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Expression of PD-L1 in tumor and immune system cells affects the survival of patients with urinary bladder cancer

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ABSTRACT

Background: The prediction of tumor malignancy is still one of the most demanding diagnostic tasks in urinary bladder cancer because of its clinicopathological heterogeneity. The aim of this study was to evaluate the expression of PD-L1 in tumor cells (TCs) and immune effector cells (IECs) as well as the pattern of distribution of PD-L1+ IECs within the tumor (dispersed or aggregated) and their association with survival of patients with pT1-pT4 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. Paraffin blocks most representative of the tumor were selected for H&E staining as well as immunostaining with the use of rabbit anti-PD-L1 (Ventana clone SP142, Roche). In each sample, the area of the tumor containing PD-L1+ IECs, as well as, the pattern of distribution (dispersed or aggregated) of PD-L1+ immune effector cells within the tumor were analyzed. In addition, the expression of PD-L1 in TCs was also assessed.

Results: Patients had a shorter survival time in pT2-pT4 cases without TCs expressing PD-L1 (p = 0.007) and/or when PD-L1+ IECs displayed a predominantly dispersed pattern of distribution (p = 0.013).

Conclusions: The expression of PD-L1 on TCs and IECs is a prognostic factor which allows for stratification of patient survival in UBC. The predominance of dispersed or aggregated pattern of distribution of PD-L1+ IECs in the tumor may be considered as a new prognostic factor in pT1-T4 UBC and indicate the functional status of the immune system.

Key words: PD-L1; urothelial bladder cancer; tumor microenvironment; immune cell distribution, immune effector cells, immune checkpoint inhibitors

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Introduction

Urothelial bladder cancer (UBC) is the seventh most frequency occurring cancers in men and may be responsible for 200 000 deaths in 2018 [1]. The assessment of the tumor malignancy requires the assessment of tumor advancement (pT), histological malignancy (G), number of nonclassic differentiation types (NDN) and the tissue invasion type (TIT) [2–4]. Unfortunately, the prediction of the tumor progression and the risk of recurrence remains a diagnostic and therapeutic challenge in UBC. Its histological and clinical heterogeneity which likely reflects its molecular heterogeneity makes understanding the biological mechanisms of UBC malignancy a challenging area of research [5–8]. Recent studies have indicated several probable mechanisms of tumor progression by the way of modulating the immune system anti-tumor response. One important mechanism is the suppression of anti-tumor response by an excess infiltration of regulatory T cells (Tregs) [9]. The over-expression of RCAS1 in tumor cells (TCs) and the surrounding cells of the cancer microenvironment were also shown to correlate with clinical and pathomorphological patterns of malignancy [5]. An

interesting new area of research is the cancer immune evasion through activation of immune checkpoints which suppress IECs [10,11]. One such mechanism is the PD-1/PD-L1 signalling pathway which suppresses the activity of T lymphocytes particularly in the effector phase of the immune response [11]. In 2017, Xingyuan et al. performed studies that showed the ability of UBC TCs to induce immunosuppression in patients through upregulation of PD-L1 expression on tumor-associated macrophages (TAMs) with the involvement of IL-10 [12]. Furthermore, PD-L1 can be expressed in both TCs and IECs. There is evidence that its expression within the tumor may in some circumstances facilitate the escape from immune surveillance and lead to tumor progression [10, 13]. The results of clinical trials have demonstrated a significant correlation between PD-L1 expression in some tumors and the effectiveness of treatment using immune checkpoint inhibitors (ICI) [14–19], especially in early stages of the disease [20], and in combination therapies [21]. However, some patients who meet the criteria for therapy do not respond well to anti-PD-L1 treatment [22, 23]. Thus, finding new eligibility criteria for immunotherapy to increase its efficacy and safety is paramount. The aim of this study is to assess the expression of PD-L1 on TCs and IECs which are present in the tumor area and to analyze the effect of PD-L1 expression on patient survival in UBC.

Materials and methods

A total of 110 patients with pT1-pT4 UBC from Oncology Centre Prof. Franciszek Łukaszczyk Memorial Hospital in Bydgoszcz (Poland) who underwent radical cystectomy (or cystoprostatectomy) in 2011–2014 were enrolled in this study. The average follow-up time after surgery was about 22 months. The clinico-pathological characteristics of the study group is presented in Table 1.

Staging and histological type of tumors were assessed according to the WHO classification [24]. Survival data were collected from the National Health Fund. The study was approved by the Committee of Ethics of Scientific Research of Collegium Medicum, Nicolaus Copernicus University, Poland (KB 587/2018).

