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Expresion paterns of various *PDCD1* and *PDL1* isoforms in prostate tumors

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Aim. To assess the relative expression (RE) levels of various isoforms of the *PDCD1* and *PDL1* immune checkpoint genes in prostate tumors, to find the clinically significant alterations in tumors and the correlations with the prostate cancer related and other immune associated genes. **Methods.** Using quantitative PCR, RE levels of different isoforms of *PDCD1* and *PDL1* were analyzed in 35 samples of prostate cancer tissues of a different Gleason score (GS) and at various grades, the paired conventionally normal prostate tissue (CNT) samples and 20 prostate adenomas. **Results.** We have assessed RE levels for 5 variants of long and short isoforms of *PDCD1* and *PDL1* in prostate cancer and noncancerous tissue samples. We detected a significant decrease of RE levels of *PDL1* long isoforms in prostate cancer with GS>7, compared with the adenoma group. Noteworthy, RE of short isoforms of *PDCD1* and *PDL1* has positive correlations with the age. The RE patterns of *PDCD1* and *PDL1* isoforms demonstrated significant correlations with the expression of 31 genes, related to tumor-associated macrophages, fibroblasts and immune cell markers in the prostate cancer group. **Conclusions.** The studied genes are involved in the prostate cancer progression, related to inflammation. The RE levels showed high dispersion in all groups of prostate tissue samples. We propose to assess the RE levels of the *PDCD1* and *PDL1* genes long isoforms, before prescribing immunotherapy methods, to analyse the putative sensitivity of tumors to such treatment. Further studies are needed to confirm the presented here results on a larger patient cohort.

K e y w o r d s: prostate tumors, relative gene expression, *PDCD1, PDL1*, long and short isoforms, pharmacological markers.

Introduction

The PDCD1 and PDL1 (CD274) proteins, together with CTLA4, are involved in the inhibitory immune checkpoint pathways. In other words, these proteins act as negative regulators of the immune response [1]. Recently, several immune checkpoint inhibitors have been developed and they are tested gradein clinical trials [2–5]. An important problem for now is how to predict the effectiveness, possible side effects, development of resistance for such type of therapy, and to propose a drug combination for the patient personalized treatment [4, 6–8].

The bioinformatics analysis of the *PDL1* and *PDCD1* sequences showed that these genes encode both long membrane-bound proteins, and the shorter isoforms. The functions of corresponding proteins in carcinogenesis were not fully elucidated yet. Among numerous mRNA isoforms of the *PDL1* gene, three are the most studied, namely **a** (NM_014143), **b** (NM_001267706), and **c** (NM_001314029). They play different roles in the development of colorectal cancer [9].

Two isoforms were annotated for the *PDCD1* gene as NM_005018 and XM_006712573. Importance of different protein isoforms in the rise and development of various tumors, their impact on the tumor microenvironment, the course of disease, and the sensitivity to therapy were shown [10, 11].

The aim of the present work was to investigate the relation between the expression patterns of various *PDL1* and *PDCD1* isoforms and the clinico-pathological characteristics of prostate tumor samples, in order to study their role in the prostate cancerogenesis.

Materials and Methods

Prostate tissue collection. The tissues of prostate cancer (T) and the paired conventionally normal prostate tissues (CNT, or N from a side, opposite to cancer) were frozen in liquid nitrogen directly after surgery. All samples were collected at National Cancer Institute (Kyiv, Ukraine). The Benign prostate tumors (prostate adenoma samples) were collected with the same procedure at the Institute of Urology (Kyiv, Ukraine) after radical prostatectomy. The samples were collected in accordance with the Declaration of Helsinki and the guidelines, issued by the Ethic Committee of the Institute of Urology and the National Cancer Institute of National Academy of Medical Sciences of Ukraine and the Ethic Committee of the Institute of molecular biology and genetics of NAS of Ukraine. 35 prostate adenocarcinomas (T) of different GS (Gleason score) and grades, 35 paired CNT (Table 1) and 20 samples of benign prostate tumors (A, adenomas) were studied. The tumors were characterized, according to the International System of Classification of Tumors based on the tumor-node-metastasis (TNM) and the World Health Organization criteria classification (Table 1).

Total RNA isolation and cDNA synthesis. 50–70 mg of frozen prostate tissues were mashed to powder in liquid nitrogen. Total RNA was extracted by TRI-reagent (SIGMA), according to the manufacturer's protocol. The total RNA concentration was analyzed by a spectrophotometer (NanoDrop Technologies Inc. USA). The quality of the total RNA was determined in a 1 % agarose gel by band intensity of 28S and 18S rRNA (28S/18S ratio).

Table 1. **Clinico-pathological characteristics of prostate tumor samples.**

Sample number	GS	Stage	PSA ng/ml	Age
1	6	2	27.3	56
2	4	3	23.6	55
3	6	2	6.5	67
4	6	2	25.2	66
5	6	2	18.6	71
6	6	2	9.3	57
7	6	2	6.0	67
8	6	2	5.0	63
9	5	2	13.3	54
10	6	2	29.1	74
11	7	2	11.7	68
12	7	2	13.9	68
13	7	2	19.8	64
14	7	2	7.1	77
15	7	1	8.2	69
16	7	2	19.3	54
17	7	2	5.6	62
18	7	2	14.3	69
19	7	2	24.6	52
20	8	3	86.3	60
21	9	2	37.8	53
22	9	4	22.6	63
23	9	2	6.9	48
24	9	3	51.0	65
25	9	3	0.5	61
26	9	2	20.3	76
27	8	2	9.7	54
28	8	2	12.1	58
29	9	2	25.1	58
30	8	3	16.0	63
31	9	2	84.2	62
32	9	3	20.9	67
33	9	2	17.0	63
34	9	2	33.0	66
35	10	3	106.0	65

GS — Gleason score, PSA — prostate specific antigen

cDNA was synthesized from 1 μg of the total RNA, treated with RNase free DNase I (Thermo Fisher Scientific, USA), using RevertAid H Minus M-MuLV Reverse Transcriptase (Thermo Fisher Scientific, USA), according to the manufacturer's protocol.

