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Expression of immunoregulatory molecules PD-L1 and PD-1 in oral cancer and precancerous lesions: A cohort study of Japanese patients

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ABSTRACT

Objective: An association of the programmed cell death-1 (PD-1) and its ligand PD-L1 with various types of malignant tumors has been established. This study aimed to investigate the role of the PD-L1/PD-1 pathway in oral squamous cell carcinoma (OSCC) and oral epithelial precursor lesions (OEPL).
Materials and methods: We examined 106 OSCC and 79 OEPL specimens for PD-L1 and PD-1 expression by immunohistochemistry. The results were compared with clinicopathological features of OSCC patients.
Results: In OSCC and OEPL specimens, PD-L1 expression was detected predominantly in epithelial or carcinoma cells, whereas PD-1 expression was found mainly in infiltrating or stromal lymphocytes. Seventy-two OSCC (67.9%) and 21 OEPL (26.6%) specimens were positive for PD-L1, and 73 OSCC (68.9%) and 23 OEPL (29.2%) specimens were positive for PD-1. PD-L1 and PD-1 expression levels were significantly different between OEPL and OSCC specimens ($P < 0.001$). There were significant positive correlations between PD-L1 and PD-1 expression in OEPL and OSCC specimens ($P < 0.001$). PD-L1 and PD-1 immunoreactivity was significantly associated with tumor size ($P < 0.05$). PD-L1 and PD-1 immunoreactivity in cases with advanced TNM staging was significantly higher than that in low staging cases ($P < 0.01$). There were significant correlations between PD-L1 and PD-1 expression in OSCC specimens and pathological variables such as stromal lymphocytic reaction ($P < 0.05$) and invasion depth ($P < 0.01$).
Conclusion: PD-L1 and PD-1 immunohistochemical status may be related to carcinogenesis, tumor progression, and prognosis in oral epithelial lesions. Agents targeting PD-1 and PD-L1 might be useful for OSCC treatment.

1. Introduction

Many investigators have mentioned that lymphocytes infiltrating and adjacent to tumor cells play an important role in immune responses that limit and correlate with tumor invasion, and these are considered to be associated with clinical outcomes of tumors including oral squamous cell carcinoma (OSCC) (Uppaluri et al., 2008; Watanabe et al., 2010). Cancer immunotherapy has entered a new phase since the discovery of drugs that are able to interfere with specific immune checkpoints such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and, more recently, programmed cell death-1 (PD-1) and its ligand PD-L1. It has been established that PD-1, an immunoinhibitory receptor that belongs to the CD28 family, plays a critical role in tumor immune escape (Greenwald et al., 2005). Furthermore, PD-L1 is involved in the negative regulation of cellular and humoral immune responses by engaging the PD-1 receptor. In addition, the involvement of PD-L1 has been demonstrated in the protection of cancer cells from cell lysis by activated T lymphocytes (Iwai et al., 2002). Previous studies have

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shown that PD-L1/PD-1 interaction inhibits T cell growth and cytokine secretion (Chen et al., 2012; Sznol and Chen, 2013; Freeman et al., 2000; Latchman et al., 2001). Nishimura et al. showed evidence that PD-1 knockout mice develop spontaneous autoimmune diseases, suggesting that PD-L1/PD-1 interaction has an inhibitory and a regulatory role in T cell responses and the maintenance of natural immune tolerance against a self-antigen (Nishimura and Honjo, 2001; Nishimura et al., 1999). In tumor cells of human carcinomas of the lung, ovary, and colon and in melanomas, PD-L1 increases apoptosis according to antigen-specific T cell clones *in vitro* (Dong et al., 2002). Atezolizumab, a fully humanized, engineered monoclonal antibody of IgG1 isotype against PD-L1, has been developed following the development of nivolumab, a human IgG4 anti-PD-1 monoclonal antibody (Roche Media Release, 2015). Moreover, Eckert et al. (2016) reported that the tumor microenvironment including hypoxia was associated with an increased immune escape of the factors that cause immune suppression in OSCC as well as immune checkpoints such as the PD-L1/PD-1 pathway (Eckert et al, 2016).

