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Original article

CORRELATION OF PD-L1 GENE EXPRESSION WITH GRADE OF THE URINARY BLADDER CANCER

КОРЕЛАЦИЈА НА ЕКСПРЕСИЈАТА НА ГЕНОТ PD-L1 СО ГРАДУСОТ НА КАРЦИНОМОТ НА МОЧЕН МЕУР

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Abstract

Introduction. Bladder cancer (BC) ranks fourth in the prevalence of malignancies in developed countries and is the eighth leading cause of cancer-related mortality in men. PD-L1, known for its role in inhibiting immune responses against malignant cells, has garnered significant attention in BC research.
Methods. This study, comprising 45 patients with histopathologically confirmed urothelial carcinoma of the urinary bladder, analyzed the connection between histological grade and PD-L1 gene expression. The patient cohort was divided into 31 classified as low-grade and 14 as high-grade, with gender and age distribution well-balanced across the groups. PD-L1 expression was notably higher in the high-grade group (p=0.005), showing its potential clinical relevance as a biomarker.
Results. Univariate logistic analysis revealed a robust correlation between histological grade and PD-L1 expression, with high-grade patients exhibiting a 7.227-fold higher likelihood of increased PD-L1 expression. A predictive model for grade determination demonstrated commendable performance, boasting an area under the curve (AUC) of 0.788.
Conclusion. These findings provide compelling evidence of a strong association between PD-L1 gene expression and the histological grade of bladder cancer. PD-L1 emerges as a potential biomarker, shedding light on a disease pathological grade, offering a significant clinical value for precise prognosis, and guiding tailored treatment strategies. These insights hold promise for improved disease management and patient outcomes.

Keywords: bladder cancer, PD-L1 gene expression, polymorphisms rs861539, biomarker

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Апстракт

Вовед. Карциномот на мочниот меур се наоѓа на четвртата позиција во преваленцата кај малигни заболувања во развиените земји и е осма водечка причина за смртност предизвикана од карцином кај мажите. PD-L1, познат по својата улога во инхибирање на имунолошките одговори против малигните клетки, привлекол значително внимание во истражувањето на карциномот на мочниот меур.
Методи. Ова истражување, кое вклучуваше 45 пациенти со хистопатолошки потврден уротелен карцином на мочниот меур, ја истражуваше поврзаноста помеѓу хистолошкиот градус и експресијата на генот PD-L1. Кохортата на пациенти беше поделена на 31 пациент (пациенти со низок градус на карцином) и 14 (висок градус на карцином), при што дистрибуцијата на полот и возраста беше рамномерна помеѓу групите. Експресијата на PD-L1 беше значително поголема кај пациентите со висок градус (p=0.005), и ова го покажува неговиот потенцијално клиничко значење како биомаркер.
Резултати. Направените анализи откриja силна корелација помеѓу хистолошкиот градус и експресијата на PD-L1, при што пациентите со висок градус покажаа 7.227-пати поголема веројатност

за зголемена експресија на PD-L1. Изработен беше предиктивен модел за одредување на градусот, кој прикажа добра предиктивност, со вредност за подрачјето под кривата (AUC) од 0.788.
Заклучок. Овие ноди обезбедуваат убедливи докази за јака поврзаност помеѓу експресијата на генот PD-L1 и хистолошкиот градус на карциномот на мочниот меур. PD-L1 се појавува како потенцијален биомаркер, овозможувајќи значителна клиничка вредност за точна прогноза и насочување на персонализирани стратегии за третман. Овие истражувања ветуваат напредок при менаџирање со болеста со цел подобар исходот за пациентите.

Клучни зборови: карцином на мочен меур, PD-L1 генска експресија, полиморфизам rs861539, биомаркер

