Dermatology Practical & Conceptual

Relationship Between PD-L1, PD-1, CD8 and Clinicopathological Factors in Primary SCCs

Preslav Vasilev[1], Savelina Popovska[2], Elitsa Petrova Kraevska[2], Martin Karamanliev[3], Dobromir Dimitrov[3], Ivelina Yordanova[1]

1 Department of Dermatology, Venereology and Allergology, Faculty of Medicine, Medical University-Pleven, Bulgaria

2 Department of General and Clinical Pathology, Faculty of Medicine, Medical University-Pleven, Bulgaria

3 Surgical Oncology Department, University Hospital "Dr. Georgi Stranski", Faculty of Medicine, Medical University-Pleven, Bulgaria

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Corresponding Author: Preslav Vasilev, Department of Dermatology and Venereology, Faculty of Medicine Medical University-Pleven 91, General Vladimir Vazov str. 5800, Pleven, Bulgaria. E-mail: preslav.vasilev@abv.bg

ABSTRACT

Introduction: Squamous cell carcinoma of the skin (SCCs) is the second most common skin cancer, with continuously increasing incidence. Programmed cell death ligand 1 (PD-L1), programmed cell death 1 receptor (PD-1), and CD8 expression in primary SCCs have not been described in many studies.

Objective: We investigated the association between PD-L1, PD-1, CD8, and clinicopathological prognostic factors for recurrence, metastasis, and mortality of SCCs.

Patients and Methods: Immunohistochemically stained sections of 100 primary SCCs divided into two groups according to diameter of the tumors (<20 mm and >20 mm) were assessed. Recombinant rabbit anti-PD-L1 antibody [SP142] - C-terminal, rabbit monoclonal anti-PD1 antibody [NAT105], and FLEX Mono Mo A-Hu CD8, cl C8/144B, RTU were used.

Results: We did not establish statistically significant differences between PD-L1, PD-1, CD8 expression, and high-risk clinicopathological features – tumor size >20 mm, depth >6 mm, poor tumor cell differentiation, perineural/lymphovascular invasion, low/absent lymphocyte stromal reaction.

Conclusions: In primary SCCs, the expression of PD-L1, PD-1, and CD8 are not associated with high-risk clinicopathological factors. We suggest that these immunohistochemical markers are more significant in advanced cases and metastatic tissues.

Introduction

Non-melanoma skin cancers, also called keratinocyte carcinomas, are a group of skin diseases which start their development in the epidermis. Squamous cell carcinoma of the skin (SCCs) accounts for about 30% of these tumors and is the second most common skin cancer [1]. A study from the Mayo Clinic following the incidence of SCCs between the periods 1976–1984 and 2000–2010 shows a 263% increase in the second period [2]. The surgical approach is a successful treatment modality, with 5-year survival rates without recurrence over 95% and mortality around 2% [3]. However, adjuvant systemic therapy is required in cases not responding to the classical treatment methods (surgery followed by radiotherapy) [4]. Immunotherapy with anti-programmed cell death-ligand 1 (PD-L1) agents is a new treatment option for management of patients with malignant melanoma, renal cancer, and lung cancer. Since 2019, it is approved for locally advanced and metastatic SCCs as well [5]. PD-L1 and programmed cell death-1 (PD-1) activation is the main pathway for the tumor cells to avoid the anti-oncogenic immune response [6]. Immunohistochemical expression analysis is the best way to determine the PD-L1/PD-1 status of a tumor [7], and while there are a lot of studies on the expression of PD-L1 and PD-1 in other malignancies, for SCCs these results are limited. CD8 lymphocyte infiltration is a good prognostic marker in most malignancies because they have the function of being direct tumor cell killers [8]. The high-risk pathological features of primary SCCs include macroscopic diameter (MD) of the tumor >20 mm, depth of invasion >6 mm, poor tumor cell differentiation, perineural and lymphovascular invasion, and low lymphocyte stromal reaction [9].

Objectives

Our study aimed to investigate the relationship between PD-L1, PD-1, CD8 expression, and the clinicopathological prognostic features for recurrence, metastasis, and mortality in patients diagnosed with primary invasive SCCs.