Although an association of PD-L1 and PD-1 expression with various types of malignant tumors such as renal cell carcinoma, esophageal cancer, stomach cancer, urothelial cancer, pancreatic cancer, oropharyngeal cancer, and malignant melanoma has been established (Thompson et al., 2004; Ohigashi et al., 2005; Wu et al., 2006; Nakanishi et al., 2007; Nomi et al., 2007; Hino et al., 2012; Kim et al., 2016), the correlation between those molecules and OSCC is uncertain. In addition, no correlation between oral epithelial precursor lesions (OEPL) and PD-L1/PD-1 expression has yet been reported. In the present study, we examined OSCC and OEPL samples for PD-L1 and PD-1 expression by immunohistochemistry to analyze the correlation of those expressions with OSCC and OEPL. Furthermore, PD-L1/PD-1 immunoreactivities were evaluated in terms of their association with clinicopathological characteristics of OSCC patients.

2. Materials and methods

2.1. Clinicopathological characteristics

Biopsy specimens were obtained from 106 patients with primary OSCC and 79 patients with OEPL by surgical resection performed at the Department of Oral and Maxillofacial Surgery, Tohoku University Hospital, from 2010 through 2013. None of the patients with OSCC received chemotherapy or radiotherapy before surgery. The TNM disease stage of OSCC obtained from medical records was classified according to the Union for International Cancer Control system (Sobin et al., 2009). Patients with OSCC attended follow-up visits at least once a month during the follow-up period (36–75 months). None of the OEPL patients were diagnosed as having OSCC in any sites before OEPL surgery.

2.2. Sample preparation

Tissue samples were fixed in 10% buffered formalin for several days, embedded in paraffin, and sliced into 3-μm-thick sections for routine histological and subsequent PD-L1 and PD-1 immunohistochemical examinations. The 106 OSCC samples were classified into well-differentiated, moderately differentiated, and poorly differentiated types according to the World Health Organization (WHO) classification of tumors of the oral cavity and oropharynx (Slootweg et al., 2005). The degree of stromal lymphocytic reaction of OSCC was classified as slight, moderate, and severe. The mode of OSCC invasion was classified as previously described (Yamamoto et al., 1984); the carcinoma grade was 2, 3, 4C, and 4D. The invasion depth of OSCC was classified as microinvasion and invasion within mucosal tissue and into submucosal tissue.

The pathological diagnosis of OEPL was classified according to the WHO classification of tumors of the oral cavity and oropharynx (Slootweg et al., 2005; Gale et al., 2005). OEPL cases were classified based on malignant pathological symptom such as irregular epithelial stratification, loss of polarity of basal cells, drop-shaped rete ridges, increased number of mitotic figures, abnormally superficial mitoses, premature keratinization in single cells, and keratin pearls within rete pegs. In all, 24 patients had leukoplakia (control group) without epithelial dysplasia (hyperkeratosis or hyperplasia: HK), 31 had low-grade epithelial dysplasia (mild to moderate dysplasia: LD), and 24 had high-grade epithelial dysplasia (severe dysplasia or carcinoma in situ: HD).

2.3. Immunohistochemistry

To determine the appropriate antibody to evaluate PD-L1 and PD-1 expression, we tested the staining patterns of several commercially available antibodies against PD-L1 in human non-small cell lung carcinoma and PD-1 in lymphoma as control specimens. To detect PD-L1 and PD-1 expression, tissue sections were processed using the antibodies against PD-L1 (CD247) (clone SP142; Spring Bioscience, Pleasanton, CA, USA) and PD-1 (clone NAT; Abcam, Cambridge, UK), and the automated immunohistochemistry/in situ hybridization slide staining system (Ventana Benchmark ULTRA; Roche, Basel, Switzerland).

The PD-L1 immunoreactivity was evaluated based on the intensity and proportion of membranous and/or cytoplasmic staining in tumor cells, according to previous studies (Koh et al., 2015; D'Incecco et al., 2015), with some modifications (Table 1 and Fig. 1). Then, the cases were defined as negative (Score 0) or positive (Score 1, 2, or 3) for PD-L1 and PD-1 expression.

2.4. Statistical analysis

All statistical analyses were performed using JMP pro software (version 11.2; SAS Institute, Inc., Cary, NC, USA). Immunohistochemistry and clinicopathological characteristic data were analyzed using the Mann–Whitney U-test for differences between 2 groups or the Kruskal–Wallis test for differences among 3 or more groups, respectively. P values of less than 0.05 were considered to indicate statistical significance.

The study protocol was reviewed and approved by the Research Ethics Committee of Tohoku University Graduate School of Dentistry (No. 26-42). We explained the study to the patients, and they provided informed consent.

Table 1
Scoring of immunohistochemical reactivity for PD-L1 and PD-1.

