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The type of distribution of PD-L1 positive immune cells and PD-L1 expression in tumor cells correlate with the development of non-classical differentiation in urinary bladder cancer

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ABSTRACT

Background: The basic diagnostic tool of urinary bladder cancer is the histopathological assessment. However, it is insufficient to accurately predict the progression of this disease. There is a need to look for new prognostic factors that will make the therapeutic process more effective. The aim of this study is to evaluate the effect of activation of a PD1 – PD-L1 immune checkpoint in immune effector cells (IECs) and tumor cells, on the development of malignancy in the form of non-classic differentiation in urinary bladder cancer.

Materials and methods: 110 patients with stage pT1-pT4 urothelial bladder carcinoma who underwent radical cystectomy/cystoprostatectomy between 2011 and 2014 were included in the study. Tumor advancement (pT stage), grade (G), as well as, non-classic differentiation frequency and number were evaluated pathologically. In each case, the area of the tumor containing PD-L1+ IECs was analyzed. The distribution of PD-L1+ immune effector cells within the tumor was also assessed as dispersed or aggregated.

Results: The frequency of non-classic differentiation was significantly lower in urothelial bladder cancer tumors with a dispersed pattern of distribution of PD-L1+ IECs. A correlation between the extent of PD-L1 expression in tumor cells and the non-classic differentiation number in UBC was identified.

Conclusions: The distribution of cells expressing the immune checkpoint biomarker PD-L1 constitutes a new prognostic factor and may play a key role in the selection of individualized immunotherapy. In addition, the evaluation of non-classic differentiation in the tumor may complement the assessment of PD-L1 expression due to its capacity to characterize the current malignant potential of the tumor, whereas the assessment of extent and distribution of PD-L1+ in tumor-associated immune cells indicates the functional status of the immune system.

Key words: PD-L1; urothelial bladder cancer; tumor microenvironment; immune cell, NDN, immune effector cells, IEC, immunological control point distribution, ICPD

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Introduction

Histopathological assessment is an important tool in the diagnostics of urinary bladder cancer (UBC). The diagnostic standard of UBC includes the evaluation of the histological type and advancement defined by

the depth of invasion (pT), lymph node status (pN), as well as the estimation of histological malignancy (G). These features are commonly acknowledged as prognostic factors [1]. However, accurate prediction of the UBC progression is still not possible. In this context, there is an urgent need to look for factors that

better characterize the development of the malignant potential of urinary bladder cancer. Literature sources have demonstrated that cases with non-classic differentiation (ND) such as nested, micropapillary, sarcomatoid or lymphoepithelial-like differentiation have a poorer prognosis. The Classification of Malignant Tumours of the Urinary System issued by the WHO in 2004, included as many as 12 types of ND that could develop in urinary bladder cancer (squamous, glandular, trophoblastic, nested, microcystic, micropapillary, lymphoepithelial-like, lymphoma-like, plasmacytoid, sarcomatoid, and giant cell differentiation, as well as the non-differentiated type), confirming the significant value of including it in routine diagnostics [2]. Since then, a new approach and methodology have been developed for assessing ND as a prognostic factor in UBC. It was demonstrated that a higher percentage of ND and the higher number of different non-classical types of differentiation in one tumor was associated with shorter survival time in patients with UBC [3, 4]. The development of ND in UBC is accompanied by various molecular phenomena, such as the expression of RCAS1, OCT4 or the increased frequency of regulatory T cells [5–7]. Recently, researchers have been interested in the issue of immune checkpoints (ICP) that influence the efficiency of effector cells of the immune system [8, 9]. One of them is the PD1-PD-L1 signalling pathway. Its activation in the neoplastic process is associated with a significant reduction or even complete elimination of the anti-cancer immune system response [9, 10]. The aim of this study is to evaluate the effect of activation of PD1 – PD-L1 immune control point in immune effector cells (IECs) and tumor cells (TCs), on the development of malignancy, in the form of ND in urinary bladder cancer.