Materials and Methods

Our study observed patients with histologically verified SCCs from the cancer registry in Pleven, Lovech region, Bulgaria, who underwent excisional resection from 1 January 2016 to 30 June 2023. The analysis found 355 SCCs cases. We performed immunohistochemical evaluation for PD-L1, PD-1, and CD8 expression of 100 primary invasive SCCs resected between 1 January 2019 and 30 June 2023. We investigated the connection between PD-L1, PD-1, and CD8 immunohistochemical expression and the MD and histopathological features of these 100 SCCs. The tumors were divided in two groups according to their MD: Group 1 consisted of 50 SCCs with a diameter <20 mm, and Group 2 included 50 tumors with a diameter >20 mm. For each tumor we also evaluated the following parameters: localization, depth of invasion, cell differentiation, perineural/lymphovascular invasion, and lymphocyte stromal reaction.

All immunohistochemical staining was performed on representative sections with 3 μm thickness obtained from the paraffin-embedded blocks. They were deparaffinised and rehydrated with xylol and alcohol in descending order 90%, 80%, and 70% EtOH.

After heat-induced antigen recovery with Dako EnVision FLEX TRS, Low pH for 20 minutes/ 95°C and blocking the endogenous peroxidase with 3% H2O2, for the evaluation of PD-1 expression a rabbit monoclonal anti-PD1 antibody [NAT105] (Abcam, 1:50 dilution; for 30 minutes incubation) was applied.

After heat-induced antigen recovery with Dako EnVision FLEX, High pH for the evaluation of PD-L1 expression, we used a recombinant rabbit anti-PD-L1 antibody [SP142] - C-terminal (Abcam, 1:100 dulition; for 30 minutes incubation).

For the CD8 expression analysis, FLEX Mono Mo a Hu CD8, cl C8/144B, RTU was applied (Abcam, 1:50 dilution, 30 minutes incubation). A Dako autostainer was used.

For positive control, tonsillar crypt epithelial cell and follicular lymphoid cell staining were considered. Staining intensity and percentage of positive cells were evaluated for each case. Staining intensity scores for PD-L1 expression were defined as: without expression (≤1%), low (2–10%), moderately high (11–49%), and high (>50%). The PD-1 expression was scored as: without expression (≤1%), low (2-10%), moderately high (11-20%), high (21-30%), and very high expression (>30%). The CD8 expression analysis was: without expression ≤1%, low (2-10%), moderately high (11-39%), and high (40%). All slides were reviewed in a blinded manner by two separate pathologists (SP and EPK) independently.

The survey data was processed through IBM SPSS (Statistical Package for Social Sciences) version 20.0.

Ethical Aspects

The study was conducted following the national and international requirements for clinical studies and according to the requirements of the Ethics Committee of Medical University of Pleven, Bulgaria: approval number No. 676/31.05.2022.

Results

We found 45 tumors without PD-L1 expression (≤1%), 35 tumors with low expression (2-10%), 12 SCCs with

moderately high expression (11-49%), and eight tumors with high expression (> 50%) (Figure 1). The analysis of the data for PD-L1 expression and the Breslow depth of invasion did not show statistically significant connection: $P < 0.05$ (Actual value $P = 0.134$) (Table 1).

According to the PD-1 expression, ten tumors were without expression (≤1%), 33 tumors with low expression (2-10%), 27 SCCs with moderately high expression (11-20%), 22 tumors with high expression (21-30%), and eight tumors with very high expression (>30%) (Figure 2). Statistical analysis did not show any significant difference between PD-1 expression and Breslow depth of invasion: $P < 0.05$ (Actual value $P = 0.453$) (Table 1). Analysis of the connection between CD8 expression and depth of SCCs invasion was also statistically insignificant: $P < 0.05$ (Actual value $P = 0.146$) (Table 1, Figure 3). However, SCCs with major depth of invasion showed higher PD-L1 and PD-1 expression, and thinner tumors showed higher CD8 expression.

