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Original Contribution

PD-L1 expression by immunohistochemistry in salivary duct carcinoma

Ameer Hamza*, Dianna Roberts, Shirley Su, Randal S. Weber, Diana Bell*, Renata Ferrarotto

The University of Texas MD Anderson Cancer Center, Houston, TX, USA

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ABSTRACT

Background: Immune checkpoint inhibitors play an increasing role in oncologic care. PD-L1 expression is associated with survival and predicts response to PD-1 or PD-L1 inhibitors in a variety of tumors. Our aim is to evaluate the frequency and prognostic significance of PD-L1 expression in salivary duct carcinoma.

Design: We retrospectively evaluated the expression of PD-L1 by two different antibodies (PD-L1 28–8 and PD-L1 22C3) in salivary duct carcinomas. PD-L1 expression in at least 1% of tumor cells was considered immunoreactive. Kaplan-Meier analysis was performed to determine the impact of PD-L1 expression on survival; differences between survival curves were assessed by the chi-square test, and pairwise comparisons of factors were assessed with the log-rank test.

Results: A total of 113 patients' specimens were evaluated. Seventy-six (76%) of the patients were male. Mean age at time of presentation was 61.2 (SD = 12.4) years. PD-L1 expression was found in 26% of the samples. Median follow-up time was 36.6 months (range = 1.4–249 months). Overall survival at 3, 5 and 10 years were 52.6%, 37.9% and 25.6%, respectively. There was no statistical difference in survival between patients with PD-L1-immunoreactive tumors and those without, regardless of which antibody was used (chi^2 result for all plots: $p = 0.53$; log rank test for pairwise comparison: $p > 0.256$).

Conclusion: In our analysis, PD-L1 expression occurred in a small proportion of salivary duct carcinomas, usually at low levels, and did not correlate with survival. Its predictive value and utility in selecting patients with salivary duct carcinoma who might benefit from PD-1/PD-L1 inhibitors warrants further investigation.

1. Introduction

Salivary duct carcinoma (SDC) is an aggressive tumor with extremely poor prognosis and 5-year survival of < 50% [1-3]. Surgical resection is the mainstay of treatment. Radiation and/or chemotherapy are considered in the adjuvant setting. A high proportion of SDC express human epidermal growth factor receptor 2 (HER2); however, the role of HER2 targeted therapy in the adjuvant setting remains undetermined [4,5]. The lack of effective therapy warrants exploration of newer adjuvant therapies such as immunotherapy.

Programmed death-ligand 1 (PD-L1) expression by tumor cells is a mechanism for evading antitumor T-cell responses and is an immunotherapy target in a variety of tumors [6]. Clinical response to immunotherapy targeting PD-1 or PD-L1 has been demonstrated in multiple types of tumors, including melanoma, lung, head and neck, breast, gastric, pancreatic and renal tumors [7-10]. From a pathologic standpoint the PD-L1 expression as detected by immunohistochemistry is being used as a surrogate marker for eligibility for anti-PD-1/PD-L1 immunotherapy in some tumor types. PD-1 antibodies have been approved for treatment of head and neck squamous cell carcinoma [11,12] but the role of PD-L1 expression and immunotherapy in non-squamous head and neck tumors, including salivary gland malignancies, has not been completely elucidated. High PD-L1 expression has been observed in aggressive salivary gland tumors such as SDC and squamous cell carcinoma and is associated with decreased disease-free survival [13]. However, previous studies examining PD-L1 expression in SDC involved small cohorts, therefore, we attempted to evaluate the frequency of PD-L1 expression in a large cohort of salivary duct carcinoma patients with available outcome data.

2. Methods

Following approval by the institutional review board, a retrospective review of the pathology database from 1983 to 2011 was performed to identify SDC cases. One hundred and thirteen consecutive patients were identified. Tissue microarrays (TMAs) were used for immunohistochemical staining. Controls were run concurrently, including Dako positive and negative cell line controls and an in-house tonsil control, serving as a positive tissue control. We evaluated the expression of PD-L1 by two different antibody clones (28–8 and 22C3; Dako,

* Corresponding authors at: University of Texas, MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA.
E-mail addresses: ahamza@mdanderson.org (A. Hamza), diana.bell@mdanderson.org (D. Bell).