Quantitative PCR (qPCR). The levels of relative expression (RE) of different isoforms of *PDCD1* and *PDL1* were assessed by qPCR, using a Maxima SYBR Green Master mix (Thermo Fisher Scientific, USA) and a Bio-Rad CFX96 Real-Time PCR Detection System (USA) under the following conditions: 95 °C — 10 min, following 40 cycles of 95 °C — 15 s, 60 °C — 30 s, 72 °C — 30 s. The primers for all genes were selected, using PRIMER3 and a primer-blast algorithm (https://www.ncbi.nlm.nih.gov/tools/primer-blast/ algorithm).

Reference gene *TBP* was used for normalization [12]. The $2^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$ methods were used for calculations [13, 14].

Statistical analysis. The Kolmogorov-Smirnov test was applied to assess the normality of distribution. The Wilcoxon Matched Pairs test was performed for comparison of RE

levels in prostate adenocarcinomas and paired normal tissues samples [13]. The Benjamini-Hochberg procedure with false discovery rate (FDR) 0.10–0.25 was used under multiple comparisons detection for genes RE levels [15]. The Kruskal-Wallis test was used to determine differences between pairs of experimental groups. The Dunn-Bonferroni (post hoc test) for multiple comparisons was performed to determine RE differences between multiple groups of prostate samples. The Spearman's rank correlation test was used to find possible correlations between gene RE and CPC of prostate tumors [14].

Results and Discussion

The schematic structures of the *PDL1* and *PDCD1* isoforms (mRNAs and proteins) were prepared, using publicly open databases (www.ncbi.nlm.nih.gov) and they are shown on Figure 1.

As was discussed earlier, the most known isoforms of *PDL1* are **a**, **b**, and **c** (Figure 1A). The first two are transmembrane receptors,

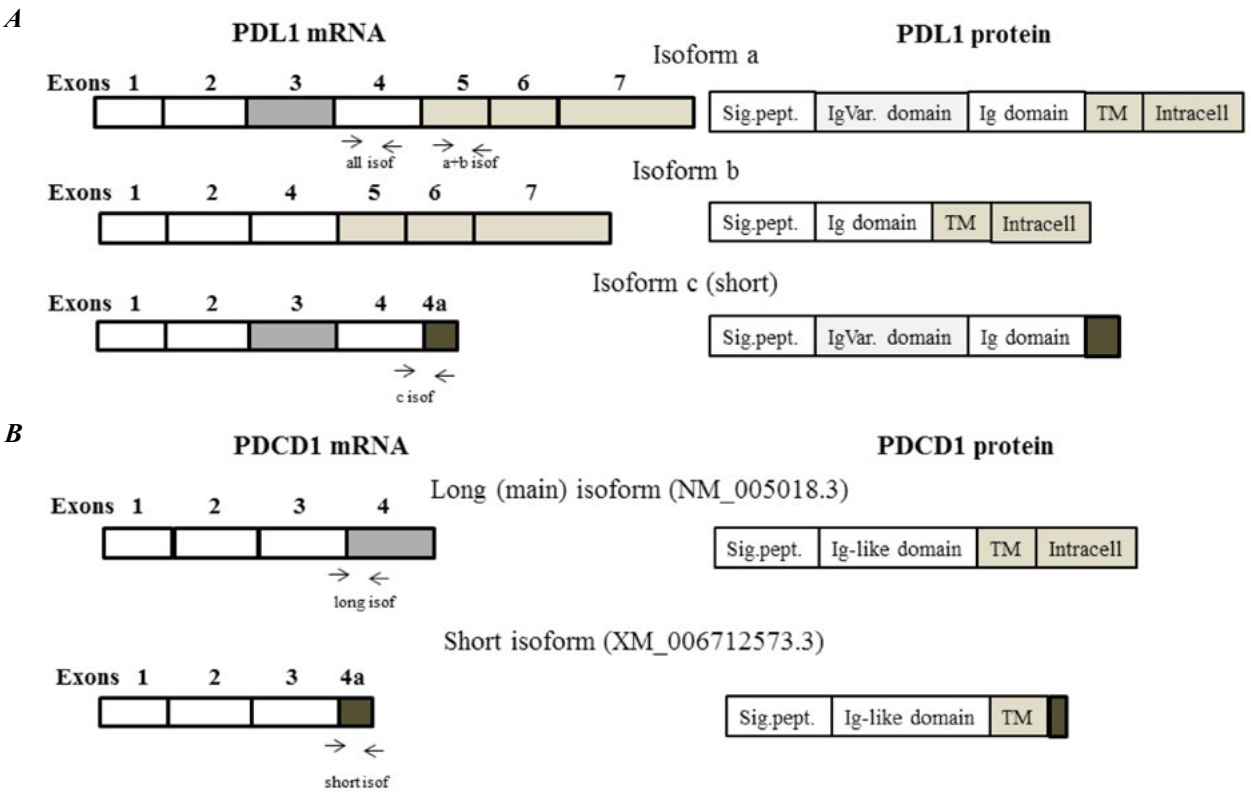


Fig. 1. Schematic representation of various mRNA and protein isoforms, and PCR products (arrows) of the *PDL1* (A) and *PDCD1* (B) genes. Protein domains: Sig. pept. — signal peptide; IgVar domain — Immunoglobulin variable domain; Ig domain — Immunoglobulin domain; Ig-like domain — Immunoglobulin-like domain; TM — transmembrane domain; Intracell — intracellular domain.

