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Scientific Article

Circulating Plasma Exosomal PD-L1 Predicts Prognosis of Head and Neck Squamous Cell Carcinoma After Radiation Therapy

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Purpose: Radiation therapy is widely used to treat head and neck squamous cell carcinoma (HNSCC). This study evaluated the association between circulating plasma programmed death-ligand 1 (PD-L1) and the outcomes of patients with HNSCC after radiation therapy.

Methods and Materials: In this retrospective observational study, plasma samples of 76 patients with HNSCC who underwent radiation therapy from June 2019 to August 2021 were analyzed. These plasma samples were obtained before radiation therapy. The median follow-up was 32.5 months. Total and exosomal PD-L1 was measured by enzyme-linked immunosorbent assay and retrospectively analyzed for association with overall survival (OS), progression-free survival (PFS), and local control (LC). Prognostic factors among patients' characteristics and circulating PD-L1 in plasma were evaluated by univariate (log-rank test) and multivariate (Cox proportional hazards model) analyses.

Results: The median concentration of total PD-L1 in plasma was 115.1 pg/mL (95% CI, 114.7-137.9 pg/mL), and the median concentration of exosomal PD-L1 was 2.8 pg/mL (95% CI, 6.0-13.0 pg/mL). Univariate and multivariate analyses showed exosomal PD-L1 as a prognostic factor for PFS and LC. Patients with high exosomal PD-L1 in plasma had poor PFS and LC compared with those with low exosomal PD-L1, indicating that 1-year PFS was 79.2% versus 33.3% ($P < .001$) and 1-year LC was 87.3% versus 50.0% ($P < .001$) in patients with high and low exosomal PD-L1, respectively. However, exosomal PD-L1 in plasma had no significant effect on OS. Total PD-L1 in plasma did not correlate with PFS, LC, and OS.

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The authors declare that all data supporting the findings of this study are available within the article or from the corresponding author upon reasonable request.

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Conclusions: The pretreatment circulating exosomal PD-L1 in plasma of patients with HNSCC was a prognostic factor after radiation therapy.

Introduction

Globally, approximately 890,000 cases of head and neck cancer (lip and oral cavity, nasopharynx, other pharynx, and larynx) were diagnosed in 2017, with 507,000 deaths.[1] Radiation therapy is widely used to treat head and neck cancers, along with surgical intervention, both in early and advanced stages.[2] Although head and neck cancer treatment has advanced, recurrence after treatment is still an important issue. Immunity against tumor cells plays a role in cancer treatment efficacy, and monitoring of patients' immunity is a good way to predict treatment efficacy. Programmed death-ligand 1 (PD-L1) is a protein expressed on the surface of cancer cells that binds to programmed cell death protein 1 on the surface of cytotoxic T cells to suppress T cells and inactivate tumor immunity.[3] In patients with metastatic or recurrent head and neck cancer, tumor PD-L1 expression was positively linked with the prognosis after nivolumab[4] and avelumab.[5]

Extracellularly, PD-L1 can be found in vesicles such as exosomes (exosomal PD-L1) or microvesicles or in a soluble state in body fluids (soluble PD-L1). The PD-L1 level in blood has been investigated as a possible biomarker to determine a patient's cancer treatment resistance, reflecting anticancer immunity.[6] It has not been well understood whether the PD-L1 level in blood is associated with outcomes of patients with head and neck squamous cell carcinoma (HNSCC) after radiation therapy. In this study, we examined total PD-L1 and exosomal PD-L1 in plasma and explored whether they were related to outcomes after radiation therapy for patients with HNSCC.

Methods and Materials

Participants

This single-institutional retrospective study was approved by the institutional review board of our institution (number: 20117). Eligible patients were aged ≥18 years with a histologically confirmed diagnosis of HNSCC of the oropharynx, hypopharynx, larynx, nasal cavity, or accessory sinus. They were treated with radiation therapy and consented to a liquid biopsy study conducted from June 2019 to August 2021. Patients with distant metastases, those who received postoperative radiation therapy, and those for whom plasma samples were unavailable were excluded from the study.