Introduction

Bladder cancer (BC) is the fourth most common malignant disease in developed countries and the eighth most common cause of cancer death among men [1,2]. The diversity in tumor phenotype and the long duration of treatment make BC one of the clinical entities with the highest financial burden on the healthcare system and has a significant impact on patients' quality of life.
The histological evaluation of bladder cancer relates to tissue differentiation and tumor aggressiveness in relation to how abnormal the cells appear under microscopic analysis [3]. There are various systems for classifying the degree of tissue differentiation, but in the papers published over the last decade, the binomial system with two grades, low and high, is most commonly used.
At diagnosis, 75% of patients have non-muscle-invasive bladder cancer (NMIBC) [4]. However, when recurrences happen, muscle-invasive bladder cancers (MIBC) are diagnosed in some of these cases, which have a high risk of metastasizing the regional lymph nodes, as well as hematogenously in distant organs. Current molecular marker sets are not secure enough to enable accurate prediction of the potential for recurrence and disease progression.
PD-L1 gene (also known as B7-H1 or CD274) codes a ligand for the PD-1 receptor and is expressed in many malignant tumors, including BC. PD-L1 can inhibit immune responses either by binding to PD-1 or other receptors, resulting in significant effects on the susceptibility of malignant cells to immune recognition and their elimination [5]. The apoptotic pathway mediated by the PD-1/PD-L1 interaction inhibits T-cell activation and plays an important role in regulating the antitumor immunity of patients with malignant neoplasms.
Immune checkpoints have an important regulatory role, i.e., they prevent an excessive immune response that would damage normal cells and tissues. These checkpoints are activated when the proteins on the surface of T-cells recognize and bind to the appropriate receptors on other cells, such as some tumor cells. When the checkpoint and partner proteins bind mutually, they send a signal to inhibit T-cells. However, this can also prevent the immune system from recognizing and lysing malignant cells. Immunotherapeutic agents called immune checkpoint inhibitors are based on blocking checkpoint proteins, thereby preventing them from connecting with their receptor proteins [6]. This prevents the blockade signal from being sent and allows T-cells to kill malignant cells.
Modern treatment with targeted drugs is based on the inhibitory action of the immune control point protein called CTLA-4. Other immune checkpoint inhibitors act inhibitory on the protein called PD-1 or its receptor protein PD-L1. Namely, some tumor cells reduce the response of T-cells by producing an excessive number of PD-L1 protein molecules on their cell membrane.
The main aim of this scientific paper was to determine the correlation of the frequency of genotypes, i.e., alleles of the examined polymorphism with susceptibility to BC.

Materials and methods

The primary aim of this study was to correlate PD-L1 expression with the grade of BC, and for that purpose we analyzed 45 bladder tissue specimens obtained via transurethral resection of the bladder at the University Clinic for Urology. Patients were diagnosed with urothelial carcinoma of the urinary bladder, and were histopathologically confirmed based on the histopathological grade of differentiation (binomial system with two classes of differentiation: low and high grade).
Demographic data, histopathological findings, as well as relevant clinical data (cystoscopic, echographic, and CT findings, clinical course of the disease, etc.) were collected for each patient. Differences in terms of gender and age distribution between the two groups of patients were analyzed using the Student's t-test. Prior to that, normal distribution of these data was assessed using the Shapiro-Wilk test.

The quantitative expression of PD-L1 gene was determined by reverse transcription of RNA samples and real-time polymerase chain reaction (qRT-PCR) using fluorescent TaqMan probes on the OneStep Real-Time PCR system (Applied Biosystems). Total RNA was isolated from tissue fragments of tumor tissue (previously stored at -80°C) using TRI reagent, and cDNA was synthesized by reverse transcription. The levels of hTERT expression were calculated using the Livak method, relative to the expression of the housekeeping gene GAPDH. The relative quantitative values (RQ) were presented as 2-ΔCt. In this study, we present the final expression values of the PD-L1 gene for each patient as the logarithm of RQ with a base of 10, i.e., log10(RQ).

The correlation between the degree of tissue differentiation of the BC tissue sample from patients and the expression of PD-L1 gene was evaluated using the univariate logistic analysis. Receiver operating characteristic (ROC) curves were evaluated, and the area under the curve (AUC) value was obtained. The odds ratio was calculated within a 95% confidence interval (CI).

Values of $p<0.05$ were considered statistically significant, while values of $p<0.01$ were considered highly significant. Statistical calculations were performed using the XLSTAT 2016 and GenAlEx 6.5 software add-ins installed on Microsoft Excel 2016.

Results

A total of 45 patients with BC were included, of which 14 had a low grade (well-differentiated histologically) and 31 had a high grade (poorly differentiated) (Table 1 and Figure 1).

Table. 1. Distribution of BC grade

Grade	n	%
Low	14	31.11
High	31	68.89
Total	45	100.00

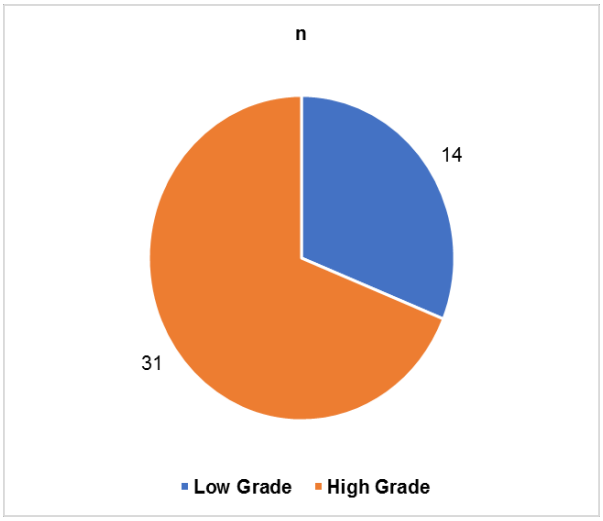

Fig. 1. Distribution of BC grade

The selected clinical data were evaluated and compared to PD-L1 expression in tumor cells.