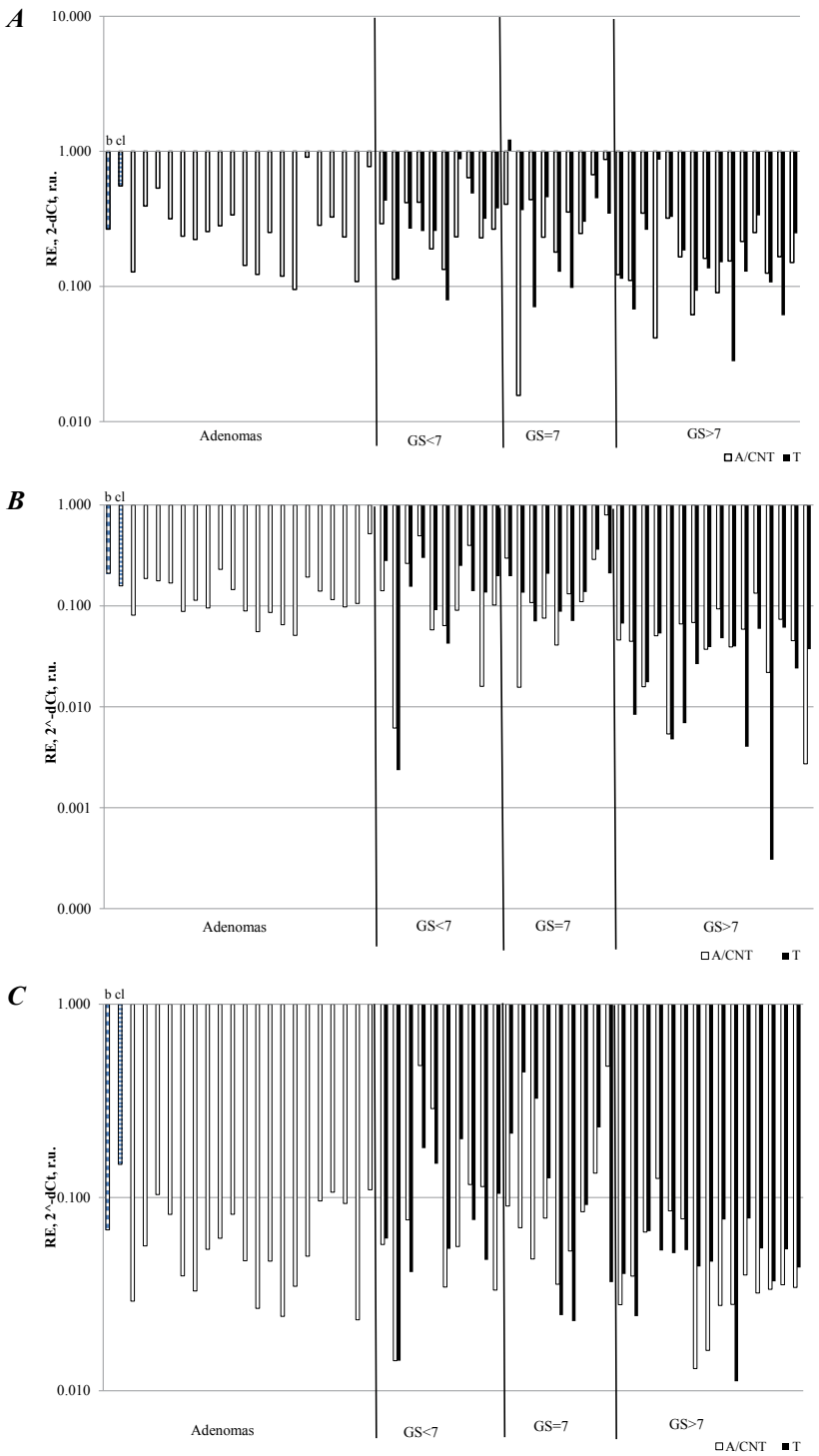


Fig. 2. Expression profiles of different *PDL1* isoforms in investigated samples: *A* — all isoforms of PDL1; *B* — a and b (long membrane-bound) *PDL1* isoforms, *C* — short **c** *PDL1* isoform

whereas the short **c** isoform has transmembrane and intracellular domains deleted. Obviously, the changes in protein structure lead to functional alterations. It is known that both *PDL1* isoforms can be secreted in a soluble form and found in exosomes [16]. The first primer pair allows detection of all known isoforms of *PDL1*, the second pair of primers detects two transmembrane isoforms **a** and **b**, and the third pair of primers is designed only for the short isoform **c**.