Materials and methods

110 patients with pT1-pT4 urothelial bladder cancer who underwent radical cystectomy/cystoprostatectomy between 2011 and 2014 were included in the study. The experimental group was comprised of patients treated at Oncology Centre Prof. Franciszek Łukaszczyk Memorial Hospital in Bydgoszcz (Poland). The mean age of patients was 65 years (65 years in women and in men). The clinico-pathomorphological characteristics of the tumors in the study group are presented in Table 1.

The staging and histological classification of tumors were assessed according to the WHO Classification of Tumors. The study was approved by the Committee of Ethics of Scientific Research of Collegium Medicum, Nicolaus Copernicus University, Poland (KB 587/2018).

Table 1. Patient clinico-pathological characteristics

Patient's characteristics	Number of patients
Sex	
Female	22
Male	88
pT	
1	12
2a	13
2b	12
3a	10
3b	31
4a	32
4b	0
G	
no available	1
1	2
2	47
3	60
NDN	
0	34
1	26
2	25
≥ 3	25

The preparation and assessment of H&E stained samples

Tissue sections were fixed in 10% buffered formalin and embedded in paraffin blocks according to a standard protocol. Tumor advancement (pT stage), histological malignancy (G), frequency of ND and the number of non-classic differentiation types (NDN) was assessed as previously described [3, 11].

The preparation and assessment of immunostained samples

Paraffin blocks most representative of the tumor were selected for immunohistochemistry staining. 4 μm sections were stained with rabbit anti-PD-L1 (Ventana clone SP142, Roche) and evaluated with OptiView DAB IHC Detection Kit and OptiView Amplification Kit using the Ventana BenchMark system according to the manufacturer's protocol. To confirm the specificity of the signal, for each sample a negative control stained without anti-PD-L1 antibody was provided. For each staining cycle, a positive control sample of human tonsil was included following the manufacturer's recommendations. In each test sample, the percent of tumor area occupied by tumor-associated immune cells exhibiting PD-L1 positive staining was assessed. IECs that were assessed included lymphocytes, macrophages, den-

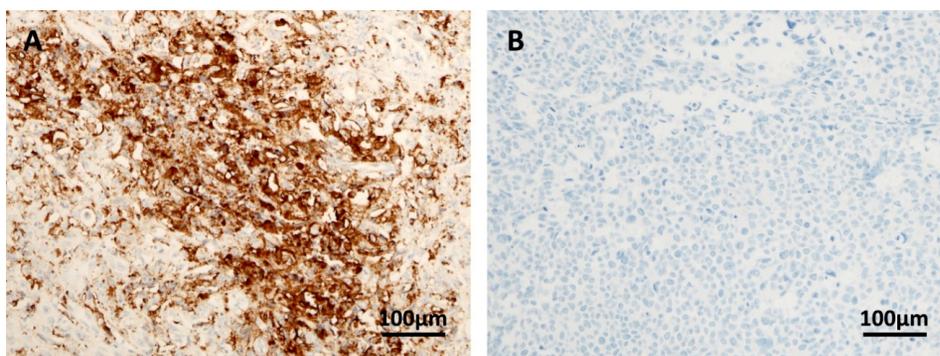


Figure 1. The sample images show the presence of PD-L1 expression in tumor cells (A) and its absence (B)