Plasma collection and storage

Blood samples were collected in EDTA-3K tubes before initiation of radiation therapy, usually 1 to 2 weeks before the beginning of treatment. After blood collection, plasma samples were immediately collected as a supernatant after 2 centrifugations in our laboratory. The first centrifugation was performed at 1300 × g for 10 minutes at 4°C, and the second was at 12,000 × g for 15 minutes at 4°C. Plasma samples were stored at −80°C in approximately 1-mL aliquots in 1.5-mL tubes and were thawed immediately before exosome purification.

Quantification of total and exosomal PD-L1

We used Total Exosome Isolation Kit (catalog #4484450, Thermo Fisher Scientific, Waltham, MA) to purify exosomes from patient plasma according to the manufacturer's instructions. Briefly, we mixed 200 μL of plasma, 100 μL of phosphate buffered saline (PBS), and 60 μL of exosome precipitation reagent and incubated the mixture at room temperature for 10 min. After incubation, the samples were centrifuged at 10,000 × g for 5 min at room temperature, and exosomes were resuspended with 200 μL of phosphate buffered saline (PBS). To detect PD-L1 in total PD-L1 and exosomal PD-L1 in plasma, we used a human PD-L1 enzyme-linked immunosorbent assay (ELISA) kit (HAK-HELPDL1-1, Hakarel, Osaka, Japan) according to the manufacturer's instructions. The plates were read at 450 nm with Varioskan LUX (Thermo Fisher Scientific). Plasma levels of total and exosomal PD-L1 were measured using ELISA and analyzed for correlation with outcomes after radiation therapy. Samples with concentrations exceeding the ELISA calibration curve range were deemed to have failed quality control and were excluded.

Clinical outcome evaluation

Patients were followed up at hospital visits every 1 to 2 months for 2 years and then every 3 months after that. The latest visit or date of contact was used to determine the mortality of patients at the time of analysis. Tumor responses after radiation therapy were evaluated using Response Evaluation Criteria in Solid Tumors, version 1.1.[7] Overall survival (OS) was defined as the duration from the start of radiation therapy to death from any cause. Progression-free survival (PFS) was defined as the

period after the start of radiation therapy before disease progression or death. Local control (LC) was defined as the absence of recurrence at the irradiated site. Treatment outcomes and the measurement of PD-L1 were blinded to each other and assessed together at analysis.

Statistics

Data analysis was performed using Prism9 (GraphPad Software, San Diego, CA) and JMP Pro, version 16.0.0 (SAS Institute Inc, Cary, NC). Concentrations of total and exosomal PD-L1 were compared using the *t* test. The cutoffs for plasma PD-L1 concentration for OS, PFS, and LC were identified by analysis of receiver operating characteristic (ROC) curves. The OS, PFS, and LC rates were calculated using the Kaplan-Meier method. Prognostic factors were analyzed using the log-rank test for univariate analysis and Cox proportional hazards models for multivariate analysis. Statistical tests were based on a 2-sided significance level, with *P* values <.05 indicating statistical significance.

Results

Patient characteristics

Out of the 102 patients initially assessed for eligibility, 21 were excluded based on the inclusion criteria, and 5 were excluded due to ELISA quality control, resulting in a final total of 76 patients for analysis (CONSORT diagram, Fig. 1). Baseline clinical characteristics of the patients are summarized in Table 1; 89.0% of patients were male, and the median age was 69.5 years (range, 37-85 years). The primary sites of cancer were the nasal cavity and accessory sinus (12 patients [15.8%]), oropharynx (24 [31.6%]), hypopharynx (22 [28.9%]), and larynx (18 [23.7%]). There were 16 patients (21.1%) with stage I, 20 (26.3%) with stage II, 8 (10.5%) with stage III, and 32 (42.1%) with stage IVA and IVB disease. Thirty (39.5%) patients received radiation therapy alone, and 46 (60.5%) received chemoradiation therapy. The median follow-up was 32.5 months.