For the *PDCD1* gene, two isoforms (Figure 1B), long and short, are annotated in the databases. The short isoform has a trun-

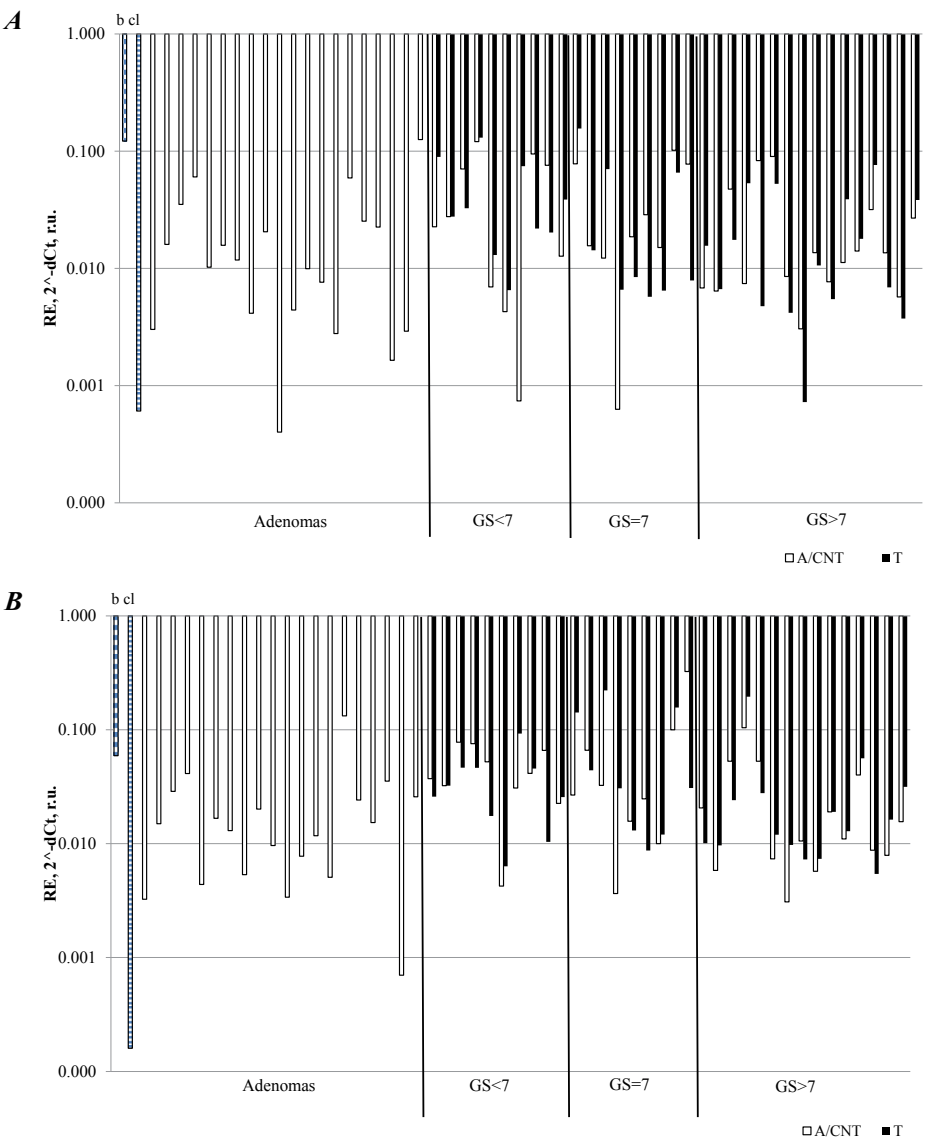


Fig. 3. Expression profiles of different isoforms of *PDCD1* in investigated samples: *A* — RE of long isoform of *PDCD1; B* — RE of short isoform of *PDCD1*.

cated 3'-end of the mRNA, i.e., no intracellular domain in the PDCD1 protein. Probably, this isoform can't conduct a signal from the receptor into the cell. The primers were selected to the last exon of both isoforms, to differentiate them.

We assessed the RE of various *PDL1* and *PDCD1* isoforms in adenomas, adenocarcinomas, and conventionally normal prostate tissues (CNT) (Figures 2A–C, 3A–B). The figures show b (column 1) — RE genes in the blood of healthy donors, cl (column 2) — RE genes in the normal prostate epithelial cell line PNT2. Next, RE levels in benign tumors of the prostate gland — adenomas. After them — paired samples of malignant tumors of the prostate gland (adenocarcinoma) and conventionally normal tissues of the prostate gland. Samples are presented with the increasing Gleason score (GS), that characterizes the change of prostate tissue. That is, tumors with GS<7 are less malignant, whereas the tumors with GS>7 are more malignant.

A high level of RE heterogeneity of all studied isoforms was observed in groups of prostate tumors and CNTs.

The RE levels of various *PDL1* isoforms in the PNT2 cell line are slightly higher than those in the adenoma group whereas the RE levels in the blood for all isoforms and isoform **c** of *PDL1* are slightly lower than in PNT2. A different situation is observed for RE levels of isoforms of the *PCDC1* gene. The RE levels of both isoforms are several folds lower in the PNT2 cell line compared to the RE levels in human blood sample.

However, no significant differences in RE for the studied gene were revealed using the paired Wilcoxon test and Fisher's exact test for paired samples of prostate tumor and CNT samples.