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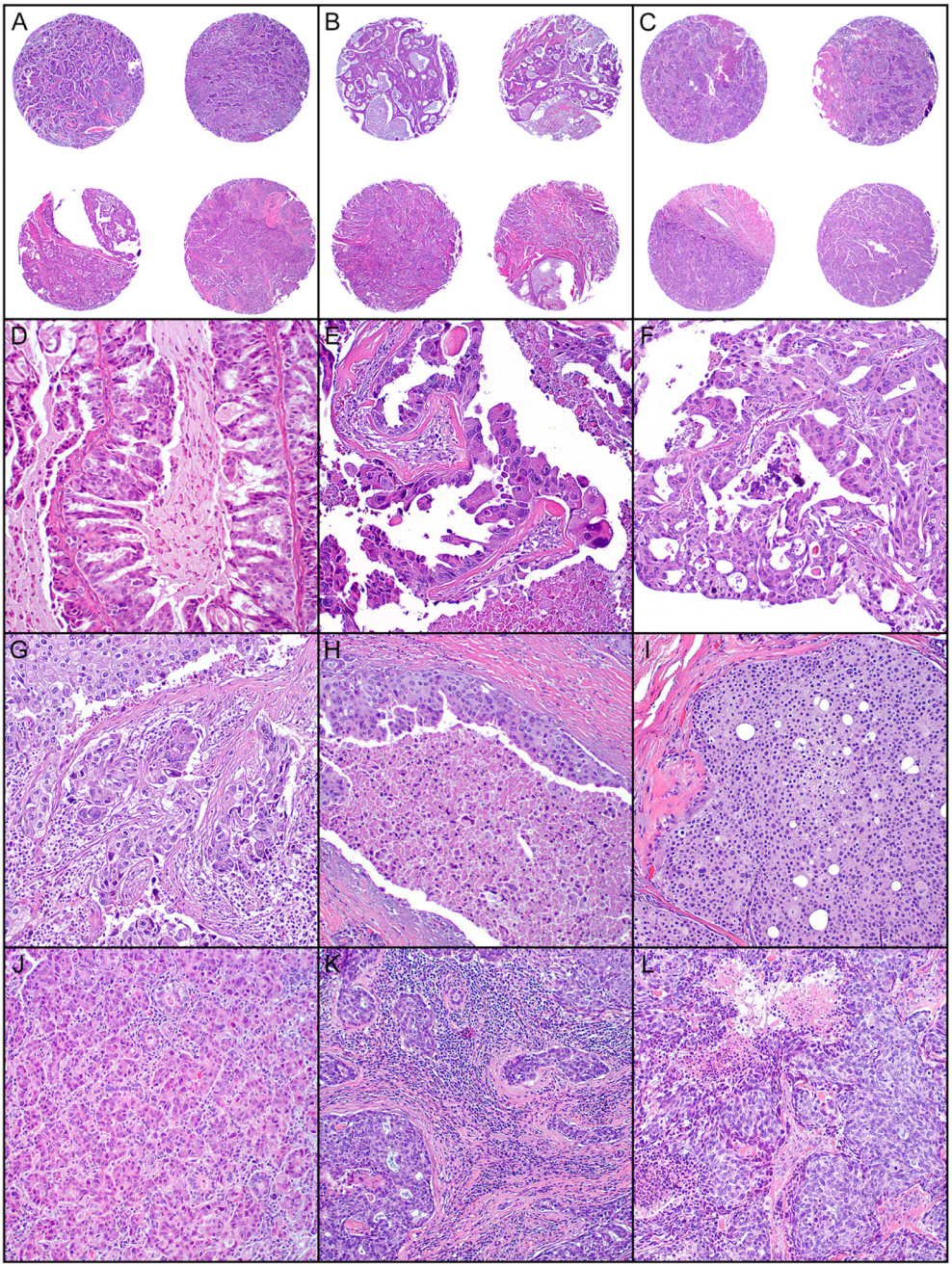

Fig. 1. Morphology and variants of salivary duct carcinoma. A, B, C– Tissue microarrays (H&E, 2×). D, E, F Apocrine type (H&E, 10×). G, H– Prominent comedonecrosis (H&E, 10×). I- Histiocytoid variant (H&E, 10×). *J*- Plasmacytoid oncocytic variant (H&E, 10×), K, L- Basaloid variant (H&E, 10×).

Agilent Technologies, Santa Clara, CA, USA). Positivity was defined as immunoreactivity of ≥1% of the tumor cells. PD-L1 expression was evaluated as the percentage of tumor cells with partial or complete membranous staining with or without cytoplasmic staining. Staining was assessed by a board-certified surgical pathology fellow and an experienced head and neck pathologist.

Kaplan-Meier analysis was performed to determine the impact of PD-L1 expression on survival. Differences between all survival curves were assessed by the chi-square test, and pairwise comparisons of factors were assessed with the log-rank test. Overall survival was defined as the time interval between date of surgery and date of death or last follow up. P values < 0.05 were considered statistically significant.

3. Results

A total of 113 patients were identified and their surgical specimens were evaluated. Seventy-six (76%) of the patients were male. The mean age at the time of presentation was 61.2 (SD: 12.4) years.