Quantification of total and exosomal PD-L1 in plasma

The median concentration of total PD-L1 in plasma was 115.1 pg/mL (95% CI, 114.7-137.9 pg/mL), and the median concentration of exosomal PD-L1 was 2.8 pg/mL (95% CI, 6.0-13.0 pg/mL). The concentration of total PD-L1 was significantly higher than that of exosomal PD-L1 (Fig. 2A). The concentrations of total and exosomal PD-L1 did not correlate with the cancer stage (Fig. 2B, C), age, sex, or primary site (data not shown).

ROC analysis for disease progression with total and exosomal PD-L1 in plasma

The ROC analysis revealed that the area under the curve for exosomal PD-L1 was greater than for total PD-

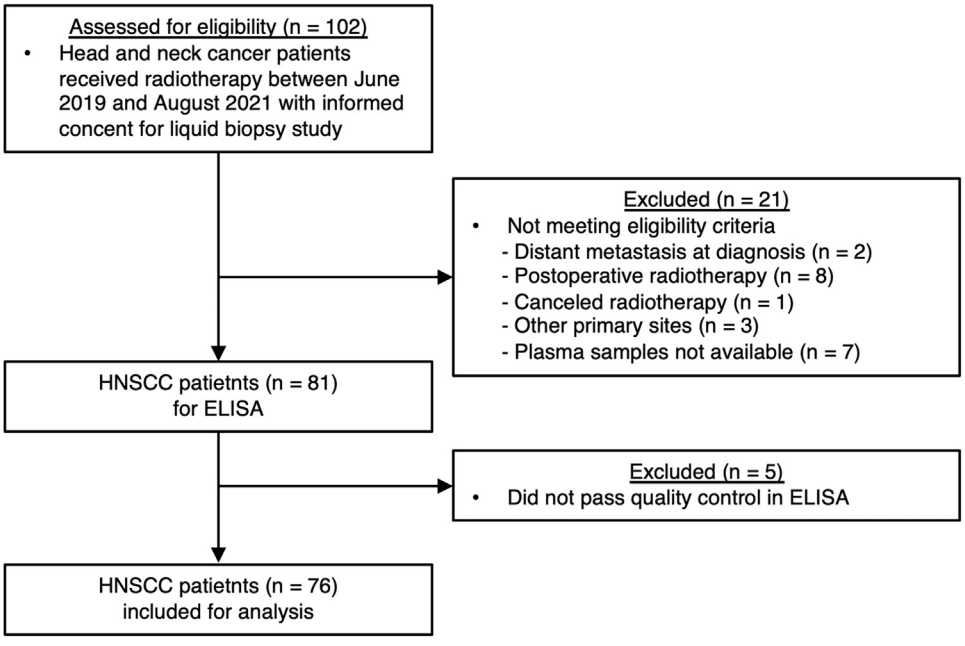

Figure 1 CONSORT diagram for this study. The flow diagram represents the number of patients with head and neck cancer eligible for this study and those excluded.

Table 1 Patient characteristics

Characteristic	Patients, No. (%)
Sex	
Male	67 (88.2)
Female	9 (11.8)
Age, median, y	69.5 (37-85)
PS score	
0	63 (82.9)
1	11 (14.5)
2-3	2 (2.6)
Primary site	
Nasal cavity, accessory sinus	12 (15.8)
Oropharynx	24 (31.6)
Hypopharynx	22 (28.9)
Larynx	18 (23.7)
Stage (UICC 8th)	
I	16 (21.1)
II	20 (26.3)
III	8 (10.5)
IV	32 (42.1)
p16	
Positive	17 (22.4)
Negative	42 (55.2)
NA	17 (22.4)
Smoking status	
Smoker	65 (85.5)
Nonsmoker	11 (14.5)
Treatment	
Radiation therapy alone	30 (39.5)
Chemoradiotherapy	46 (60.5)
Total dose	
70 Gy / 35 Fx	69 (90.8)
66 Gy / 33 Fx	6 (7.9)
60 Gy / 30 Fx	1 (1.3)

Abbreviations: Fx = fractions; NA = not assessed; PS = performance status; UICC = Union for International Cancer Control.