Immunohistochemical reactivity for PD-L1	
Score 0	Negative
Score 1	Weakly positive (scatteredly positive in <25% of the epithelial/tumor cells)
Score 2	Moderately positive (scatteredly positive in ≥25% of the epithelial/tumor cells)
Score 3	Strongly positive (continuously positive in ≥25% of the epithelial/tumor cells)
Immunohistochemical reactivity for PD-1	
Score 0	Negative
Score 1	Weakly positive (scatteredly positive in <10% of infiltrating lymphocytes)
Score 2	Moderately positive (scatteredly positive in ≥10% of infiltrating lymphocytes)
Score 3	Strongly positive (diffusely positive in ≥10% of infiltrating lymphocytes)

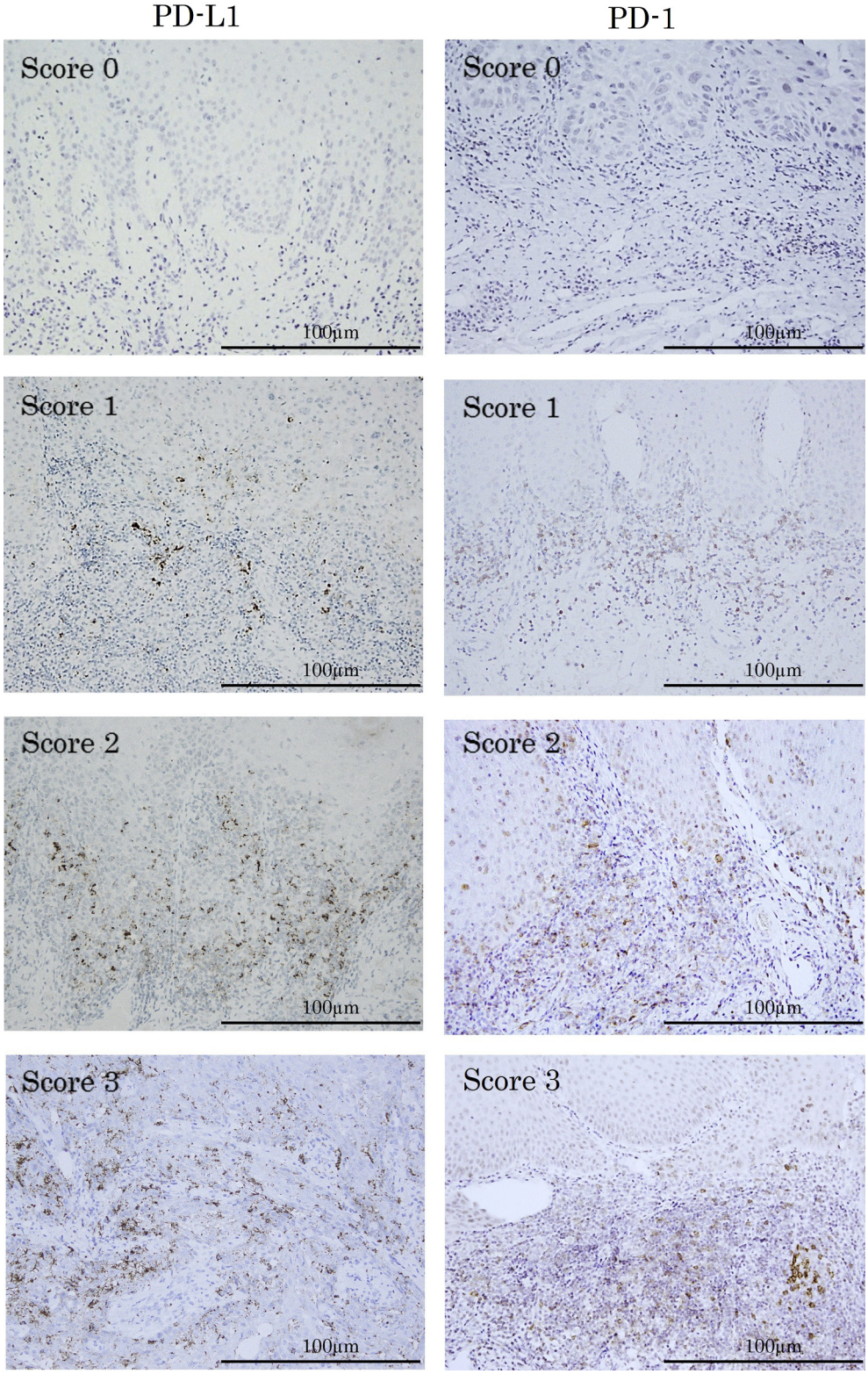

Fig. 1. Immunohistochemical reactivity for PD-L1 and PD-1. PD-L1 was expressed in the cell membrane and cytoplasm of epithelial cells and a few immune cells. PD-L1 immunostaining was graded as Score 0, Score 1, Score 2, and Score 3. PD-1 was immunostained in the cell membrane of infiltrating lymphocytes. PD-1 immunostaining was graded as Score 0, Score 1, Score 2, and Score 3 (× 200). Abbreviations: PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1.