A comparative analysis between the groups of adenomas (A), adenocarcinomas (T) and CNTs of the prostate gland by the Kruskal-Wallis tests with FDR = 0.2 and the Dunn-Bonferroni post-hoc test for multiple comparisons also found no significant differences between the groups (Figure 4). Of note, the significant level of data dispersion is observed, especially in the prostate tumor groups.

When GS and cancer grades were analyzed, together with RE levels a number of significant differences in RE between the studied groups were calculated (Figure 5A–E). A significant decrease in the RE levels was found for the **a** and **b** (long) isoforms of the *PDL1* gene (Figure 5B, Table 2A) in tumors samples with GS > 7 (and corresponding CNTs), compared with adenomas. The RE levels of long isoforms were lower in tumors with GS > 7, compared to tumors with GS < 7.

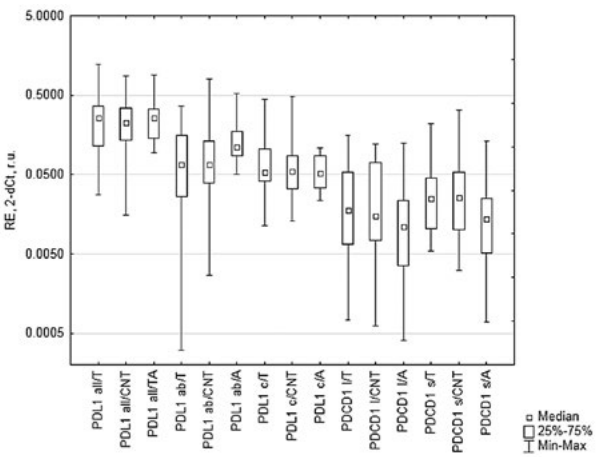
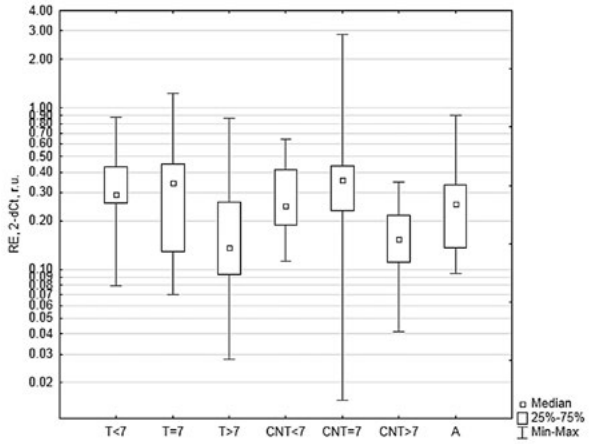
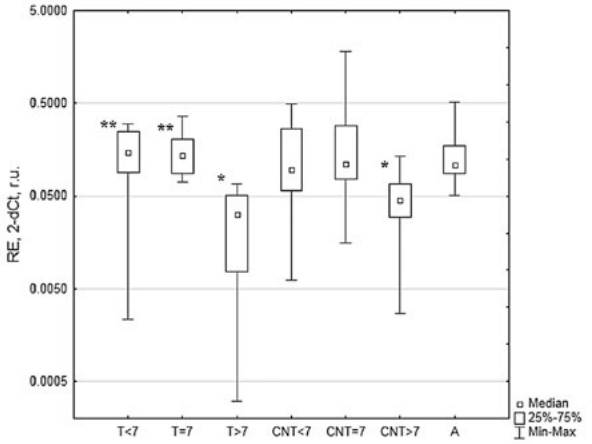


Fig. 4. Boxplots of descriptive statistical data of RE levels of long and short isoforms of *PDL1* and *PDCD1* for T, CNT and A groups of prostate samples

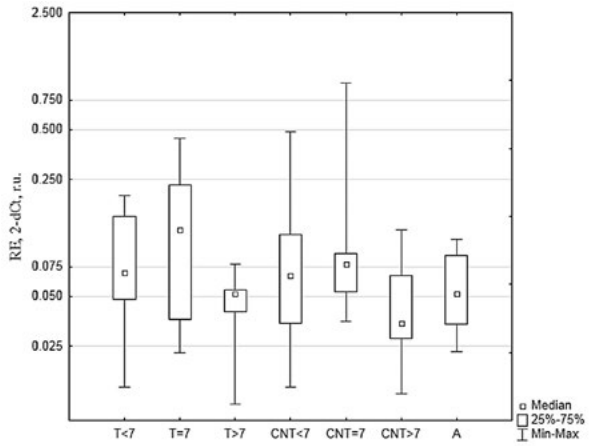
A. Gene expression levels of *PDL1* all isoforms



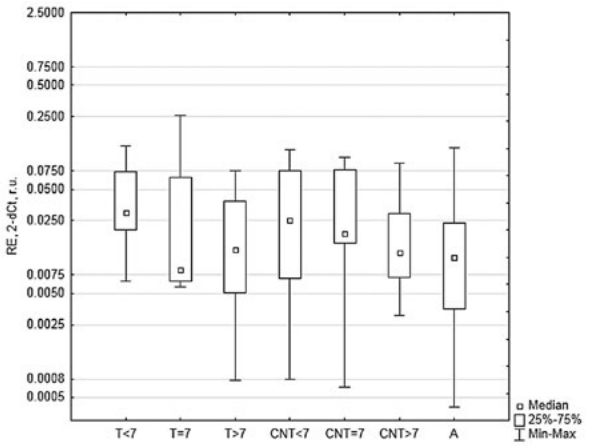
B. Gene expression levels of *PDL1* ab (long) isoforms