L1 for disease progression (0.672 vs 0.572) and for local progression (0.669 vs 0.536), although neither difference was statistically significant ($P = .318$ and $P = .236$ respectively) (Fig. 3A, B). Based on ROC analysis, the cutoffs for OS were determined to be 171 pg/mL for total PD-L1 and 23 pg/mL for exosomal PD-L1; for PFS and LC they were 173 pg/mL for total PD-L1 and 15 pg/mL for exosomal PD-L1.

Analysis of prognostic factors for OS, PFS, and LC

Univariate analysis identified primary site ($P < .001$); Union for International Cancer Control (UICC), 8th edition, stage ($P = .005$); and total PD-L1 in plasma ($P = .038$) as significant prognostic factors for OS. However, none of these factors remained significant in multivariate analysis for OS (Table 2). For PFS, UICC stage ($P = .045$), p16 status ($P = .002$), total PD-L1 in plasma ($P = .012$), and exosomal PD-L1 in plasma ($P < .001$) were significant prognostic factors in univariate analysis. In multivariate analysis, only exosomal PD-L1 in plasma ($P = .007$) remained a significant prognostic factor for PFS. For LC, p16 status ($P = .002$), total PD-L1 in plasma ($P = .005$), and exosomal PD-L1 in plasma ($P < .001$) were significant prognostic factors in univariate analysis. In multivariate analysis, only exosomal PD-L1 in plasma ($P = .029$) remained a significant prognostic factor for LC (Table 3).

Prognostic effect of exosomal PD-L1 in plasma on PFS and LC

Patients with high concentrations of exosomal PD-L1 in plasma had poor PFS and LC compared with those with low concentrations. One-year PFS was 79.2% versus 33.3% ($P < .001$) and 1-year LC was 87.3% versus 50.0% ($P < .001$) in high exosomal PD-L1 and low exosomal PD-L1, respectively (Fig. 4A, B).

Discussion

Although biologic examination of tumor tissue is essential for treatment selection and prognostic prediction, there are several issues with tissue-based biopsy. These problems include challenges in obtaining tumor tissue, challenges in repeating tissue acquisition, invasive nature, and tumor heterogeneity. Nevertheless, liquid biopsy, which overcomes tumor heterogeneity, is simple, repeatable, and less invasive and uses blood, urine, saliva, and other biofluids.[8]

Our study found pretreatment exosomal PD-L1 in plasma as a new prognostic factor for PFS and LC in patients with head and neck cancer. Total PD-L1 concentrations were higher than exosomal PD-L1 concentrations because total PD-L1 in plasma reflects soluble PD-L1, exosomal PD-L1, and microvesiclar PD-L1. Exosomal PD-L1 was interestingly more closely related with outcomes than was total PD-L1. Moreover, given that the concentrations of exosomal PD-L1 in plasma have no considerable differences in cancer stages, exosomal PD-L1 may be released not only from cancer cells but also from