The presented data and the results of the statistical analysis of the gender and age distribution in the two groups (patients with low and high grades) indicated statistically insignificant differences ($p>0.05$) (Tables 2 and 3 and Figures 2 and 3). The two groups were well balanced in terms of gender and age, which ensured a reliable comparison regarding gene expression.

Table. 2. Gender distribution

Gender	Low grade		High grade		Fisher exact test*
	n	%	n	%	*p*
male	12	85.71	26	83.87	
female	2	14.29	5	16.13	1.000
total	14	100.00	31	100.00	

* two-tailed

Table. 3. Age distribution

Parameter (years)	Low grade	High grade	Student's t-test *
n	14	31	
Average	65.14	62.16	
SD	11.27	7.87	0.311
min. age	47	46	
max. age	80	76	

* two-tailed

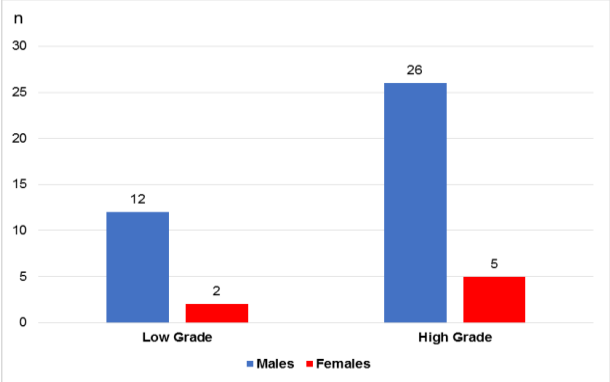

Fig. 2. Gender distribution

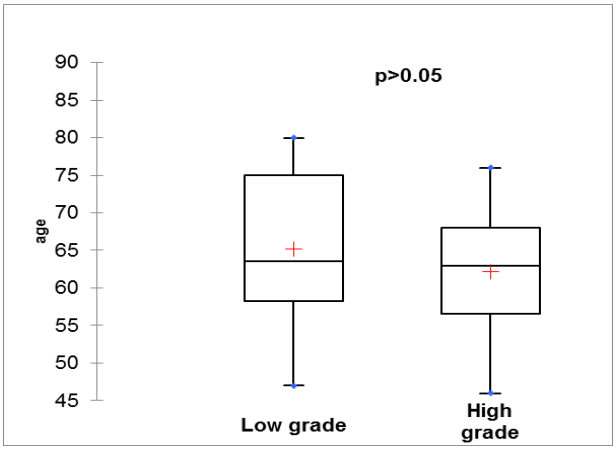

Fig. 3. Age distribution

Table. 4. Expression of PD-L1 gene according to BC grade

PD-L1	Low grade	High grade
n	14	31
average	0.779	1.398
SD	0.548	0.569
min. value	0.221	0.141
max. value	1.753	2.481

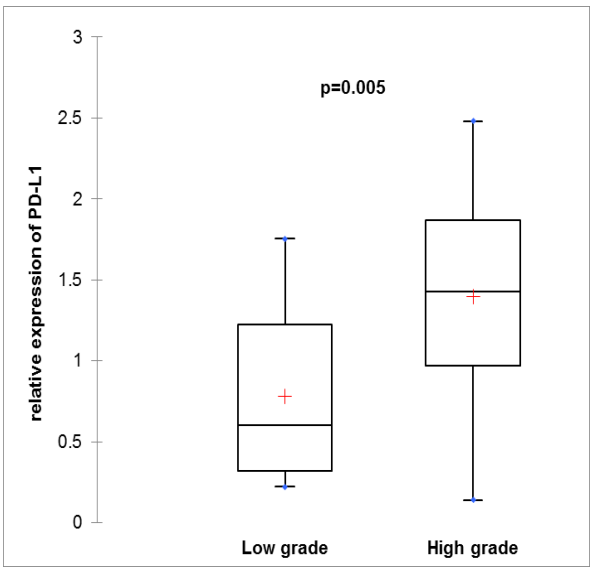

Fig. 4. Expression of PD-L1 gene according to BC grade

Table. 5. Univariant logistic analysis for correlation with expression of gene PD-L1 in relation with the grade

Parameter	β-coefficient	Standard error (SE)	Wald's χ^2	p	OR (95% CI)
Grade	0.676	0.241	7.879	0.005	7.227(1.816 - 28.757)

Furthermore, the data obtained from quantitative determination of PD-L1 gene expression were analyzed. The values referred to the expression of PD-L1 gene for each patient as log10(RQ). Descriptive results are presented in Table 4 and Figure 4.