C. *PDL1* c (short) isoform



D. *PDCD1* long isoform



E. *PDCD1* short isoform

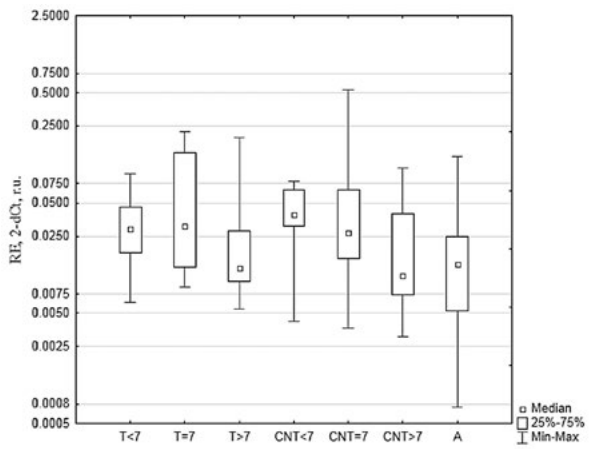


Fig. 5. Boxplots with descriptive statistical data of RE levels of investigated gene transcripts for prostate adenocarcinoma (T), CNT groups taking into account GS, and A group: A. *PDL1* all isoforms; B. *PDL1* ab (long) isoforms; C. *PDL1* c (short) isoform, D. *PDCD1* long isoform; E. *PDCD1* short isoform. * — $p < 0.05$ comparing to adenoma group: ** — $p < 0.05$ comparing to T > 7 group (Dunn-Bonferroni test for multiple comparisons).

Similar patterns of RE were also found for the long **a** and **b** *PDL1* isoforms, when samples were grouped by a cancer grade. The significant RE decrease was calculated for tumors of a grade 3–4, compared to grade 1–2 ($p = 0.0016$) and tumors of grade 3–4, compared with adenomas ($p = 0.0002$) (Table 2B). The same pattern was found in the CNT group, taken from patients with tumors of grade 3–4 compared to adenomas ($p = 0.0019$).

Table 2. **Significant differences of gene expression levels between (vs) groups of prostate samples for *PDL1* ab (long) isoforms:**

Cancer GS grouping

Groups	p-value
T>7 vs A	0.0001
T<7 vs T>7	0.0017
T=7 vs T>7	0.0006
T<7 vs CNT>7	0.0458
CNT>7 vs A	0.0108

Cancer grade grouping

Groups	p-value
T 1-2 st vs T 3-4 st	0.0016
T 3-4 st vs A	0.0002
CNT 3-4 st vs A	0.0019

It has been shown that protein PDL1 is differentially expressed among different cancer types [17–19], in particular, in the primary prostate cancer patients. The high PDL1 expression has been associated with a poor prognosis [20] on one hand, but with high sensitivity to immunotherapy [19, 21] on the other hand. The low PDL1 expression in tumors may be an adverse prognostic factor [22].

It is known that three isoforms of PDL1 proteins (**a, b, c**) have different effects in cancerogenesis. This was shown for colorectal tumors. The isoform **b** has more significant inhibitory effect on T cells, than the other two isoforms. Additionally, the **c** isoform may contribute to the progression of colorectal tumors by regulating the epithelial-mesenchymal transition [9].

Analysis of Spearman's rank correlation (r^s) of gene RE and clinico-pathological characteristics revealed significant correlations ($p < 0.05$) between these parameters (Table 3).

Table 3. **Spearman's rank correlation coefficients (r^s) between the RE of the *PDL1* and *PDCD1* isoforms in prostate tumors and clinico-pathological characteristics (CPC)**

	PDL1 all isof	*PDL1* ab isof	*PDL1* c isof	*PDCD1* long isof	*PDCD1* short isof
GS	-0.2739	**-0.6263**	-0.2120	-0.2861	-0.2248
Stage	-0.0970	**-0.6003**	-0.2391	0.1822	0.1201
PSA ng/ml	***-0.3702***	-0.2487	-0.1288	-0.0801	-0.1774
Age	0.2861	0.2546	**0.5284**	0.1597	***0.3525***

Note: ***p < 0.05*** — bold italic, **p < 0.01** — bold; FDR = 0.2

Significant inverse (negative) correlations with GS and grades have the long **a** and **b** *PDL1* isoforms ($p<0.01$). This means that in the process of tumor progression, expression of these isoforms decreases, while no such relationships were found for the short isoform **c**. However, for short isoforms of the *PDCD1* and *PDL1* genes, positive correlations with the age of patients were found. It is known, that the age is one of the important pathogenetic factors in the development of prostate cancer [23, 24]. Additionally, for colorectal cancer, it was shown that the **c** isoform of the PDL1 protein played a pro-meta-

static role and is the new potential target for the therapy of this type of cancer [9].

For all studied *PDL1* and *PCDC1* isoforms, a high level of dispersion of RE levels was found, especially in groups of prostate tumors, which may indicate the different sensitivity of individual tumors to modern immunotherapy. This confirms the necessity and importance of creating a PCR test system for evaluating gene expression as potential pharmacological markers for evaluating the sensitivity and effectiveness of cancer treatment. Detection of PDL1 and PDCD1 proteins, regardless of isoforms, is already possible by immunohistochemical method [25].