The preparation and evaluation of H&E stained samples

Tissue sections were fixed in 10% buffered formalin and embedded in paraffin blocks according to a standard protocol. The evaluation of HE (Hematoxylin-Eosin) stained sections from each urinary bladder was performed and one representative paraffin-embedded block was chosen for immunohistochemical staining.

Table 1. Patient clinico-pathological characteristics

Patient's characteristics	Number of patients
Age (years)	
30–50	2
51–60	24
61–70	53
71–80	29
81–90	2
Sex	
Female	22
Male	88
pT	
1	12
2a	13
2b	12
3a	10
3b	31
4a	32
4b	0
pN	
Not available	2
0	61
1	12
2	33
3	2
Recurrence	
Yes	16
No	94

Immunohistochemical staining and evaluation of samples

Immunohistochemical staining of $4\,\mu$m sections was performed with rabbit monoclonal anti-PD-L1 (Ventana clone SP142, Roche) and visualization system OptiView DAB IHC Detection Kit and OptiViewAmplification Kit using VENTANA BenchMark system, according to the manufacturer's protocol. To confirm the specificity of the signal, the same protocol but without the use of anti-PD-L1 was performed as a negative control for each sample. For each staining cycle, a positive control sample of human tonsil was included following the manufacturer's recommendations. In each test sample, the percentage of tumor area occupied by tumor-associated immune cells exhibiting PD-L1 positive staining was assessed, regardless of staining intensity or the number of ICs present [25] (Fig. 1A, B). Furthermore, the pattern of distribution of PD-L1+ IECs in the tumor was evaluated as either dispersed or aggregated (with cell aggregates of 10 cells or more in a high-power field of view) (Fig. 1C, D).
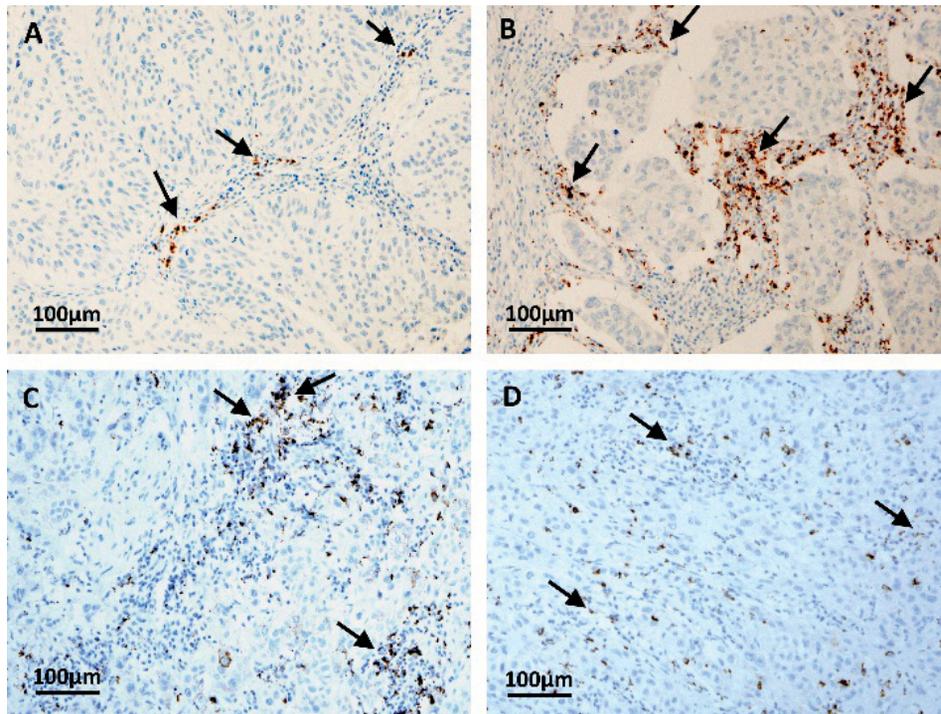
Figure 1. The panel gives an example of the extent of the presence of PD-L1+ IECs in the tumor: < 5% (**A**) and ≥ 5% (**B**). The aggregated and dispersed presence of PD-L1+ IECs is shown in figures C and D, respectively. Arrows point to immune cells
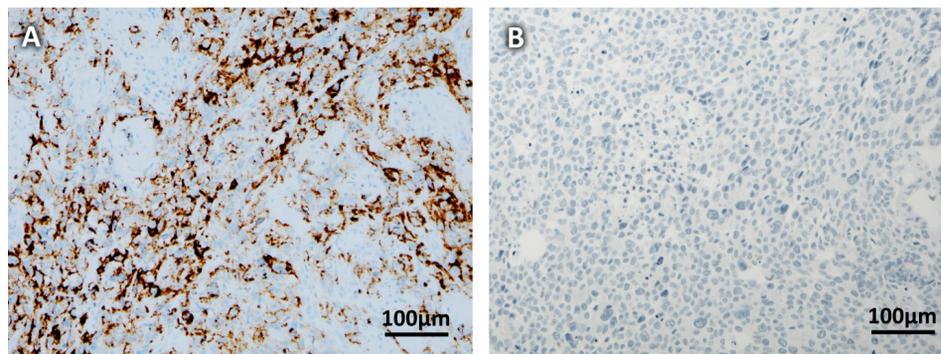

