced by chemotherapy in esophageal cancer patients. It has been demonstrated that tumor cells express PD-L1 not only constitutively with oncogenic transformation but also adaptively with immunological stimulation, including inflammatory cytokines.⁽⁶⁾ However, the relationship between tumor cell expression of PD-L and other factors, including chemotherapy, has not been fully investigated. Few *in vitro* studies have reported on this issue and the results are somewhat inconsistent. Qin *et al.*,⁽²⁷⁾ for example, report that cisplatin induce PD-L1 in hepatoma cells via the Erk/MAPK signaling pathway. In contrast, Lesterhuis *et al.*⁽²⁸⁾ report that platinum dephosphorylates STAT6, resulting in decreased PD-L2 expression on both human DC and human tumor cells. Furthermore, Guo *et al.*⁽²⁹⁾ report that chemotherapeutic treatments do not directly induce PD-L1 expression but indirectly induce PD-L1 expression through the promotion of IFN- γ production from the immune-related components. Examination on the detailed mechanisms for PD-L upregulation appears to be necessary to better understand our results.

Esophageal squamous cell carcinoma is one of the most refractory cancers among those with gastrointestinal origin and

is associated with poor prognosis. Systemic chemotherapy is believed to be one of the most effective means to treat esophageal squamous cell carcinoma, and is frequently included in the multimodal treatment for those in advanced and metastatic stages.^(30–32) However, the objective response rates, especially the complete response rates in esophageal cancer patients, are still unsatisfactory.^(13,33) The results presented in the current study might be used as evidence warranting the clinical development of novel combination approaches using conventional cytotoxic drugs and PD-1 blockades. Such approaches have recently been shown to have very promising clinical potency in multiple types of cancer, including squamous cell carcinoma of the lung.⁽³⁴⁾

Disclosure Statement

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Distribution of the immune-reactivity (IR) scores: (a) PD-L1; (b) PD-L2.

Fig. S2. Overall survival curves for 180 patients with esophageal cancer according to expression level of PD-L1 and PD-L2 determined by percentage of the stained cells in the tumor area (a, b), predominant intensities of the staining (c, d) and score calculated by adding the values of percentage and intensities (e, f). Thick line, negative expression group; dotted line, positive expression group. (a, c, e) The PD-L1 positive groups divided using these criteria showed significantly shorter overall survival ($P = 0.0011$, $P = 0.0117$, and $P = 0.0056$, respectively) than those of the corresponding PD-L1 negative groups. (b, d, f) The PD-L2 positive groups divided using these criteria showed significantly shorter overall survival ($P = 0.0083$, $P = 0.0234$ and $P = 0.0056$) than those of the corresponding PD-L2 negative groups.

Table S1. Tumor staging of the patients at diagnosis with respect to neoadjuvant chemotherapy (NAC) history.

Table S2. Pathological responses and expression of PD-L in the tumors.