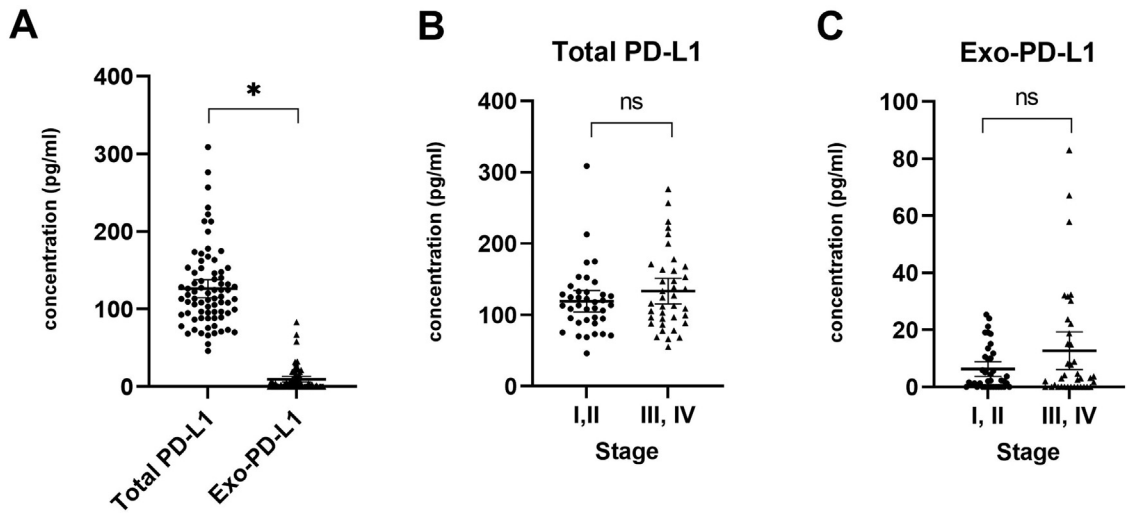

Figure 2 Total and exosomal PD-L1 in plasma of patients with head and neck cancer. (A) Plot of total and exosomal PD-L1 in plasma and (B, C) comparisons of total and exosomal PD-L1 in plasma according to cancer stage. Means and 95% confidence intervals are indicated. *Abbreviation:* ns = not significant. *$P < .001$.

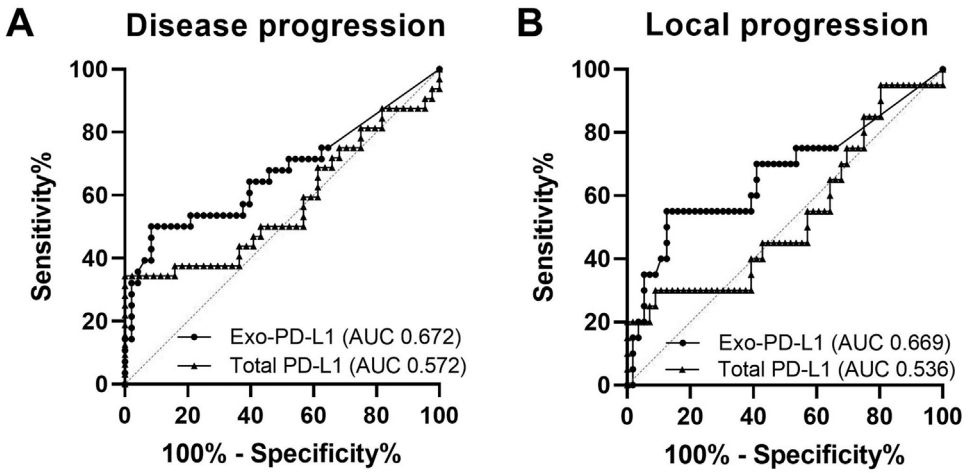

Figure 3 ROC analysis for disease progression after radiation therapy with PD-L1 in plasma. ROC curves of total and exosomal PD-L1 in plasma for (A) disease progression and (B) local progression. The AUCs are indicated. *Abbreviations:* AUC = area under the curve; ROC = receiver operating characteristic.

normal cells, because PD-L1 is represented in hematopoietic cells, heart and skeletal muscle, and the placenta, lung, kidney, and liver.[9] Currently, there is disagreement about the correlation between PD-L1 expression in tumor tissue immunohistochemistry and exosomal PD-L1 in blood. For example, there are no prior reports for head and neck cancer, to our knowledge, but there is a correlation for pancreatic cancer[10] and no correlation for lung cancer.[11] Additional studies are needed to clarify the mechanisms of exosomal PD-L1 production.