Morphologically the tumors resembled high-grade ductal carcinoma of the breast with cribriform architecture and comedonecrosis. Cytologically most cases were apocrine characterized by oncocytic cells with abundant cytoplasm and large nuclei with coarse chromatin and prominent nucleoli. Additionally, some cases showed varying proportions of sarcomatoid, micropapillary, mucin rich and histiocytoid components. For basaloid SDC (basal-like phenotype) comedonecrosis was present, while other mimickers were excluded (high-grade adenoid cystic carcinoma, solid or with high-grade transformation). Various morphologies are shown in Fig. 1. Histologically all cases were high-

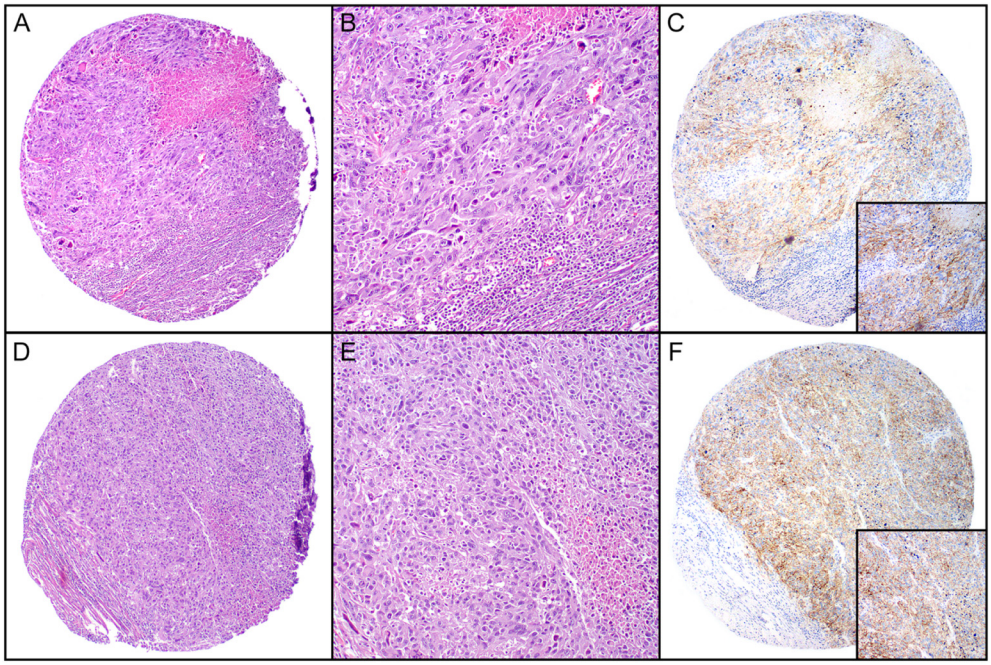

Fig. 2. Salivary duct carcinoma PD-L1 expression (PD-L1 22C3 clone, 100×).

grade and most were high-stage.

PD-L1 expression in at least 1% of tumor cells was found in 26% of the specimens. The degree of expression varied from weak expression in barely 1% of tumor cells to strong expression in 100% of tumor cells (Fig. 2). Considering the PD-L1 antibody clones separately, 25 specimens showed staining with clone 28–8, while only 15 showed staining with clone 22C3; 11 specimens showed staining with both clones.

The median follow-up time was 36.6 months (range: 1.4–249). Overall survival at 3, 5 and 10 years were 52.6%, 37.9% and 25.6%, respectively. There was no statistically significant difference in survival between patients with PD-L1-immunoreactive tumors and those with non-immunoreactive tumors, regardless of which antibody was used (chi^2 result for all plots: $p = 0.53$; log rank test for pairwise comparison: $p > 0.256$). (Fig. 3).

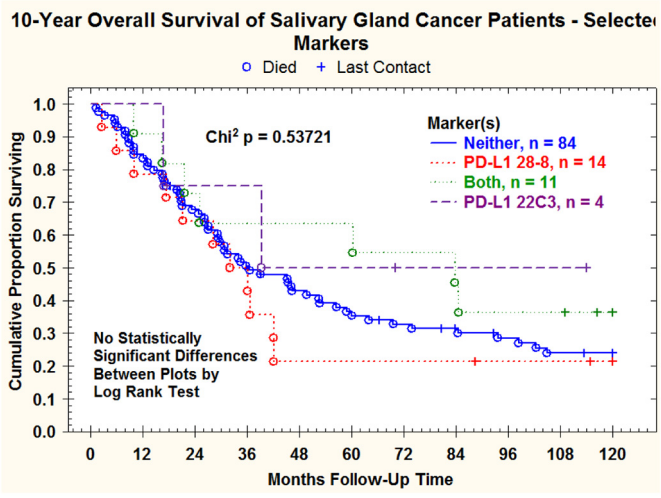

Fig. 3. Kaplan-Meier curves for overall survival by PD-L1 expression in patients with salivary duct carcinoma.