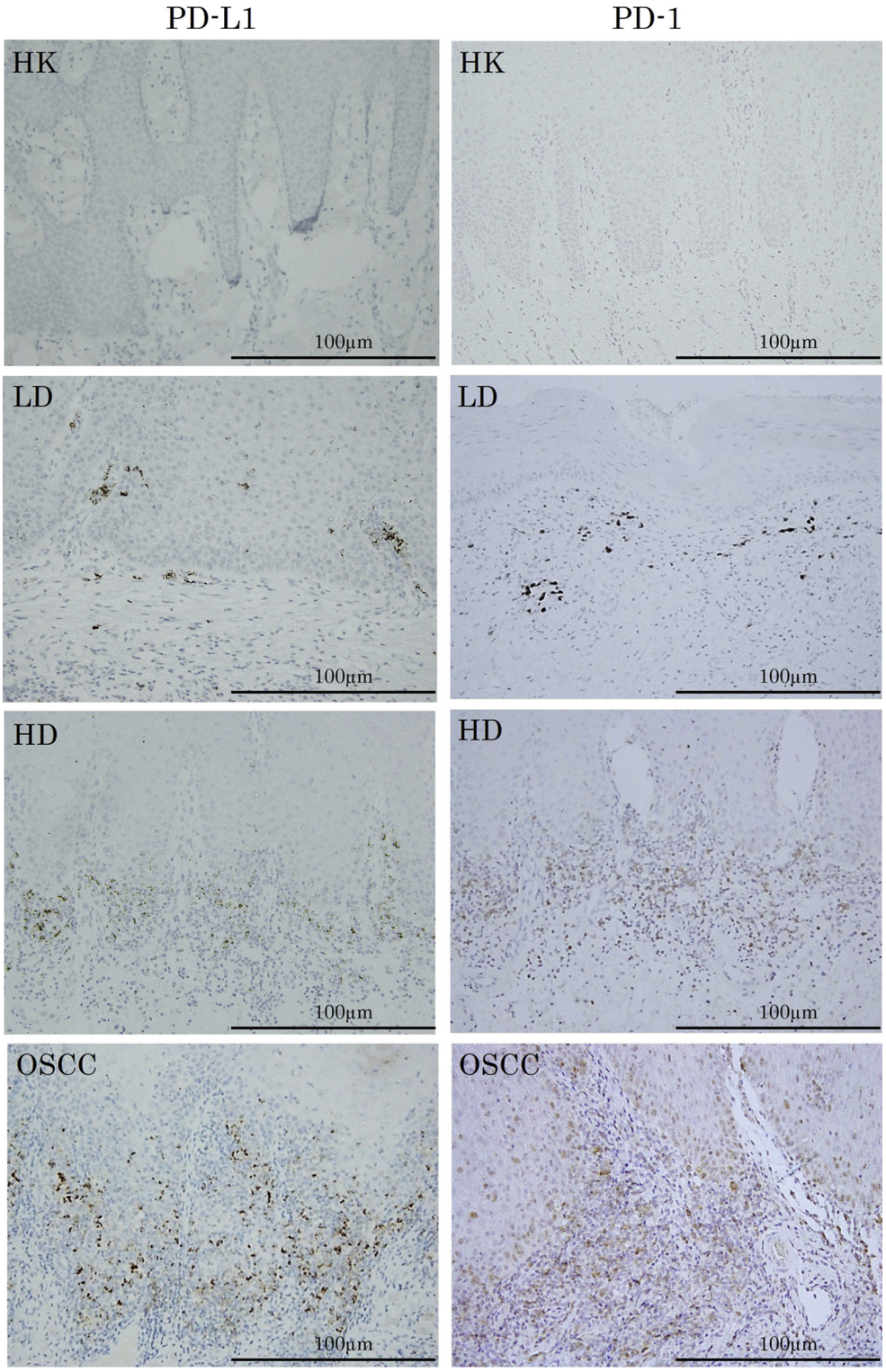

Fig. 2. Representative images of PD-L1 and PD-1 immunohistochemistry in OEPL and OSCC. Leukoplakia without epithelial dysplasia (HK) showing no reactivity for PD-L1 (Score 0) or PD-1 (Score 0). Low-grade epithelial dysplasia (LD) showing scattered PD-L1 expression in epithelial cells adjacent to the subepithelial lymphocytes (Score 1) and scattered PD-1 expression in infiltrating lymphocytes adjacent to dysplastic epithelium (Score 1). High-grade dysplasia (HD) showing PD-L1 expression in many epithelial cells adjacent to subepithelial lymphocytes (Score 2) and PD-1 expression in many infiltrating lymphocytes neighboring the dysplastic epithelium (Score 2). OSCC showing PD-L1 expression in many carcinoma cells adjacent to the stromal lymphocytes (Score 3) and PD-1 expression in many infiltrating lymphocytes near the carcinoma cell nests (Score 3) (× 200). Abbreviations: HD, severe dysplasia or carcinoma in situ; HK, hyperkeratosis or hyperplasia; LD, mild to moderate dysplasia; OEPL, oral epithelial precursor lesions; OSCC, oral squamous cell carcinoma; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1.

3. Results

3.1. Immunoreactivity for PD-L1 and PD-1 in OEPL and OSCC

Various levels of PD-L1 expression in OEPL and OSCC specimens were observed mainly in the cell membrane and cytoplasm of epithelial or tumor cells as well as some lymphocytes and a few macrophages neighboring the epithelial tissues (Fig. 1). PD-1 expression in OEPL and OSCC specimens was detected in the cell membrane of lymphocytes infiltrating and adjacent to dysplastic epithelium or tumor cell nests (Fig. 1). Levels of PD-L1 and PD-1 expression tended to increase in association with malignant degrees of the oral epithelial lesions (Fig. 2).

The results of immunohistochemical reactivity for PD-L1 and PD-1 in OEPL and OSCC specimens are summarized in Table 2. PD-L1 and PD-1 expression levels demonstrated significant differences between OEPL and OSCC specimens ($P < 0.001$), as well as between HK/LD/HD and OSCC specimens ($P < 0.001$).