It was evident that the expression of PD-L1 gene was statistically significantly higher in the group of patients with a high grade compared to the low grade (p=0.005).

From the results obtained, it can be seen that there was a correlation between the histological grade of differentiation (low and high) and the quantitative levels of PD-L1 gene expression. Patients with a high grade (poor differentiation) had a 7.227-fold higher probability of having increased levels of PD-L1 gene expression compared to patients with a low grade (good differentiation) in BC. The results of the univariate logistic analysis are presented in Table 5.

The constructed model for predicting the grade based on the expression levels of PD-L1 gene is depicted by the ROC curve in Figure 5.

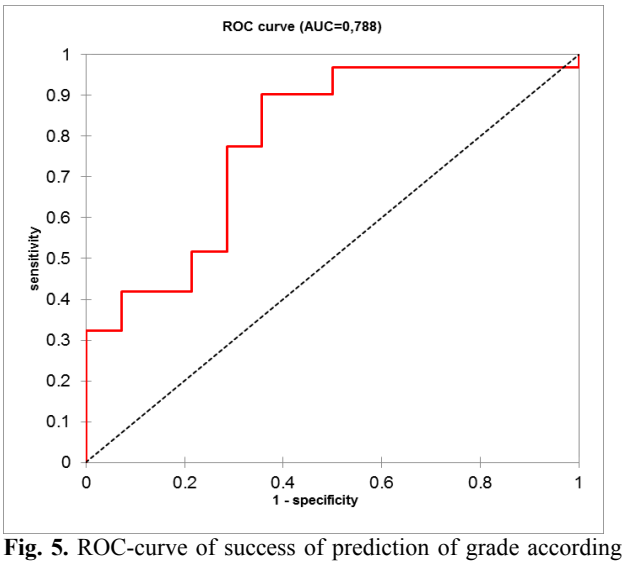

Fig. 5. ROC-curve of success of prediction of grade according to expression of PD-L1 gene in patients with BC

The value of the area under the curve (AUC) was 0.788, indicating a good predictive performance of the model.

Discussion

The results presented in this scientific paper indicated higher levels of transcriptional activity, specifically the expression of PD-L1 gene, in tissue samples from patients with BC with a higher histological grade (well-differentiated) compared to the lower grade (poorly differentiated), and the difference was statistically highly significant (p=0.005).

The expression of PD-L1 gene is present in antigen-presenting cells (APCs), such as monocytes, activeted dendritic cells, and others [7]. PD-L1 is a regulatory ligand that can inhibit immune responses by binding to the PD-1 receptor on the surface of T lymphocytes, leading to apoptosis or anergy of antigen-specific T cells. However, malignant cells can exploit these checkpoint pathways by overexpressing PD-L1, protecting themselves from detection and elimination by cytotoxic T cells of the immune system. Thus, excessive expression of PD-L1 is a mechanism by which tumors develop tolerance to immune responses.

The role of PD-L1 in the progression of bladder cancer, as well as many other malignancies, is undeniable. The correlation between PD-L1 expression in tumor cells and poor clinical outcomes was first reported in a study conducted by Nakanishi *et al.* that included 65 patients with BC [8].

Sharma *et al.* demonstrated that PD-L1 expression in tumor cells was not a good predictor of prognosis [9].

However, most published studies have demonstrated a correlation between elevated expression of PD-1/PD-L1 and a poor prognosis in bladder cancer. According to Kawahara (2018), PD-L1 expression may serve as a novel biomarker that correlates with the pathological grade of bladder cancer [10].

The results of this study also support the existence of a clear correlation and potential clinical utility of PD-L1 expression in predicting disease progression and selecting patients suitable for immune checkpoint inhibitor therapy.

Conclusion

The findings of this study, based on data and specimens from 46 patients, offer compelling evidence of the strong correlation between PD-L1 gene expression and the histological grade of bladder cancer. This correlation suggests that PD-L1 could potentially serve as a novel biomarker, shedding light on the pathological grade of bladder cancer. Such a biomarker could hold a significant clinical value, helping in establishing a more precise prognosis and guiding tailored treatment strategies.

Additionally, the link between PD-L1 and disease stage progression in bladder cancer adds a layer of importance to these discoveries. Elevated PD-L1 expression in high-grade tumors, often indicative of aggressive clinical behavior, implies that PD-L1 may influence the disease progression. This observation could pave the way for the development of targeted therapies aimed at modulating PD-L1 expression, potentially altering the course of bladder cancer at a crucial juncture.

Conflict of interest statement. None declared.

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