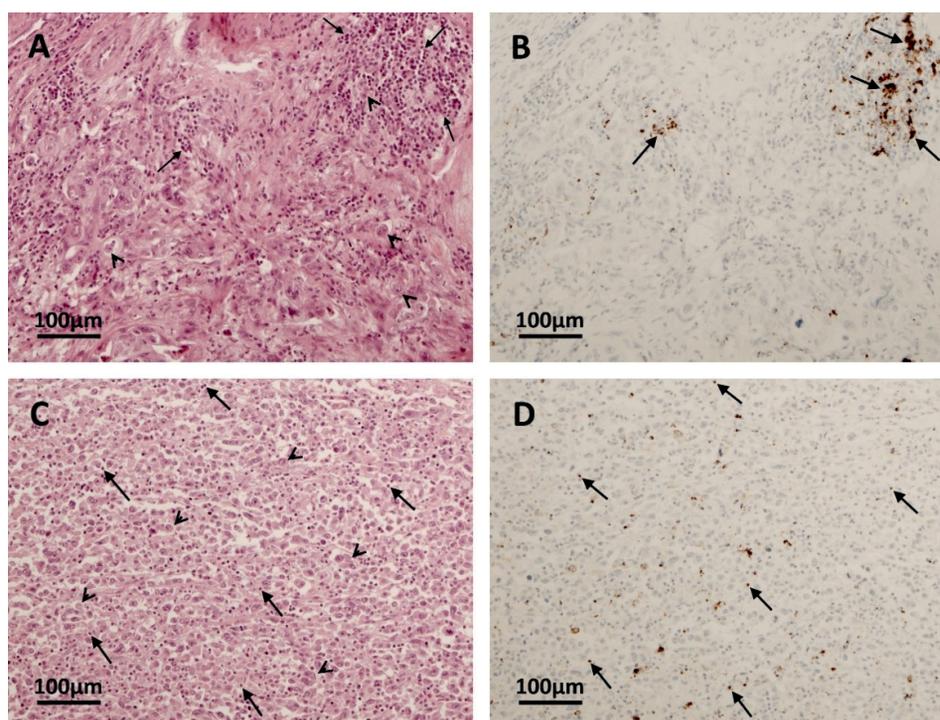


Figure 2. A low percentage of non-classic differentiation in the tumor (A) (case with ND=10%) correlates with a dispersed distribution pattern of PD-L1 + IECs (B). An aggregated distribution pattern of PD-L1 IECs (D) correlates with a high percentage of non-classic differentiation in the tumor (C) (case with ND=85%). Arrows indicate PD-L1+ IECs, arrowheads indicate tumor cells

driftic cells and granulocytes. The pattern of distribution of PD-L1+ IECs within the tumor was determined as either dispersed (Fig. 2D) or aggregated (Fig. 2B) [12]. In tumor cells, the expression of PD-L1 was assessed as either positive (Fig. 1A) or negative (Fig. 1B).

Statistical analysis

The relationships between expression of PD-L1 on IECs and TCs and variables such as pT, G, the extent of non-classic differentiation and NDN were analyzed using T-test for independent samples. The statistical analyses were performed using STATISTICA data analysis software (version 8.0; StatSoft, Inc., Tulsa,

OK, USA). A p-value < 0.05 was considered to be significant.

Results

PD-L1 positive immune cells and non-classic differentiation

A correlation between the extent of non-classic differentiation and the distribution pattern of IECs expressing PD-L1 in UBC was observed (Fig. 2). ND frequency was significantly lower in tumors with a dispersed distribution pattern of PD-L1 + IECs (Fig. 3).

PD-L1 expression in tumor cells and NDN

The expression of PD-L1 on tumor cells was correlated with NDN in UBC samples (Fig. 4). In tumors

with the presence of non-classic differentiation types (NDN>0), the extent of PD-L1 expression in tumor cells was significantly higher compared to tumors without non-classic differentiation type (NDN = 0) (Fig. 5).

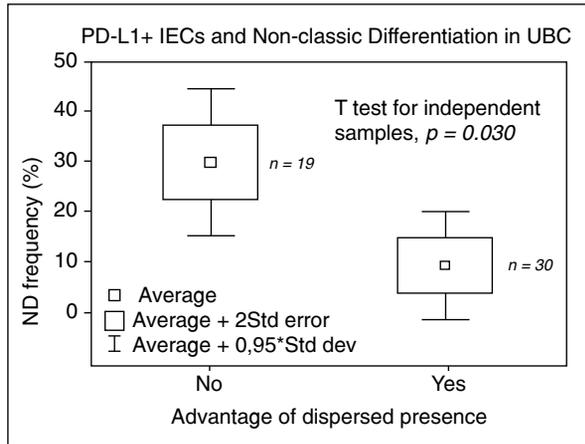


Figure 3. In tumors presenting high dynamics of invasion, in which the expression of PD-L1 includes at least 80% of tumor cells (n=49), the extent of non-classical differentiation in tumors with the advantage of the aggregated presence of PD-L1 + IECs was almost twice as much as in tumors with a predominance of dispersed PD-L1 + IECs presence