The correlations between immunohistochemical reactivity for PD-L1 and PD-1 in OEPL and OSCC specimens are shown in Table 3. There were significantly positive correlations between PD-L1 and PD-1 expression in OEPL and OSCC specimens ($P < 0.001$).

3.2. Correlation between clinical characteristics and immunoreactivity for PD-L1 and PD-1 in OSCC

The correlations between PD-L1 and PD-1 expression and the clinical features of OSCC are summarized in Table 4. PD-L1 and PD-1 immunoreactivity in the 106 OSCC specimens did not significantly correlate with demographic and clinical variables such as age, sex, tumor site, M classification, recurrence, postoperative metastasis, or survival. PD-L1 and PD-1 immunoreactivity was significantly associated with tumor size (T classification) ($P < 0.05$). Additionally, the PD-L1 and PD-1 immunoreactivity in the cases with advanced TNM staging was significantly higher than that in cases with low staging ($P < 0.01$). Incidentally, there was no apparent association between PD-L1 or PD-1 immunohistochemistry and the clinicopathological characteristics of OEPL patients, including age at disease onset, sex, or occurrence site.

3.3. Correlation between pathological characteristics and immunoreactivity of PD-L1 and PD-1 in OSCC

The correlations between the pathological characteristics and expression of PD-L1 and PD-1 in OSCC specimens are summarized in Table 5. Although PD-L1 and PD-1 immunoreactivity in the 106 OSCC specimens did not significantly correlate with the degree of differentiation or mode of invasion, high PD-L1 and PD-1 expression was frequently observed in OSCC specimens with poor differentiation and diffuse invasion. There were significant correlations between PD-L1 and PD-1 expression in OSCC specimens and pathological variables such as stromal lymphocytic reaction and invasion depth ($P < 0.05$ and $P < 0.01$, respectively).