4. Discussion

Salivary duct carcinoma is an aggressive salivary gland tumor that resembles high-grade ductal carcinoma of the breast. It accounts for upto 10% of all salivary gland malignancies, has a predilection for elderly males and most commonly arises in the parotid gland [5]. It has variable histologic manifestations including the, oncocytoid, sarcomatoid, mucin-rich and micropapillary, in addition to the typical apocrine morphology [5,14-17].

The therapeutic and prognostic role of PD-L1 expression in head and neck squamous cell carcinoma is well established [11,12]; however, its role in other head and neck tumors, including salivary gland tumors, has not been studied thoroughly. In a recent study of recurrent or metastatic salivary gland carcinomas with PD-L1 expression in ≥1% of tumor or stromal cells, pembrolizumab demonstrated some anti-tumor activity, with a confirmed objective response rate of 12% (95% confidence interval, 2%–30%), after a median follow-up of 20 months [18]. With respect to SDC only, a recent study of 18 patients found that high PD-L1 expression (defined in the study by positivity in > 10% of tumor cells) was strongly associated with unfavorable prognosis and shorter overall survival (log-rank test: $p = 0.0045$) [19]. In a study of 219 salivary gland tumors, which included 31 cases of SDC, PD-L1 expression was associated with reduced 5 year disease free survival as well as overall survival ($p \leq 0.001$) [13]. In our cohort of 113 patients, PD-L1 expression occurred in only 26% of SDCs, usually at low levels and did not correlate with survival. Table 1 compares our study with two other studies describing relation of PD-L1 expression and survival in SDC.

There are a few differences between our study and the two previously mentioned studies [13,19]. Firstly, we did not stratify the PD-L1 expression into high and low; and simply considered immunoreactivity of ≥1% as positive. Secondly, we found PD-L1 immunoreactivity in just over a quarter (26%) of cases, as compared to nearly 50% immunoreactivity in the other studies. The potential reasons for this difference are manifold. First, there are at least six PD-L1 antibody clones, including 22C3, 28–8, and 73–10 (from Dako, Agilent Technologies, Santa Clara, CA, USA); SP263 and SP142 (from Ventana Medical Systems, Tucson, AZ, USA); and E1L3N (from Cell Signaling Technology, Danvers, MA) [17]. We used the 22C3 and 28-8clones in our study; Mukaigawa et al. [13] and Sato et al. [15] both used clone E1L3N. We

Table 1
Summary of literature on relation between PD-L1 expression and survival in SDC.

Study	Number of patients	Tissue section type	PD-L1 Cut off	Survival data
Sato et al. [19]	18	Whole tissue section	10%	Shorter OS ($p = 0.0045$)
Mukaigawa et al. [13]	31	Tissue microarray	1%	Shorter 5 year DFS ($p < 0.001$)
Current	113	Tissue microarray	1%	No difference in 10 year OS ($p > 0.256$)

and Mukaigawa et al. [13] used tissue microarrays, while whole tissue sections were used by Sato et al. [19]. Another factor that may have contributed to the low level of PD-L1 positivity in our study was the use of TMAs rather than whole tissue sections; however, Mukaigawa et al. [13] also used TMAs and about 50% of their cases demonstrated staining. Aside from technical factors, another possible explanation for the different positivity rates in various studies is interobserver variability. A recent study by Wang et al. [20] demonstrated that in hypopharyngeal squamous cell carcinoma, interobserver agreement in the evaluation of PD-L1 expression using clones SP263 and SP142 was only moderate ($\kappa = 0.469$, 95% CI 0.251–0.687; $p < 0.001$ and $\kappa = 0.591$, 95% CI 0.373–0.809; $p < 0.001$; respectively). They found overall inter-observer agreement to be 80% for SP263 and 88% for SP142.

Other pertinent biomarkers with respect to SDC include androgen receptor (AR) and Her-2/neu. Multiple studies in literature have addressed this aspect [21-23]. Since the sole object of our study was to investigate the PD-L1 expression in SDC, we did not analyze the expression of AR and Her-2/neu.

In conclusion, there is limited data in the literature with respect to PD-L1 expression and its association with prognosis in SDC. This is further limited by only moderate reproducibility of PD-L1 immunohistochemistry interpretation. In our experience, PD-L1 expression did not correlate with overall survival when using two different PD-L1 clones (28-8 and 22C3). Its predictive value and utility in selecting SDC patients who might benefit from PD-1/PD-L1 inhibitors warrants further investigation. Exploration of other pathways and checkpoint inhibitors, such as CTLA-4 and LAG-3, may also be helpful.

Conflict of interest

All authors declare no conflict of interest.

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