According to the macroscopic diameter of the tumors, we did not find any statistically significant difference between the immunohistochemical expression of PD-L1 ($P = 0.464$), PD-1 ($P = 1.000$), and CD8 ($P = 0.359$) in Group 1 (MD < 20 mm) and Group 2 (MD > 20 mm). As for the histopathological subtypes, predominance of nonspecific (classical) type SCCs was discovered (76 tumors). Fifteen SCCs were diagnosed as invasive keratoacanthoma-like, four tumors were acantholytic. We found three verrucous tumors, one clear-cell carcinoma, and one pigmented SCCs. Comparing the histopathological subtype of SCCs (nonspecific, classical type SCCs:76 tumors, invasive keratoacanthoma-like: 15 SCCs, acantholytic: four tumors, verrucous tumors: three, clear-cell carcinoma: one, and pigmented SCCs:1) and immunohistochemical expression of PD-L1, PD-1, and CD8, we did not find any statistically significant difference: $P < 0.05$ (Actual value $P = 0.304$). However, we found that only the classical and the acantholytic subtypes of SCCs express PD-L1.

According to the tumor cell differentiation, ten of the SCCs were poorly differentiated, 19 were moderately differentiated, and 71 were well differentiated. Data analysis did not find any statistically significant connection between the tumor cell differentiation and the PD-L1 ($P = 0.277$), PD-1 ($P = 0.552$), and CD8 expression ($P = 0.889$). Still, of the well-differentiated SCCs, only 49% showed positive PD-L1 expression, and 90% of the poorly differentiated tumors were PD-L1-positive.

Studying the lymphovascular and perineural invasion, we found seven tumors with lymphovascular invasion and five SCCs with perineural invasion.

Data analysis revealed that there was no statistically significant difference between the perineural invasion and the expression of PD-L1 ($P = 0.284$), PD-1 ($P = 0.710$), and CD8 ($P = 0.327$).

There was also no statistically significant connection between the lymphovascular invasion and the PD-L1 ($P = 0.065$), PD-1 ($P = 0.825$), and the CD8 immunohistochemical results ($P = 0.548$).

We analyzed the inflammatory response around the tumor area. The lymphocyte stromal reaction was scored as: missing (N=2), poor (N=9), moderate (N=21), and high (N=68). The data showed no statistically significant connection between the lymphocyte stromal reaction and the PD-L1 ($P = 0.637$) and PD-1 expression ($P = 0.491$) and a positive correlation with the CD8 expression: $P < 0.05$ (Actual value $P = 0.011$).

Discussion

PD-L1, also named B7 homologous protein (B7-H1) or cluster of differentiation 274 (CD274), was first described in 1999. It is a very important co-stimulatory molecule of the immune response which induces immune tolerance in the tumor microenvironment [11]. PD-L1 is rarely expressed on normal tissues. It is found in tumors such as malignant melanoma, lung cancer, breast cancer, pancreas, kidney, bladder, tumors of the esophagus, colon, and rectum [11]. The PD-L1/PD-1 bind induces T cell death and leads to poor prognosis

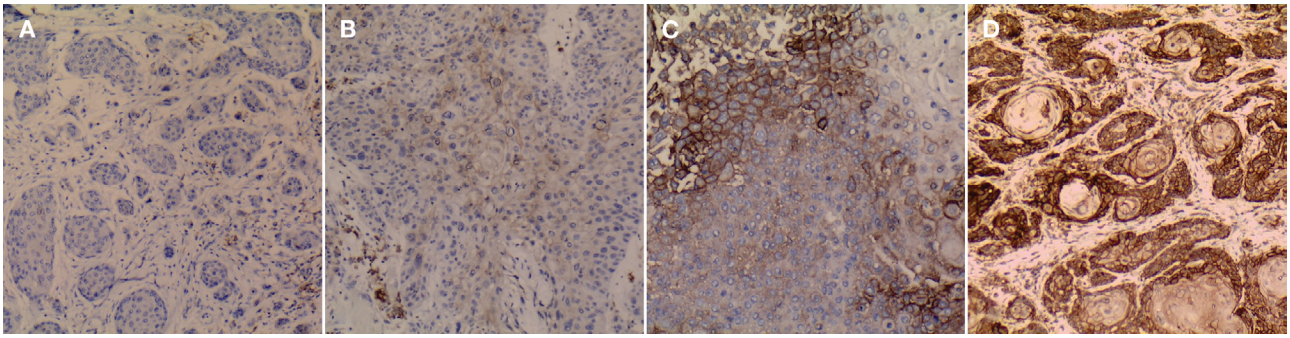

Figure 1. SCCs with different PD-L1 expression: A – without expression ≤1%, B – with low expression 2–10%, C – with moderately high expression 11–49% and D – with high expression > 50% (Anti-PD-L1 antibody [SP142] - C-terminal, Abcam)

Table 1. Correlation analysis between PD-L1, PD-1, CD8 expression, and Breslow depth of invasion.