4. Discussion

In the present study, we showed that the expression of immune checkpoints within the PD-L1/PD-1 pathway differs according to clinical and pathological characteristics (Ishida et al., 1992), and PD-1 is expressed mainly on T cells as well as B cells and myeloid cells. Its ligand PD-L1 (B7-H1) has been identified as a lymphocyte or tumor cell-surface glycoprotein belonging to the B7 family (Dong et al., 1999; Freeman et al., 2000; Tseng et al., 2001; Latchman et al., 2001). Our results also showed that various levels of PD-L1 expression in OSCC specimens were observed mainly in the cell membrane and cytoplasm of tumor cells, and PD-1 expression in OSCC specimens was detected in the cell membrane of lymphocytes infiltrating tumor cell nests as well as other solid tumors (Thompson et al., 2004; Ohigashi et al., 2005; Wu et al., 2006; Nakanishi et al., 2007; Nomi et al., 2007; Hino et al., 2012; Koh et al., 2015; D'Incecco et al., 2015). Malaspina et al. (2011) demonstrated that PD-1 and PD-L1 molecules were present in the blood of patients with OSCC as well as lesion samples. In the present study, there were significantly positive correlations between the degree of PD-L1 and PD-1 expression in OSCC specimens, and both PD-L1−positive tumor cells and PD-1−positive lymphocytes were localized in each specimen.

Our analyses indicated that immunohistological levels of PD-L1 and PD-1 expression significantly increased in association with malignant degrees of the oral epithelial lesions, and there were significantly positive differences in PD-L1 and PD-1 expression between OEPL and OSCC. However, our results showed that there was no significant difference among the three OEPL groups (HK, LD, and HD). In particular, we placed great importance on the boundary of immunohistological levels between HD and OSCC. We consider that the PD-1/PD-L1 checkpoint may have an impact on the invasive capacity of OSCC more than malignant transformation or dysplasia of carcinomas *in situ*. This result indicated that the expression of PD-L1 and PD-1 was useful as a diagnostic and disease-specific marker. In a previous study, PD-L1 mRNA was elevated and PD-1 expression was significantly higher in laryngeal papillomas that were premalignant lesions with the potential for malignant conversion, compared with that of control laryngeal tissues (Hatam et al., 2012). Although the correlation between the PD-L1/PD-1 pathway and other premalignant lesions has not previously been reported, an abnormality of the PD-L1/PD-1 pathway

Table 2
Immunohistochemical reactivity for PD-L1 and PD-1 in OEPL and OSCC.

	Number of cases	PD-L1					PD-1				
		Score0	Score1	Score2	Score3		Score0	Score1	Score2	Score3	
OEPL	79	58(73.4%)	4(5.1%)	14(17.7%)	3(3.8%)		56(70.8%)	11(13.9%)	10(12.7%)	2(2.5%)	
Leukoplakia without epithelial dysplasia (Hyperkeratosis or hyperplasia)	24	19(79.2%)	2(8.3%)	2(8.3%)	1(4.2%)	$P=0.001$	18(75.0%)	2(8.3%)	4(16.7%)	0(0%)	$P=0.001$
Low-grade epithelial dysplasia (Mild to moderate dysplasia)	31	21(67.7%)	2(6.5%)	6(19.4%)	2(6.5%)		20(64.5%)	6(19.4%)	3(9.7%)	2(6.5%)	
High-grade epithelial dysplasia (Severe dysplasia or CIS)	24	18(75.0%)	0(0%)	6(25.0%)	0(0%)		18(75.0%)	3(12.5%)	3(12.5%)	0(0%)	
OSCC	106	34(32.1%)	11(10.4%)	43(40.5%)	18(17.0%)	$P=0.001$	33(31.1%)	9(8.5%)	50(47.2%)	14(13.2%)	$P=0.001$

Abbreviations: OEPL, oral epithelial precancerous lesions; CIS, carcinoma in situ ; OSCC, oral squamous cell carcinoma.

Table 3
Correlation between PD-L1 and PD-1 immunoreactivity in OEPL and OSCC.

		Number of cases	PD-L1 in OEPL							Number of cases	PD-L1 in OSCC				
			Score 0	Score 1	Score 2	Score 3					Score 0	Score 1	Score 2	Score 3	
Total		79	58 (73.4%)	4 (5.1%)	14 (17.7%)	3 (3.8%)		Total		106	34 (32.1%)	11 (10.4%)	43 (40.5%)	18 (17.0%)	
PD-1 in OEPL	Score 0	56 (70.8%)	54	1	1	0	$P < 0.001$	PD-1 in OSCC	Score 0	33 (31.1%)	31	2	0	0	$P < 0.001$
	Score 1	11 (13.9%)	4	2	5	0			Score 1	9 (8.5%)	1	5	2	1	
	Score 2	10 (12.7%)	0	1	8	1			Score 2	50 (47.2%)	2	4	38	6	
	Score 3	2 (2.5%)	0	0	0	2			Score 3	14 (13.2%)	0	0	3	11	

Table 4
Correlation between clinical variables and immunoreactivity for PD-L1 and PD-1 in OSCC.