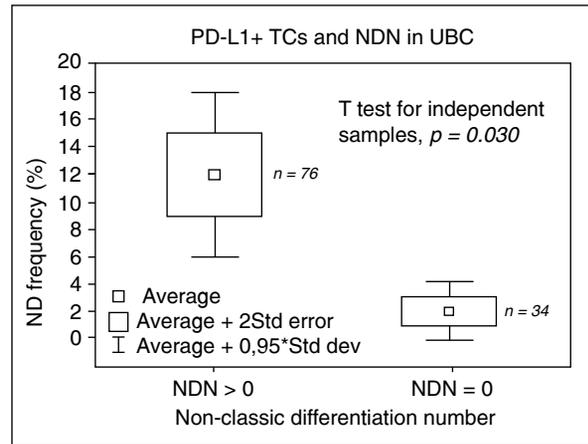


Figure 5. The extent of PD-L1 expression in tumor cells was at least six times higher in tumors with non-classical differentiations

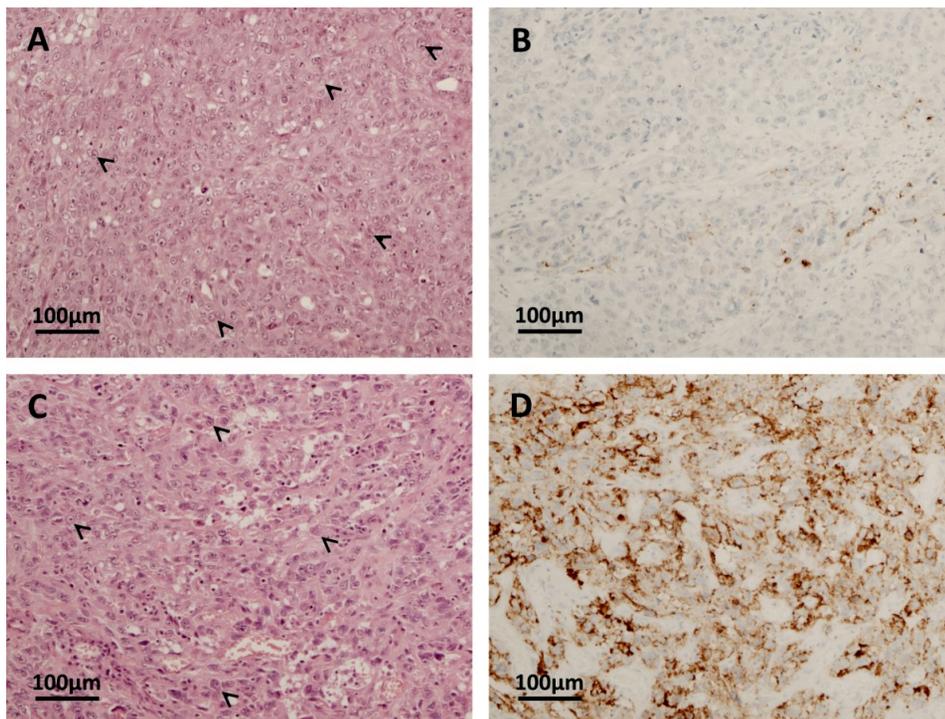


Figure 4. A low number of non-classic differentiations (A) is associated with a low frequency of tumor cells expressing PD-L1 (B) (case with NDN = 0 and %TCs-PD-L1+ = 1%). A higher frequency of PD-L1+ tumor cells (D) can be observed in tumors with a higher number of non-classic differentiation types (C) (case with NDN = 2 and %TCs-PD-L1+ = 80%)

PD-L1 expression in neoplastic cells and tumor stage (pT)

There was no significant correlation between PD-L1 frequency and tumor staging (not shown).

