ORIGINAL RESEARCH

ASSESSMENT OF TUMOUR INFILTRATING LYMPHOCYTES AND PD-L1 EXPRESSION IN ADENOID CYSTIC CARCINOMA OF THE SALIVARY GLAND

VOL 44 NO 1 MARCH 2021

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Submitted 16th July 2020, Accepted 6th December 2020

Clin Invest Med 2021;44(1):E38-41; DOI: 10.25011/cim.v44i1.35218

ABSTRACT

Purpose: Early phase clinical studies are ongoing to evaluate the role of immune checkpoint inhibitors in adenoid cystic carcinoma (ACC) despite a paucity of information on the immune microenvironment. This study aims to better characterize the immune microenvironment of ACC tumours and evaluate survival outcomes based on tumour infiltrating lymphocyte (TIL) and programmed death-ligand 1 (PD-L1) expression.

Methods: Patient characteristics, treatment and outcome data were collected for 24 ACC patients. The CD8+ (cluster of differentiation 8) TIL and PD-L1 expression were quantified by immunohistochemistry. Marker expression and survival outcomes were evaluated by Kaplan-Meier analysis.

Results: All cases were negative for PD-L1 expression; four cases had focal high, eight cases had focal moderate and 12 cases had low TIL expression. Based on TIL expression, there was no difference in disease-free or overall survival.

Conclusion: Adenoid cystic carcinoma tumours were found to be associated with a poor immunogenic microenvironment, with absent PD-L1 expression and low CD8+ TILs. There was no association between TIL expression and survival. These data suggest that PD-L1 and TIL expression are unlikely to be useful as predictive biomarkers for response to immunotherapy.

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INTRODUCTION

Immune evasion is an important feature of cancer pathogenesis [1] and the inhibition of pathways involved in immune evasion with the use of immune checkpoint inhibitors has become an exciting area of cancer therapy [2]. Various factors, such as programmed death-ligand 1 (PD-L1) expression, tumour mutation burden and tumour infiltrating lymphocytes (TIL), likely contribute to the response to immune checkpoint inhibitors. It has been suggested that PD-L1 expression by tumour/immune cells and the presence of CD8+ (cluster of differentiation 8) TILs might be associated with a favourable response to anti-PD1 immunotherapy [3, 4]. Early data from clinical trials evaluating pembrolizumab in advanced salivary

gland carcinoma showed promising anti-tumour activity in PD-L1-positive cases [5].

Adenoid cystic carcinoma (ACC) is a high-grade malignant tumour with a dismal prognosis and minimal response to traditional treatment. Early phase clinical trials are ongoing to evaluate the efficacy of immune checkpoint inhibitors in the treatment of ACC (NCT03087019, NCT03172624 and NCT03146650); however, there is a paucity of information regarding the immune microenvironment of ACC tumours. Vital et al. studied the expression of PD-L1 in 167 salivary gland carcinoma cases, with 28 cases (16%) showing PD-L1 expression in tumour cells (three cases were adenoid cystic carcinoma) [6]. Their study also showed that PD-L1 positivity

was associated with more TILs. Sridharan et al. studied 28 cases of primary and metastatic ACC of the salivary gland and showed low tumour expression of PD-L1 in tumour cells but a relatively higher programmed death-ligand 2 (PD-L2) expression [7]. A study by Mosconi et al. showed low intra-tumoural CD8+ TILs and absent PD-L1 expression in ACC tumours [8]. Similarly, a recent study by Wolkow et al. showed low PD-L1 and PD-L2 expression, as well as sparse tumour infiltrating lymphocytes, in 23 cases of adenoid cystic carcinoma of the orbit [9]. In the current study, we aimed to further characterize the immune microenvironment of ACC tumours and evaluate survival outcomes based on TIL and PD-L1 expression.