Figure 2. The picture shows sample images of the presence of PD-L1 expression in tumor cells (**A**) and its absence (**B**)

The presence or absence of PD-L1 expression was evaluated in the TCs regardless of the intensity and extent of the expression.

The microscopic assessment was performed with the use of Nikon Eclipse 80i microscope. Pictures were taken with Nikon Digital Sight DS Fi1-U2 camera and with NISElements BR 3.0 software (Nikon Instruments Europe B.V., Badhoevedorp, The Netherlands).

Statistical analysis

The relationship between PD-L1 expression in TCs and IECs was analyzed using T-test for independent sam-ples. PD-L1 expression in TCs and IECs and the probability of survival was assessed with Kaplan–Meier curves. 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

Expression of PD-L1 and survival

The analysis of PD-L1 expression in TCs showed that the probability of survival was higher in cases where
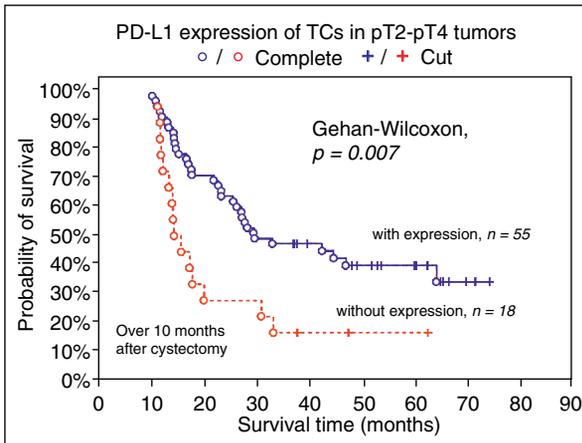
Figure 3. The survival probability 20 and 50 months after cystectomy for UBC was about two and a half times and twice higher, respectively, in patients with tumor PD-L1 expression
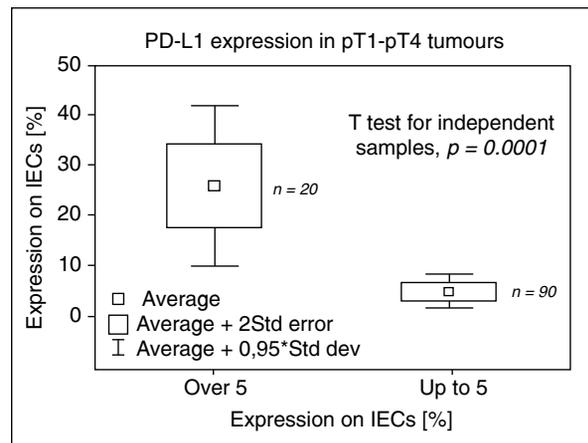

Figure 5. The average extent of PD-L1 expression in tumor cells is at least 5 times as high in tumors in which PD-L1 + immune cells occupy over 5% of their surface area
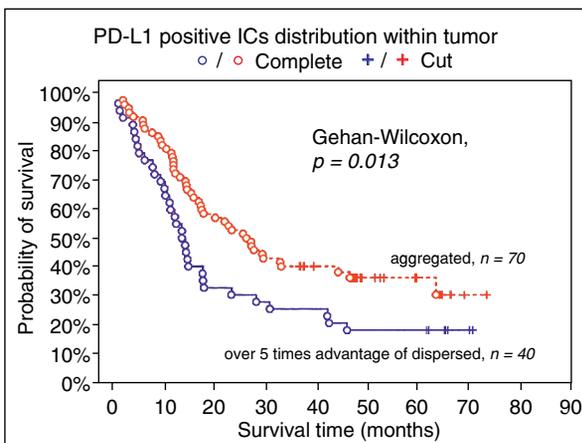
Discussion