		Number of cases	PD-L1			PD-1		
			Negative	Positive	*P* value	Negative	Positive	*P* value
Total		106	34 (32.1%)	72 (67.9%)		33 (31.1%)	73 (68.9%)	
Age	Mean	67.56 ± 12.66	66.94 ± 12.96	67.86 ± 12.51	0.7300[a]	67.73 ± 12.40	67.49 ± 12.80	0.9306[a]
	30–39	4	0	4	0.999	0	4	0.999
	40–49	6	4	2		3	3	
	50–59	11	4	7		4	7	
	60–69	34	11	23		11	23	
	70–79	31	6	25		6	25	
	80–89	19	9	10		9	10	
	90–99	1	0	1		0	1	
Sex	M	45	16	29	0.5105[b]	16	29	0.3994[b]
	F	61	18	43		17	44	
Site	Tongue	59	23	36	0.4601[c]	22	37	0.3851[c]
	Upper gingiva	10	3	7		3	7	
	Lower gingiva	13	1	12		1	12	
	Hard palate	1	0	1		0	1	
	Buccal mucosa	12	4	8		4	8	
	Floor of mouth	6	2	4		2	4	
	Lower lip	5	1	4		1	4	
T classification	1	31	17	14	0.0177[b]	15	16	0.0425[b]
	2	41	9	32		8	33	
	3	12	3	9		5	7	
	4	22	5	17		5	17	
N classification	0	88	31	57	0.472[c]	30	58	0.5163[c]
	1	13	3	10		3	10	
	2	4	0	4		0	4	
	3	1	0	1		0	1	
M classification	0	102	33	69	0.7522[b]	32	70	0.7831[b]
	1	4	1	3		1	3	
TNM Stage	I	30	17	13	0.0100[b]	15	15	0.0464[b]
	II	37	9	28		8	29	
	III	14	3	11		5	9	
	IV	25	5	20		5	20	
Recurrence	Negative	85	28	57	0.6986[b]	27	58	0.7759[b]
	Positive	21	6	15		6	15	
Postoperative metastasis	Negative	92	31	61	0.3446[b]	31	61	0.1203[b]
	Positive	14	3	11		2	12	
Survival	Alive	82	29	53	0.3800[d]	27	55	0.1332[d]
	Dead	15	2	13		3	12	
	Dead of other disease	4	1	3		1	3	
	Unknown	5	2	3		2	3	

[a] Student t test.
[b] χ^2 test.
[c] Fisher's exact test.
[d] Fisher's exact test: Alive vs Dead.

might already be present at a certain point before premalignant lesions become invasive or malignant.

Our correlation analyses indicate that there was no apparent association between PD-L1 or PD-1 immunohistochemistry and the clinical characteristics of OSCC patients, including age at disease onset, sex, and disease location. PD-L1 and PD-1 expression was significantly higher in cases with large tumor size or advanced TNM staging. In previous reports of other malignant tumors, there were significant correlations of PD-L1 expression with tumor size and survival rate in renal cell carcinoma, esophageal cancer, stomach cancer, urothelial cancer, pancreatic cancer, oropharyngeal cancer, and malignant melanoma (Thompson et al., 2004; Ohigashi et al., 2005; Wu et al., 2006; Nakanishi et al., 2007; Nomi et al., 2007; Hino et al., 2012; Kim et al., 2016). In particular, PD-L1 expressions in renal cell carcinoma, esophageal cancer, pancreatic cancer, and oropharyngeal cancer significantly correlated with tumor staging, lymph node, and distant metastasis. In addition, our data showed that PD-L1 and PD-1 expression tended to be higher in patients with recurrence, postoperative metastasis, or death caused by complications than in patients without those characteristics.

Table 5
Correlation between pathological variables and immunoreactivity for PD-L1 and PD-1 in OSCC.

