PROGRAMMED CELL DEATH LIGAND-1 EXPRESSION AS A PROGNOSTIC INDICATOR IN ORAL SQUAMOUS CELL CARCINOMA.

DR. SAMAR SAEED KHAN B.D.S, M.D.S, F.P.F.A ASSISTANT PROFESSOR, DEPARTMENT OF ORAL AND MAXILLOFACIAL SURGERY AND DIAGNOSTIC SCIENCES; DIVISION OF ORAL AND MAXILLOFACIAL PATHOLOGY

COLLEGE OF DENTISTRY, JAZAN UNIVERSITY, JAZAN, SAUDI ARABIA

Background of the Study

The programmed-death 1 receptor (PD-1)/programmed-death ligand 1 (PD-L1, also called B7-H1, and CD274) pathway regulates T-cell responses and the balance between T-cell activationand tolerance, and acts as an immunologic checkpoint (1). In normal tissues, PD-L1 is expressed on T cells, B cells, dendritic cells, macrophages, mesenchymal stem cells, and mast cells, as well as several hematopoietic cells (2, 3). PD-L1 is also expressed in multiple types of cancers (4), including head and neck squamous cell carcinomas (HNSCC) and, among them, in oral squamous cell carcinomas (OSCC; refs. 3, 5, 6). In the tumor microenvironment, PD-L1 expressed in tumor cells binds to the inhibitory receptor PD-1, a member of the B7 family of receptors (7), on activated T cells reaching the tumor [tumor-infiltrating lymphocytes (TIL)], thereby delivering an inhibitory signal to those T cells that ultimately prevents tumor elimination from the immune system (3, 8, 9).

Oral Squamous Cell Carcinoma (OSCC) is the most common type of cancer in the head and neck area. It belongs to the ten most common cancers, and approximately 300,000 new cases are diagosed annually worldwide (10). OSCC is associated with poor prognosis and higher morbidity when diagnosed at advanced stages (11), and it has been considered to be a very immunosuppressive cancer. Immunen tolerance is mediated by inhibitory signaling pathways called immune checkpoints. One of the most important immunologic checkpoints is the axis PD-1/PD -L1 that has been proposed as a potential mechanism facilitating the immunosuppressive phenotype in OSCC (5, 6). Consequently, at least from a theoreticalpoint of view, blockade of the PD-1/PD-L1 pathway could effectively reduce tumor growth and improve survival (6). Positive tumor PD-L1 expression determined by immunohistochemistry (IHC) is thought to be predictive of clinical response (12), and thus, the PD-1/PD-L1 checkpoint inhibitors, such as nivolumab and pembrolizumab have demonstrated clinical efficacy in the treatment of advanced HNSCC .

The expression of PD-L1 is up-regulated in many cancers, such as lung, ovary, colon, skin, brain, kidney, esophagus, stomach and breast cancers. Moreover, PD-L1 expression has been associated with poor prognosis in many malignancies, such as nasopharynx, kidney, esophagus, stomach, pancreas, breast and salivary gland carcinomas, as well as malignant melanoma.

Aim & Objectives of the Study

The purpose of the present study was to investigate the expression of PD-L1 in OSCC and to establish correlations of PD-L1 expression and clinicopathologic grades , and their impact on patients' s survival.

Materials & Methodology

GROUPS	SAMPLE SIZE
Well differentiated squamous cell carcinoma	21
Moderately differentiated squamous cell carcinoma	20
Poorly differentiated squamous cell carcinoma	12

A retrospective study was designed. Surgical tissue specimens from 53 patients with OSCC who underwent surgical treatment with curative purposes were retrospectively collected, in accordance to approved institutional review board guidelines. Clinicopathologic data were collected from medical records. Tissue specimens were obtained and representative tissue sections were obtained from archival, formalin-fixed paraffin-embedded blocks and the histologic diagnosis was confirmed by an experienced Pathologist.