METHODS

A retrospective analysis of 24 ACC patients diagnosed at the Tom Baker Cancer Centre (TBCC) in Calgary, Canada between 2010 and 2018 was undertaken. Patient characteristics, treatment and clinical outcome data were collected. The pathologic diagnosis of ACC was confirmed by two independent reviewers. The CD8+ TILs were quantified by immunohistochemistry utilizing a CD8+ antibody (RTU Dako clone C8/144B in high TRS 20-X-20 on Dako Omnis) and classified as low (<10/HPF) (HPF; high power field), moderate (10–50/HPF) and high (>50/

HPF). Immunohistochemistry for PD-L1 was performed using the Dako pharmaDx 22C3 PD-L1 antibody. The PD-L1 staining was considered positive if $\geq 1\%$ of membranous staining in either tumour cells or immune cells and was graded as negative (<1%), weak (1–10%), moderate (10–50%) and strong (>50%). The TIL expression and survival outcomes were analyzed by Kaplan-Meier curves.

RESULTS

Patient characteristics

Twenty-four patients with ACC were identified. Baseline patient characteristics are summarized in Table 1. Mean age at diagnosis was 58 years, with 50% females, 83.3% node-negative and 12.5% of patients with metastatic disease (M1). Seventeen patients (70.8%) received multimodality treatment with surgery and radiation. Ten patients (41.7%) recurred, with 20.8% (five patients) recurring distantly and 16.7% (four patients) with both local and distant recurrence.

TIL and PD-L1 expression and survival outcomes

All 24 cases had absent staining for PD-L1 in either tumour cells or immune cells (Figure 1). The positive controls (tonsil) had patchy positivity—using the same antibody and same immunohistochemistry assay. Four

FIGURE 1.
QUANTIFICATION OF PD-L1 EXPRESSION AND TUMOUR INFILTRATING LYMPHOCYTES (CD8 POSITIVITY: LOW <10/HPF, MODERATE 10–50/HPF AND HIGH >50/HPF).

TABLE 1.
BASELINE PATIENT CHARACTERISTICS IN THE TOTAL POPULATION AND BY TUMOUR INFILTRATING LYMPHOCYTE EXPRESSION.

Patient characteristics		Total population n= 24 (%)	<10 HPF n= 12 (%)	10–50 HPF n= 8 (%)	>50 HPF n= 4 (%)
Age at diagnosis in years, mean (SD)		58.3 (13.3)	60.8 (13.0)	56.9 (14.5)	53.7 (13.8)
Overall survival in years, mean (SD)		5.0 (2.6)	5.1 (2.6)	4.6 (2.4)	5.7 (3.2)
Disease free survival in years, mean (SD)		4.1 (2.7)	4.6 (2.9)	4.1 (2.7)	2.5 (2.2)
Gender	Female	12 (50.0)	7 (58.3)	3 (37.5)	2 (50.0)
	Male	12 (50.0)	5 (41.7)	5 (62.5)	2 (50.0)
Tumour site (n=24)	Parotid gland	14 (58.3)			
	Submandibular gland	2 (8.3)			
	Nasal cavity	1 (4.2)			
	Nasopharynx	1 (4.2)			
	Oral cavity	5 (20.8)			
	Lung metastasis	1 (4.2)			
T stage	1	5 (20.8)	3 (25.0)	2 (25.0)	0 (0.0)
	2	7 (29.2)	4 (33.3)	1 (12.5)	2 (50.0)
	3	3 (12.5)	1 (8.3)	1 (12.5)	1 (25.0)
	4	9 (37.5)	4 (33.3)	4 (50.0)	1 (25.0)
N stage	0	20 (83.3)	9 (75.0)	7 (87.5)	4 (100.0)
	1	1 (4.2)	1 (8.3)	0 (0.0)	0 (0.0)
	2	3 (12.5)	2 (16.7)	1 (12.5)	0 (0.0)
M stage	0	21 (87.5)	11 (91.7)	6 (75.0)	4 (100.0)
	1	3 (12.5)	1 (8.3)	2 (25.0)	0 (0.0)
TNM stage (8th edition)	I	5 (20.8)	3 (25.0)	2 (25.0)	0 (0.0)
	II	7 (29.2)	4 (33.3)	1 (12.5)	2 (50.0)
	III	2 (8.3)	0 (0.0)	1 (12.5)	1 (25.0)
	IVa	6 (20.8)	4 (33.3)	1 (12.5)	1 (25.0)
	IVb	1 (4.2)	0 (0)	1 (12.5)	0 (0.0)
	IVc	3 (12.5)	1 (8.3)	2 (25.0)	0 (0.0)
Primary treatment	Surgery alone	2 (8.3)	1 (8.3)	1 (12.5)	0 (0.0)
	Surgery +radiation	17 (70.8)	10 (83.3)	4 (50.0)	3 (75.0)
	Radiation alone	4 (16.7)	0 (0.0)	3 (37.5)	1 (25.0)
	Declined treatment	1 (4.1)	1 (8.3)	0 (0.0)	0 (0.0)
Recurrence	Yes	10 (41.7)	3 (25.0)	4 (50.0)	3 (75.0)
	No	13 (54.2)	8 (66.7)	4 (50.0)	1 (25.0)
	N/A (M1 disease)	1 (4.2)	1 (8.3)	0 (0.0)	0 (0.0)
Site of recurrence	Local	1 (4.2)	0 (0.0)	0 (0.0)	1 (25.0)
	Distant	5 (20.8)	2 (16.7)	1 (12.5)	2 (50.0)
	Both	4 (16.7)	1 (8.3)	3 (37.5)	0 (0.0)
	Unknown	14 (58.3)	9 (75.0)	4 (50.0)	1 (25.0)