Theodoraki et al conducted a study using plasma-derived exosomes from 40 patients with HNSCC and revealed that levels of PD-L1 in exosomes, not total PD-L1 levels, related to disease activity, stage, and lymph node metastases.[12] Because their study did not show the timing of sample collection, treatment details, patient survival, and exosome isolation techniques that were different from ours, we cannot simply compare the results. However, their results together with our data suggest that exosomal PD-L1, not total PD-L1 in plasma, plays a role in HNSCC.

The importance of exosomal PD-L1 in cancer treatment has been studied. Chen et al showed that high plasma exosomal PD-L1 enhances tumor progression in mice and predicted the treatment efficacy of immune checkpoint inhibitors among patients with melanoma.[13] Fan et al retrospectively assessed the prognostic value of exosomal PD-L1 and soluble PD-L1 in plasma of patients with gastric cancer.[14] They demonstrated that OS was significantly lower in the high exosomal PD-L1 group

Table 2 Univariate log-rank and multivariate Cox proportional hazards models for overall survival

Factor	Patients, No.	OS univariat *P* value	OS multivariate	
			HR (95% CI)	*P* value
Age, y				
≥70	38	.820	-	-
<70	38		-	
PS score				
0-1	74	.051	-	-
2-3	2		-	
Sex				
Male	67	.216	-	-
Female	9		-	
Primary site				
NC, AS	12	<.001	Reference	.075
Oropharynx	24		0.375 (0.085-1.646)	
Hypopharynx	22		0.062 (0.006-0.598)	
Larynx	18		0.101 (0.010-0.945)	
Stage, UICC, 8th ed				
I, II	36	.005	Reference	.091
III, IV	40		6.454 (0.741-56.170)	
p16				
Negative	42	.402	-	-
Positive	17		-	
NA	17		-	
Smoking status				
Smoker	65	.676	-	-
Nonsmoker	11		-	
Treatment				
RT	30	.112	-	-
CRT	46		-	
Total PD-L1 in plasma				
Low, <171 pg/mL	64	.038	2.79 (0.762-10.193)	.121
High, ≥171 pg/mL	12			
Exo−PD-L1 in plasma				
Low, <23 pg/mL	66	.115	-	-
High, ≥23 pg/mL	10		-	

Abbreviations: CI = confidence interval; HR = hazard ratio; NA = not assessed; NC, AS = nasal cavity and accessory sinus; OS = overall survival; PD-L1 = programmed death ligand 1; exo−PD-L1 = exosomal programmed death ligand 1; PS = performance status; RT = radiation therapy; UICC, Union for International Cancer Control.

compared with the low exosomal PD-L1 group. Interestingly, soluble PD-L1 revealed no correlation with OS. Therefore, exosomal PD-L1, not total PD-L1 in plasma, could correlate with patient outcomes. Poggio et al conducted a unique experiment to support this idea.[15] When Rab27a, a protein essential for exosome production, was knocked out from MC38 and TrampC2 and transplanted into mice, circulating exosomal PD-L1 was reduced and tumor growth was delayed. Intravenous administration of exosomal PD-L1 to these mice promoted tumor growth, showing that exosomal PD-L1 is crucial for tumor growth.