The presence of soluble forms of PD-1/PD-L1 proteins [26] and PD-L1 protein in exosomes was demonstrated. These proteins play an important role in tumor progression, increasing the composition and functional complications of the PD-1/PD-L1 signaling pathway [16]. Additionally, exosomal PD-L1 acts as a biomarker for tumor progression and as the predictive biomarker for response to immunotherapy. We can assume that the short isoforms of *PDL1* and *PDCD1* could be the source of these proteins.

In addition to the correlations with the clinico-pathological characteristics of prostate tumors, we calculated the correlations between RE of the *PDL1* and *PDCD1* isoforms and genes of various functional groups (more than 55 genes), associated with prostate cancer, the expression of which we previously studied in these tumor samples [13, 14, 27]. We found 31 genes, which have significant correlations with the RE levels of the studied transcripts. The largest number of gene correlations was found in the gene groups of tumor-associated macrophages (TAM), cancer-associated fibroblasts (CAF), and immune-associated genes (IAG) (Table 4).

Among the RE levels of three variants of PDL1 isoforms, the correlations for all *PDL1* isoforms showed mainly intermediate results between r^s data for *PDL1* **a** and **b** and *PDL1* **c** isoforms. The highest positive correlations of RE levels of *PDL1* **a** and **b** were found with the expression of genes *HIF1A* ($r^s = 0.8174$), *CCR4* ($r^s = 0.7179$), *CD68* ($r^s = 0.6342$), *CCL22* ($r^s = 0.6311$).

Hypoxia-inducible factor 1 alpha (*HIF1A*) is one of the transcription factors that modulates the activity of up to 1.5 % of genes under hypoxia in various cell types and in the development of many types of cancer [28–30]. In addition to tumor cells, increased expression of *HIF1A* also occurs in the main components of the tumor microenvironment — fibroblasts, which affects the deregulation of the extracellular matrix, increased vascularization, and suppression of the immune response [31].

The next three genes belong to the TAM group whereas the *NOS2A* gene ($r^s = 0.5608$) is a known marker of M1, or tumor suppressor macrophages [32]. It encodes NO synthase 2 [33], the expression of which is a positive predictor for survival in lung cancer patients [34].

The *CD68* gene encodes a glycoprotein of the LAMP family, which is a known marker of anti-inflammatory M2 macrophages [35]. The protein encoded by the *CCL22* gene is secreted by TAM. This causes inhibition of cytotoxic T-lymphocytes and allows tumors to evade the immune response [36]. In this chain, the *CCR4* gene encodes a receptor that is affected by secreted CCL22, enhancing tumor

development and metastasis [37]. So, TAM promote the prostate cancer migration through activation of the CCL22-CCR4 axis [38].

Proteins encoded by the genes that have high positive correlations with the expression of the *PDL1* **a** and **b** and *PDL1* **c** isoforms are *CTGF* (connective tissue growth factor) and the chemokine gene *CXCL12* (C-X-C motif of chemokine ligand 12), that belong to the CAF secretome. They mediate tumor-stroma interactions, increasing tumor proliferation and metastasizing [39, 40]. Activation of the CXCL12/CXCR4 pathway was shown to mediate the phenotypic transition from myofibroblasts to CAF through effects on non-canonical EGFR/MEK/ERK signaling [41]. Another CAF marker — *ACTA2* has positive correlations with almost all studied variants of the *PDL1* and *PDCD1* genes. These data once again emphasize the important connections between the studied genes and the functioning of the CAF in tumor development.

The gene from IAG group, for which five significant positive correlations with the expression of the studied transcripts were found, is *CIAS1*. It is known that the *CIAS1* (NLRP3) gene encodes the protein cryopyrin, which is a component of inflammasomes, a special protein complexes capable of activating caspase activity and the activity of the NF-kB pathway in macrophages and neutrophils [42]. Inflammatory reactions are triggered upon contact with microorganisms, uric acid, and a number of toxins [43, 44]. In the processes of cancerogenesis, a violation of the functioning of cryopyrin inflammasomes was found, which has a contradictory role in the development and progression of various types of cancer [45].

The next gene of the IAG group is the *CTLA4* gene. It has four out of five variants (excluding the isoform **c** of *PDL1*) of significant positive correlations with RE levels of the studied gene transcripts. It encodes immunoglobulin — a protein associated with cytotoxic T-lymphocytes 4, which transmits an inhibitory signal to T cells [46, 47]. Gene *CTLA4*, together with *PDCD1/PDL1*, is one of the most well-known target proteins for immunotherapy of various types of tumors, and an immune checkpoint inhibitor and a marker of sensitivity to this type of therapy, respectively [47–49]. We have shown the potential for determining the diapasons of *CTLA4* expression levels in prostate tumors for the detection of tumor sensitivity to immunotherapy [50].

The *IL1RL1* gene has the highest correlation of the RE level with the *PDL1* **c** isoform. The *IL1RL1* gene encodes interleukin-like receptor 1 and belongs to a family of Toll-like receptors [51]. IL1RL1 is a marker of type 2 T-helper cells (Th2), which take an active part in inflammatory reactions in tumors [52, 53]. Therefore, it is possible to conclude about participation of the *PDL1* **c** isoform in these processes, considering that inflammatory processes are activated with age, having the name inflammaging [54].