PD-L1 expression in neoplastic cells and tumor grade (G)

There was no significant correlation between PD-L1 frequency and tumor grading (not shown).

Discussion

Evaluation of PD-L1 expression in cells within a tumor area is a multifaceted source of information on the developmental status of a tumor. Activation of the PD-1 – PD-L1 signalling pathway may result in not only a reduction of anti-cancer immune system response [9, 10] but also drive tumor progression and malignancy as evidenced by increased non-classic differentiation in UBC [13]. Our results suggest that the effectiveness of the PD-1 – PD-L1 immune checkpoint might depend not only on the expression of the ligand protein PD-L1 on IECs but also the pattern of distribution of those cells within the tumor. A dispersion of PD-L1 positive immune cells does not favour the frequency of non-classic differentiation in the tumor (Fig. 2A, B). Conversely, ND is significantly higher in tumors with an aggregated pattern of distribution of PD-L1+ IECs (Fig. 2C, D, Fig. 3). This pattern of expression is consistent with the proposed mechanism of immune checkpoints and may be of key importance in planning immunotherapy strategies as the upregulation of PD-L1 expression on tumor cells is often accompanied by an increased expression on IECs as well [14]. Suppression of IECs activity by the activation of immune checkpoint signalling pathways may promote the progression of UBC [9, 15]. According to our knowledge, the pattern of distribution of PD-L1+ cells has not been evaluated in UBC before. The second parameter describing non-classic differentiation we assessed was NDN which is independent of the extent of ND in the tumor [3]. In our study, we observed an increase in NDN within tumors with a higher frequency of PD-L1 positive TCs (Fig. 4, Fig. 5). An increase in the number of non-classic histological types of differentiation underlies an increase in tumor malignancy even if the extent of ND as a whole constitutes only a small percentage of the tumor area [3, 4, 11]. Pichler et al. demonstrated that the assessment of PD-L1 expression on IECs is the most reliable prognostic marker of tumor response to therapy. That study also highlighted high heterogeneity of PD-L1 expression which may help explain divergent results [16]. Altogether, an assessment of activated immune checkpoint pathways, expression

of PD-L1 as well as the distribution of PD-L1 positive cells within the tumor should be a part of a standard evaluation of tumor immunological status in urothelial bladder cancer. In our study, the expression of PD-L1 and distribution of PD-L1+ cells was not correlated with tumor advancement (pT) and grade (G). There is no consensus on that matter in literature. In a study on lung adenocarcinoma, a correlation between high histological differentiation and PD-L1 expression on TCs and IECs was found [17]. Jabbour et al. found a correlation between PD-L1 expression on TCs and IECs and tumor advancement [18]. However, other studies did not confirm these findings [19] in line with our own results. Numerous studies confirm the efficacy of inhibitors of control points in cancer immunotherapy [20–26]. However, not all patients respond to anti-PD-L1 therapy to the same extent which highlights the need to search for novel biomarkers to further stratify the treatment groups [27, 28].

Conclusions

The pattern of expression of immune checkpoint biomarkers such as PD-L1 in tumors is a new predictive tool and a potential prognostic factor in patients with UBC. An assessment of dispersion or aggregation patterns of cells expressing immune checkpoint biomarkers may play a major role in tailoring individualized immunotherapy. Moreover, the evaluation of ND in the tumor may complement the assessment of PD-L1 expression due to its capacity to characterize the current malignant potential of the tumor, whereas the assessment of extent and distribution of PD-L1+ in tumor-associated immune cells indicates the functional status of the immune system.

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Conflicts of interest

The authors declare no conflicts of interest.

List of abbreviations

IEC – immune effector cells
 TC – tumour cells
 PD-L1 – programmed death ligand 1
 PD-1 – programmed death 1
 ICI – immune checkpoint inhibitors
 UBC – urothelial bladder cancer

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