Table 3 Univariate log-rank and multivariate Cox proportional hazards models for progression-free survival and local control

Factor	Patients, No.	Univariate PFS *P* value	Multivariate PFS		Univariate LC *P* value	Multivariate LC	
			HR (95% CI)	*P* value		HR (95% CI)	*P* value
Age, y							
≥70	38	.990	-	-	1.000	-	-
<70	38		-			-	
PS							
0-1	74	.380	-	-	.467	-	-
2-3	2		-			-	
Gender							
Male	67	.757	-	-	.631	-	-
Female	9		-			-	
Primary site							
NC, AS	12	.131	-	-	.867	-	-
Oropharynx	24		-			-	
Hypopharynx	22		-			-	
Larynx	18		-			-	
Stage, UICC, 8th ed							
I, II	36	.045	Reference	.175	.154	-	-
III, IV	40		1.832 (0.764-4.392)			-	
p16							
Negative	42	.002	Reference	.475	.034	Reference	.452
Positive	17		0.647 (0.205-2.045)			0.615 (0.173-2.184)	
NA	17		0.131 (0.017-1.018)			0.175 (0.022-1.398)	
Smoking status							
Smoker	65	.476	-	-	.359	-	-
Nonsmoker	11		-			-	
Treatment							
RT	30	.117	-	-	.091	-	-
CRT	46		-			-	
Total PD-L1 in plasma							
Low, <173 pg/mL	65	.012	Reference	.144	.005	-	.139
High, ≥173 pg/mL	11		2.142 (0.771-5.953)			-	
Exo−PD-L1 in plasma							
Low, <15 pg/mL	58	<.001	Reference	.007	<.001	Reference	.029
High, ≥15 pg/mL	18		3.238 (1.378-7.611)			3.165 (1.127-8.885)	

Abbreviations: CI = confidence interval; Exo−PD-L1 = exosomal programmed death ligand 1; HR = hazard ratio; LC = local control; NA = not assessed; NC, AS = nasal cavity and accessory sinus; PD-L1 = programmed death ligand; PFS = progression-free survival; PS = performance status; 1.

Because exosomal PD-L1 reduces immunity against tumors in patients with HNSCC after radiation therapy, targeting PD-L1 agents such as atezolizumab, durvalumab, and avelumab might be the treatment approach for patients with high exosomal PD-L1 in plasma. In contrast, avelumab, an anti−PD-L1 antibody, with chemoradiation therapy for locally developed head and neck cancer demonstrated no improvement compared with standard

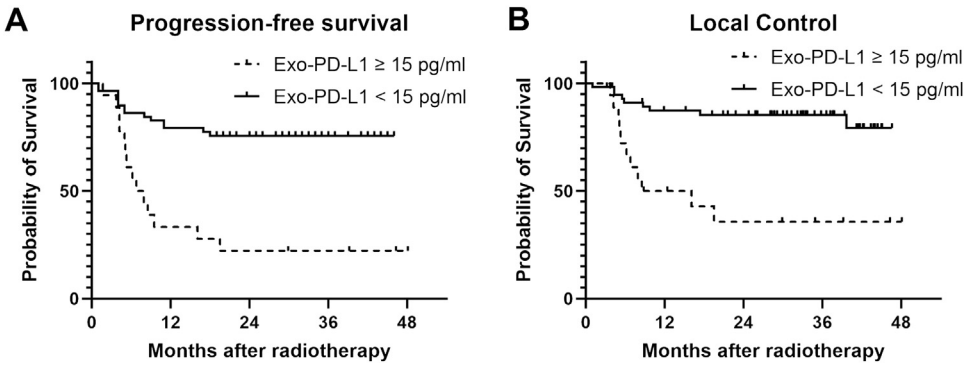

Figure 4 Pretreatment levels of circulating exosomal PD-L1 in plasma was a prognostic factor after radiation therapy in patients with head and neck cancer. (A, B) Progression-free survival and local control curves compared by pretreatment circulating exosomal PD-L1 (Exo−PD-L1) levels. The associated *P* values are shown. *Abbreviation:* PD-L1 = programmed death-ligand 1.

chemoradiation therapy.[16] Considering our result that high exosomal PD-L1 was negatively related with outcomes after radiation therapy, combination therapy of radiation therapy and anti−PD-L1 antibodies might be efficient in the high exosomal PD-L1 group. Further studies are required.