PD-L1	N	Breslow depth of invasion				
		Mean	SD	Min	Max	
Without expression ≤1%	45	6.16	3.3	1	18	*P*=0.134
Low expression 2-10%	35	5.37	2.24	2	10	
Moderately high expression 11-49%	12	6.83	3.41	2	11	
High expression > 50%	8	6.5	4.85	3	16	
PD-1						
Without expression ≤1%	10	5.9	3.28	2	11	*P*=0.453
Low expression 2-10%	33	6.12	2.58	3	13	
Moderately high expression 11-20%	27	5.37	2.44	1	11	
High expression 21-30%	22	6.32	4.54	1	18	
Very high expression >30%	8	5.71	2.06	4	9	
CD8						
Without expression ≤1%	2	10	0	10	10	*P*=0.146
Low expression 2-10%	13	7.15	2.79	4	13	
Moderately high expression 11-39%	48	5.6	3.17	1	18	
High expression > 40%	37	5.78	3.03	2	16	

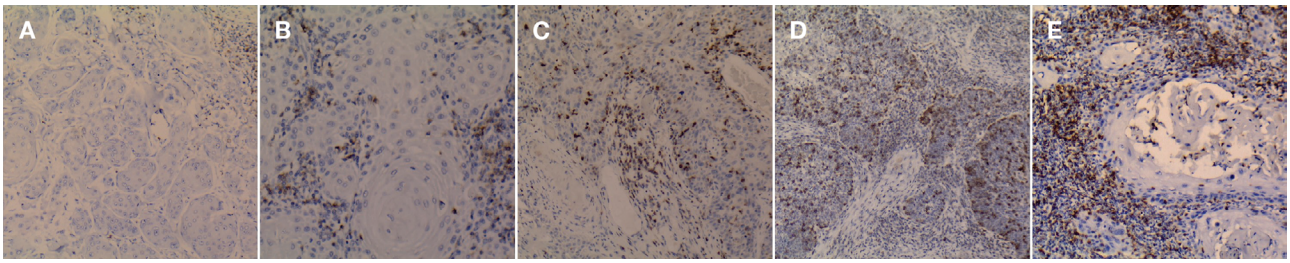

Figure 2. SCCs with different PD-1 expression: A–without expression ≤1%, B–with low expression 2–10%, C–with moderately high expression 11–20%, D–with high expression 21–30%, and E–with very high expression >30% (Anti-PD1 antibody [NAT105], Abcam)

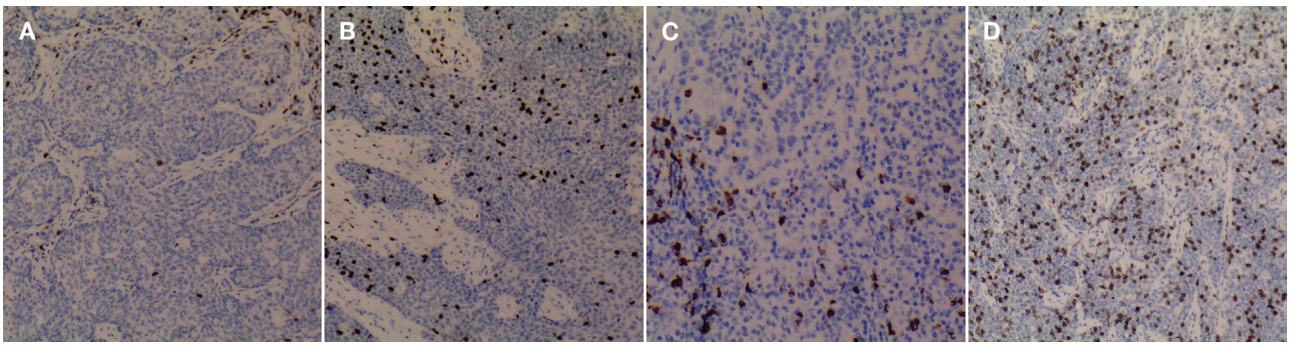

Figure 3. SCCs with different CD8 expression: A–without expression ≤1%, B–with low expression 2–10%, C–with moderately high expression 11–39%, and D–with high expression > 40% (FLEX Mono Mo a Hu CD8, cl C8/144B, RTU, Abcam)