		Number of cases	PD-L1			PD-1		
			Negative	Positive	*P* value	Negative	Positive	*P* value
Total		106	34 (32.1%)	72 (67.9%)		33 (31.1%)	73 (68.9%)	
Degree of differentiation	Well	88	30	58	0.0742***	29	59	0.0696***
	Moderate	16	3	13		3	13	
	Poor	2	1	1		1	1	
Stromal lymphocytic reaction	Slight	13	8	5	0.0330***	8	5	0.0299***
	Moderate	90	26	64		25	65	
	Severe	3	0	3		0	3	
Mode of invasion	2	22	11	11	0.0287***	11	11	0.0616***
	3	66	20	46		20	46	
	4C	14	2	12		1	13	
	4D	4	1	3		1	3	
Invasion depth	Microinvasion	7	3	4	0.0028***	3	4	0.0202***
	Mucosal invasion	53	24	29		23	30	
	Submucosal tissue	46	7	39		7	39	

*** Fisher's exact test.

Another study using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis demonstrated a significant correlation between PD-L1 and OSCC tumor size and lymph node metastasis (Oliveira-Costa et al., 2015). These features have suggested that malignant tumors with a deviation of the PD-L1/PD-1 pathway are associated with a more malignant clinical condition, including tumor progression and prognosis.

Histologically, well-differentiated OSCCs are the most common (Slootweg et al., 2005), and the mode of invasion in OSCC is assessed based on the tumor–host boundary and is considered closely related to recurrence and metastasis rates (Yamamoto et al., 1984). In the present study, although we did not detect a significant correlation between PD-L1/PD-1 immunoreactivity and invasion mode or degree of differentiation, PD-L1 and PD-1 immunoreactivity in the cases with low differentiation or prominent invasion mode tended to be higher than in cases with high differentiation or slight invasion. Moreover, there was a significant correlation between PD-L1 and PD-1 expression in OSCC and invasion depth, indicating an association with invasive behavior. Koh et al. (2015) have reported that the PD-L1 expression rate in poorly differentiated pulmonary adenocarcinoma is especially high. These findings suggest that the PD-L1/PD-1 pathway may be correlated with high malignant potential of OSCC cells, and PD-L1/PD-1–activated OSCCs have a more malignant phenotype and aggressive invasiveness. Stromal lymphocytic reactions play an important role in immune responses that limit tumor invasion and are considered to be an indicator of clinical outcomes (Koh et al., 2015). A previous study of oral cancerous lesions showed that inflammatory reactions, neovascularization, and the up-regulation of extracellular matrix-related protein expression are observed in moderate and higher grades of dysplasia (Yokoyama, 2011). Our study revealed that PD-L1- and PD-1–positive OSCC cases have significantly moderate to severe stromal lymphocytic reaction.

In general, chemoradiotherapy is applicable to inoperable patients as the definitive treatment, and in patients with advanced OSCC, postoperative adjuvant chemoradiotherapy is often used in conjunction with surgery. Unfortunately, even after the above comprehensive treatment, the survival outcomes for these patients with advanced OSCC are still unsatisfactory. Progress in the treatment of individuals with recurrent/metastatic OSCC has been slow; thus, these patients still have significant morbidity and mortality (Vermorken and Specenier, 2010). Nivolumab, a fully human IgG4 antibody that blocks the PD-1 receptor, produced durable objective responses in patients with melanoma, renal-cell cancer, and non-small cell lung cancer (Topalian et al., 2014). Recently, available effectiveness data on nivolumab immunotherapy in combination with ipilimumab, an antibody against CTLA-4, has been reported in advanced melanoma (Wolchok et al., 2013). Neither drug trials of ipilimumab nor of nivolumab dedicated to OSCC have been completed, although it is an active area of investigation with some preliminary safety data reported (Ferris et al., 2017). A phase Ib study of pembrolizumab (MK-3475), another anti–PD-1 antibody, provided preliminary safety data specifically for head and neck cancer patients (Tanguy et al., 2014). Although further studies are needed to clarify their role in OSCC management, the present study supports the potential of these targeted molecular therapies using specific antibody agents.

5. Conclusion

In this study, OSCC specimens that were positive for PD-L1 and PD-1 had more malignant clinicopathological features and showed a poor prognosis. Particularly, the PD-L1 and PD-1 immunoreactivity in the cases with advanced TNM staging was significantly higher than that in cases with low staging. In addition to these results, the higher immunoreactivity of PD-L1 and PD-1 in OSCC than in OEPL suggests that the PD-L1/PD-1 pathway might be correlated with oncogenesis and tumor progression in the oral cavity among Japanese patients. The application of immunotherapy using antibodies against these molecules in OSCC patients is expected to improve the treatment of advanced OSCC patients.

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Conflict of interest

The authors declare that there are no conflicts of interest with regard to this work.

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