This study has several limitations. First, the best approach of exosome collection has not been developed. Several methods were known to obtain exosomes from plasma, such as differential ultracentrifugation, ultrafiltration, precipitating agents such as polyethylene glycol, immunoaffinity capture, microfluidics, and size-exclusion chromatography.[17] In this study, we used precipitating agents to obtain exosomes, as explained previously.[13] Second, we calculated the cutoff value after identifying PD-L1 concentrations in our patients. Because few studies have assessed PD-L1 levels in the blood and the assay methods are not identical, the cutoff values were established based on the measurements in this study. Third, this study did not analyze fluctuations in exosomal PD-L1 levels during and after radiation therapy, and their effect on outcomes was not examined in this study; therefore, it requires further investigation. Despite these drawbacks, our study examined the prognostic factors for radiation therapy in patients with HNSCC, including clinical parameters, and demonstrated that the pretreatment level of circulating exosomal PD-L1 in plasma was an independent prognostic factor for PFS and LC.

Conclusions

We found that a high concentration of circulating exosomal PD-L1 in plasma before radiation therapy was negatively associated with outcomes among patients with HNSCC and proposed 1 of the liquid biopsy methods for plasma biomarkers in head and neck cancer. Further prospective studies with a larger population are needed to validate the results of this study.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Fitzmaurice C, Abate D, Abbasi N, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. *JAMA Oncol*. 2019;5:1749.
2. Chow LQM. Head and neck cancer. *N Engl J Med*. 2020;382:60-72.
3. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. *Immunity*. 2018;48:434-452.
4. Ferris RL, Blumenschein G, Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2016;375:1856-1867.
5. Burtness B, Harrington KJ, Greil R, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): A randomised, open-label, phase 3 study. *Lancet*. 2019;394:1915-1928.
6. Daassi D, Mahoney KM, Freeman GJ. The importance of exosomal PDL1 in tumour immune evasion. *Nat Rev Immunol*. 2020;20:209-215.

7. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228-247.
8. Yu W, Hurley J, Roberts D, et al. Exosome-based liquid biopsies in cancer: Opportunities and challenges. *Ann Oncol*. 2021;32:466-477.
9. Dai S, Jia R, Zhang X, Fang Q, Huang L. The PD-1/PD-Ls pathway and autoimmune diseases. *Cell Immunol*. 2014;290:72-79.
10. Park SJ, Park JY, Shin K, et al. Clinical significance of serum-derived exosomal PD-L1 expression in patients with advanced pancreatic cancer. *BMC Cancer*. 2023;23:389.
11. Li C, Li C, Zhi C, et al. Clinical significance of PD-L1 expression in serum-derived exosomes in NSCLC patients. *J Transl Med*. 2019;17:355.
12. Theodoraki MN, Yerneni SS, Hoffmann TK, Gooding WE, Whiteside TL. Clinical significance of PD-L1+ exosomes in plasma of head and neck cancer patients. *Clin Cancer Res*. 2018;24:896-905.
13. Chen G, Huang AC, Zhang W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature*. 2018;560:382-386.
14. Fan Y, Che X, Qu J, et al. Exosomal PD-L1 retains immunosuppressive activity and is associated with gastric cancer prognosis. *Ann Surg Oncol*. 2019;26:3745-3755.
15. Poggio M, Hu T, Pai CC, et al. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. *Cell*. 2019;177. 414-427.e13.
16. Lee NY, Ferris RL, Psyrri A, et al. Avelumab plus standard-of-care chemoradiotherapy versus chemoradiotherapy alone in patients with locally advanced squamous cell carcinoma of the head and neck: A randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. *Lancet Oncol*. 2021;22:450-462.
17. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: Is size exclusion chromatography the best option? *Int J Mol Sci*. 2020;21:6466.