for the patients. Monoclonal antibodies targeted against PD-L1 and/or PD-1 block the interaction between PD-1 and PD-L1, which activates the T-cytotoxic lymphocytes and leads to suppression of tumor cell differentiation and proliferation [12]. Immunohistochemical expression of PD-L1, PD-1, and CD8 in primary invasive SCCs may be used as biomarkers for prediction of response to anti-PD-L1/PD-1 immunotherapy, since tumors that respond to the immunotherapy usually show higher expression. They can also be prognostic markers for recurrence and metastasis [13]. The higher CD8 expression in SCCs may be a positive prognostic marker [14]. The prognostic value of PD-L1 is controversial. Studies show that high PD-L1 expression usually correlates with poor prognosis in gastric cancer, hepatocellular, and

renal carcinoma [15-19], but in Merkel cell carcinoma and breast cancer, it is also a marker for better prognosis [20,21].

There are not many studies in the literature providing information about the PD-L1/PD-1 and CD8 expression in non-melanoma skin cancers [22]. Slater and Googe report PD-L1 positivity in 20% of low-risk SCCs and in 70% of high-risk tumors. Their work analyzed 40 primary SCCs cases and found statistically significant connection between the PD-L1 expression profile and histopathological features for increased metastasis risks, such as tumor thickness >6 mm, MD >20 mm, and poor histopathological grade [23]. Our study did not find any statistically significant connection between PD-L1/PD-1 expression and the parameters mentioned above, but we found that SCCs with greater depth of invasion tended to show higher PD-L1, PD-1 expression, and thinner tumors showed higher CD8 expression. According to our results, we did not find any statistically significant connection between PD-L1/PD-1 expression status and the tumor grade, which is similar to the data reported by Oh et al. [24]. Roper et al. revealed that PD-L1 expression predicts longer disease-free survival in high-risk head and neck SCCs. This study revealed a statistically significant connection with PD-L1 expression >5% using the sp263 clone [25]. On the other hand, a study by Garcia-Diez et al. showed increase in the risk of metastasis in SCCs with higher PD-L1 expression [26]. They used the same anti-PD-L1 clone as in our study – SP142. A study by Varki et al. using the SP142 clone of anti-PD-L1 reported positive staining (>5%) in 26% of 66 primary SCCs cases [27]. As opposed to this study, 64% of our PD-L1-positive cases demonstrated 2–10% PD-L1 expression in the tumor cells, and 36% showed >10%. Schaper et al. reported a positive correlation between the inflammatory response and the PD-L1 expression [28]. Unlike these authors, our study revealed that 98 of the tumors had various inflammatory response, but we did not find any statistically significant relationship between PD-L1 expression and the inflammation around the primary tumor. Similarly to Schaper et al., our study did not find any statistically significant connection between PD-L1 expression and SCCs tumor diameter and cell differentiation. Studying the difference between PD-L1 expression status in primary SCCs and lymph node metastases, Amoils et al. demonstrated higher expression in metastatic tissues. Their results did not show any statistically significant connection between the expression of PD-L1 and clinicopathological features, similar to the analysis of our results [2].

Conclusion

According to our results in early stages of SCCs, the expression of PD-L1, PD-1, or CD8 is not associated with high-risk clinicopathological factors. Therefore, we suggest that the immunohistochemical examination is more meaningful in advanced SCCs and in metastatic tissues, since they show higher PD-L1/PD-1 expression. Our study presents data on the immunohistochemical expression of PD-L1, PD-1, and CD8 in primary invasive SCCs. Clinicians should be suspicious for high-risk patients and for clinicohistopathological features for recurrence and metastasis.

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