Immune mechanisms play a key role in tumor development and progression. Inhibition of anti-tumour response of the immune system and induction of immune tolerance are observed in the early and late stage of UBC development, respectively [5, 9, 26–28]. Recent studies have focused on immune checkpoint modulation of IECs activity by means of a PD1-PDL1 signalling pathway in UBC [11, 29]. We have demonstrated that the expression of PD-L1 in TCs of UBC is associated with a higher probability of survival (Fig. 3). Contrary findings were obtained by Chun-Te et al. and also by Yide et al. [30, 31]. However, the results of several other authors are not in accord. Bellmunt et al. and Davickai et al. confirmed such relation in patients with UBC [32, 33], likewise, Kim et al. detected an association in head and neck tumors [34]. We cannot rule out that the difference in findings may be due to an adopted selection of samples (i.e. non-epithelial bladder cancer included) and due to differences in methodology (a type of antibody, representative section sampling). Some other authors report that the expression of PD-L1 on TCs may be associated with the increase of PD-L1 + IECs numbers within the tumor [31]. In our study, we have observed that the increase of the extent of the tumor with PD-L1 + IECs is associated with the increase of PD-L1 expression in TCs (Fig. 5). This may suggest a similar or even synergistic involvement of these two cellular components (TCs and IECs) in downregulation of anti-tumor response through PD-L1. There is no clear consensus on that matter in literature [35, 36]. The studies on the role of PD-L1 molecule in ovarian cancer demonstrated that PD-L1 expression in tumor infiltrating macrophages

Figure 4. The probability of survival 4 years after cystectomy for UBC was more than twice lower in patients with the advantage of dispersed PD-L1+ IECs in tumor

TCs expressed PD-L1 compared to those where such expression was not found (Fig. 3).

Further, we observed that the presence of PD-L1+ IECs with a dispersed distribution pattern (n = 40) was associated with significantly lower survival probability (Fig. 4).

Expression of PDL1 in tumor and immune cells

We observed a strong correlation between the extent of PD-L1 expression in TCs and the extent of expression of PD-L1 in IECs in close vicinity (p < 0.05; r = 0.60; n = 110). The extent of PD-L1 expression in TCs was particularly high in tumors where PD-L1+ IECs occupied more than 5% of the whole tumor area (Fig. 5).

may confer them protection from the immune system. The macrophages remove debris of tumor cells which in effect leads to high levels of tumor antigens in them which may result in them being targeted by IECs. PD-L1 expression on the surface of macrophages may protect them against this autoimmune response [37, 38]. Thus, a parallel increase of PD-L1 expression in immune and tumor cells suggests a mechanism in which the immune system tries to activate a protective mechanism for its own effector cells. Ottenhof et al. in their study on the prognostic value of PD-L1 expression in penile cancer discovered an association between the dispersed pattern of ligand expression in tumor cells and a lower probability of survival [39]. In our study, the probability of survival in patients with UBC in stages pT1-T4 was reduced twice with a dispersed distribution of PD-L1+ IECs (Fig. 4). The findings we obtained suggest that the effectiveness of immune checkpoints linked to PD-L1 expression on IECs dispersed within the tumor is higher in comparison with PD-L1 located on aggregated IECs in UBC. Pichler et al. concluded that outcome prediction in UBC is most effective when the expression of PD-L1 on IECs is assessed. Their studies indicated, however, that high heterogeneity of expression dependent on the type of cells expressing PD-L1 cells may lead to unreliable results [40]. Recent studies suggest that not only the level of PD-L1 expression in IECs may have a prognostic value but also their location and aggregation within the tumor may significantly affect survival [41, 42]. Our findings indicate that there is a significant prognostic value of assessing the dispersed and aggregated patterns of distribution of PD-L1+ IECs within the tumor. The diagnostic assessment standards for UBC proposed by other authors do not include the assessment of the distribution of PD-L1+ IECs within the tumor [25]. In our opinion, dispersion or aggregation of immunocompetent cells may be an indication of the status of the immune system and as such should be included in the eligibility criteria for immunotherapy in UBC.

Conclusions

The assessment of PD-L1 expression in TCs allows for prognostic stratification of patients with UBC in all stages. The predominance of dispersed or aggregated type of distribution of PD-L1+ IECs in the tumor may be considered as a new prognostic factor in pT1-pT4 UBC and may also indicate the status of the immune system of a patient. The assessment of the PD-L1 expression in TCs and IECs as well as of the type of distribution of IECs PD-L1+ in the tumor should be considered for inclusion in immunotherapy eligibility criteria in patients with UBC.

Conflicts of interest

The authors declare no conflicts of interest.

List of abbreviations

IECs – immune effector cells
TCs – tumor cells
PD-L1 – programmed death ligand 1
PD-1 – programmed death 1
ICI – immune checkpoint inhibitors
UBC – urothelial bladder cancer

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