Abbreviations: HPF, high power field; M, presence of distant metastasis; N, lymph node involvement; T, primary tumour site; TNM, tumour, node, metastasis

cases (16.7%) had focal high CD8+ TILs (>50/HPF, Figure 1-bottom panel), eight cases (33.3%) had focal moderate TILs (10-50/HPF, Figure 1-middle panel) and 12 cases (50.0%) had low TILs (<10/HPF, Figure 1-top panel).

Mean disease free survival was 4.1 years and mean overall survival was 5.0 years. There was no difference in disease free survival or overall survival based on TIL expression (Table 1 and Figure 2).

FIGURE 2.
KAPLAN-MEIER SURVIVAL ESTIMATES: NO DIFFERENCE IN OVERALL SURVIVAL BASED ON TIL EXPRESSION.

DISCUSSION

Our data are consistent with previous studies that suggest that ACC tumours are associated with low immunogenicity and with minimal/absent PD-L1 expression in tumour cells [8-9]. However, in contrast to results published by Sridharan et al., we did not find PD-L1 expression in TILs, which may be attributed to the use of different antibody clones. We also found that the level of TIL expression in ACC tumours was not significantly associated with differences in survival outcomes. Our data suggest that PD-L1 and TIL expression are unlikely to be useful as predictive biomarkers for response to immunotherapy in ACC tumours.

A potential limitation of our study is the lack of quantification of PD-L2 expression and tumour mutation burden. The PD-L2, another ligand that can bind PD-1, has been shown to be a strong prognostic indicator for poor disease-free survival/progression-free survival in multiple solid cancers [10]. Studies by Sridharan et al. showed PD-L2 expression in tumour cells in 60% of the cases [7]. Recent studies by Nakano et al. showed that PD-L2 expression, rather than PD-L1 expression, reflected aggressive phenotypes in salivary gland carcinomas [11]. Future studies should be considered to evaluate PD-L2 and tumour mutation burden as potential biomarkers to help predict response to immunotherapy in ACC tumours [12].

FINANCIAL SUPPORT

This research is funded by “resident research grant” in the Department of Pathology, University of Calgary.

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