DISCUSSION

HNSCC has been defined as one of the most highly immune-infiltrated cancer types, and in fact, the most highly NK-cell and Treg-infiltrated cancer type, although immune infiltration varies depending on several clinical and genetic features, such as HPV infection, tumor location within the head and neck region, molecular subtype, mutational smoking signature, and genomic instability. PD-L1 is a cell-surface glycoprotein primarily expressed by antigen-presenting cells and tumor cells that induces T-cell anergy and apoptosis by engaging its PD-1 receptor. Binding of PD-L1 to its receptor PD-1 inhibits the proliferation of activated T cells, leading to apoptosis or downregulation of cytotoxic T lymphocytes (CTL), called "T-cell exhaustion", which results in an escape of tumor cells from T-cell–mediated immune surveillance (2). It is assumed that tumor cells upregulate PD-L1 expression to evade the host immune reaction, thereby increasing their survival rate (31).

Tumor PD-L1 expression is induced intrinsecally by oncogenic signaling pathways, like the MAPK signaling pathways, and extrinsecally by factors from the tumor microenvironment, such as hypoxia, through the induction of the hypoxia-associated transcription factor HIF1a and cytokine production.

The prognostic relevance of PD-L1 was confirmed by multivariate analysis in this study, therefore suggesting that patients with OSCC with high PD-L1 expression might require PD-L1– targeted immunotherapy to improve prognosis and clinical outcome. Thus, even though the mechanisms leading to PD-L1 overexpression are not yet fully understood (36), clinical data from treatment with PD-1/PD-L1 inhibitors have shown response rates ranging from 10% to 50% in HNSCC and other tumor types (11). In a recent meta-analysis, Gandini and colleagues (12) found that the IHC evaluation of tumor PD-L1 expression correlated with clinical response to PD-1/PD-L1 immunotherapy in various cancers, and Topalian and colleagues (44) reported that response to anti-PD-1/PD-L1 targeted therapy was only observed in PD -L1–positive tumors.

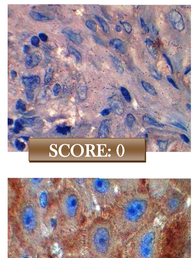
IHC Staining and Protocol

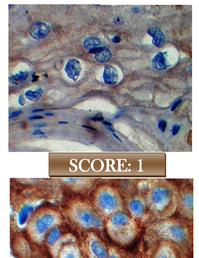
1. The sections were deparaffinized with standard xylene and hydrated through graded alcohols into water. Antigen retrieval was performed by heating the sections with Envision Flex Target Retrieval solution, high pH (Dako). Staining was done at room temperature on an automatic staining workstation (Dako Autostainer Plus, Dako).
2. Rabbit monoclonal PD-L1 antibody (clone E1L3N, 1:200 dilution; Cell Signaling Technology #13684), by using the Dako EnVision Flex þVisualization System (Dako Autostainer). Counterstaining with hematoxylin was the final step.
3. PD-L1 immunostainings were manually evaluated by three independent observers , with a high level of interobserver concordance (>95%).

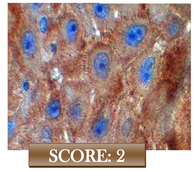
Degree of staining was scored as:

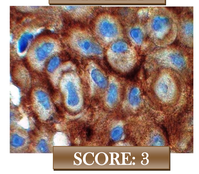
- 3+: extensive staining of the tumour including the invasion front towards the connective tissue
- 2+: more than 50% of positive staining
- 1+: less than 50% of positive staining
- 0: almost negative

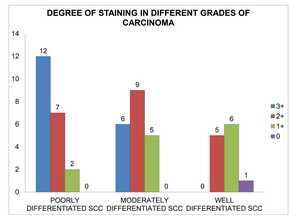
RESULTS

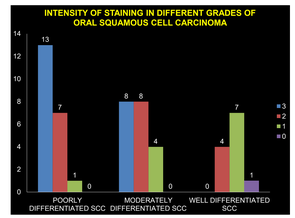












CONCLUSION

In conclusion, patients with OSCC with high PD-L1 expression showed a poor prognosis probably due to the activation of PD-1/PD-L1 pathway that enhances an aggressive tumor phenotype by allowing tumor cells to evade the host immune system, thus establishing a T-cell exhaustion, which ultimately induces a specific tolerance. Accordingly, this group of patients with OSCC emerges as an ideal candidate for clinical trials of anti-PD-L1 targeted therapy.

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