The obtained data confirm that *PDL1* is expressed not only in tumor cells, but also in tumor infiltrating lymphocytes [55], CAF [56], thus, it might participate in the processes related to the polarization of macrophages in TAMs in tumors [57]. According to the literature data, *PDCD1* expression is closely associated with TAM [58] and infiltration of various immune cells in tumors of different types [59].

Table 4. **The Spearman's rank correlation (r^s) analysis of RE of different isoforms of *PDCD1* and *PDL1* and genes, associated with prostate cancer, EMT and tumor microenvironment**

Groups	Genes/ Isoforms	*PDL1* all isoforms	*PDL1* ab isoforms	*PDL1* c isoform	*PDCD1* long isoform	*PDCD1* short isoform
EMT-associated genes	*VIM*	0.0225	0.0241	0.2358	*0.3566*	0.1615
	FN1	0.2764	0.1409	*0.4231*	0.1843	*0.4090*
	MMP2	*0.3995*	0.1426	0.2596	*0.4171*	0.2370
	MMP9	0.2975	0.1415	0.1951	***0.4697***	0.2636
	OCLN	-0.1670	0.1387	-0.0952	-0.3123	*-0.3406*
PrCa-associated genes	*VDR*	***0.4173***	***0.4720***	*0.3992*	0.1454	0.1349
	PCA3	***-0.4860***	-0.0725	-0.3335	-0.3255	-0.2953
	PSA	***-0.4368***	-0.1011	-0.3335	***-0.4812***	***-0.5532***
	HOTAIR	0.1924	-0.0880	*0.3892*	0.1238	0.3259
	SCHLAP1	***-0.5316***	***-0.5384***	***-0.4393***	-0.2347	***-0.4545***
TAM	*CD68*	0.3876	**0.6342**	***0.4154***	0.1787	0.1826
	CD163	0.2993	-0.1518	0.1444	*0.3773*	*0.3867*
	CCR4	***0.5007***	**0.7179**	***0.4316***	0.3067	0.1792
	CCL17	0.1911	-0.2095	0.0680	*0.4098*	0.1450
	CCL22	***0.4808***	**0.6311**	*0.3442*	0.1874	0.0958
	NOS2A	0.2856	***0.5608***	0.1835	0.0521	0.0426
CAF	*THY1*	*0.3598*	*0.3720*	***0.4536***	0.3185	***0.4139***
	ACTA2	***0.4304***	***0.4193***	***0.4765***	0.3090	***0.4692***
	CXCL12	0.3791	***0.4552***	***0.5172***	***0.4182***	0.3152
	CXCL14	-0.2086	-0.2008	0.0903	-0.0655	0.1355
	CTGF	***0.4002***	**0.6056**	*0.3516*	0.2745	*0.3870*
	HIF1A	***0.4824***	**0.8174**	***0.4442***	0.0675	0.1991
	S100A4	*0.3592*	-0.1134	0.1957	0.2246	0.2568
IAG	*CIAS*	***0.5160***	***0.4700***	***0.4387***	*0.3532*	***0.4423***
	CTLA4	***0.4912***	***0.5342***	0.2318	***0.4333***	*0.3775*
	KLRK	*0.3467*	0.3210	0.3387	0.1266	0.1603
	IRF1 (T1)	***0.4973***	***0.4882***	***0.4741***	0.2535	*0.3775*
	IL1RL1 (T2)	***0.4044***	0.1608	***0.5676***	0.0487	0.2144
	IL1R1 (T17)	0.2620	***0.4050***	0.2388	-0.1989	0.0130
	IL2RA (Treg)	***0.4399***	***0.4888***	0.3189	*0.3913*	0.1826
	HLA-G	0.1312	-0.0120	0.0524	***0.4798***	0.1426

Note: $p < 0.05$ — italic; ***p < 0.01*** — bold italic; **p < 0.001** — bold; EMT-associated (related) genes — epithelial-to mesenchymal transition associated genes, PrCa — prostate cancer associated genes, TAM — tumor-associated macrophages, CAF — cancer-associated fibroblasts, IAG — immune-associated genes

Therefore, the assessment of the expression pattern of *PDCD1* and *PDL1* isoforms, along with the characterization of IAG, associated with inflammation, can be used to comprehensively evaluate both, the potential effectiveness of immunotherapy and the possibility of immune escape. This will allow obtaining the greatest clinical benefit for the patient [60].

The largest number of correlations with the tumor-associated genes involved in prostate carcinogenesis was found for isoforms of the *PDL1* gene. This indicates a complex system of regulation of both, its expression in different cell types and its effects on the properties of these tumors, their aggressiveness, sensitivity to therapy and the disease prognosis.

The data obtained show the importance of determining the expression ranges of the *PDL1* and *PDCD1* isoforms for the development of PCR test systems that will assist in prediction of the tumor sensitivity to immunotherapy.

Conclusions

The studied genes are involved in the prostate cancer progression, related to inflammation. The RE levels showed high dispersion in all groups of prostate tissue samples. We propose to assess the RE levels of *PDCD1* and *PDL1* genes long isoforms, before prescribing immunotherapy methods, to analyse the putative sensitivity of tumors to such treatment. Further studies are needed to confirm the presented results, on a larger patient cohort.

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Патерни експресії різних ізоформ *PDCD1* та *PDL1* у пухлинах передміхурової залози

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Мета. Оцінити рівні відносної експресії (ВЕ) різних ізоформ генів імунних контрольних точок *PDCD1* і *PDL1* у пухлинах передміхурової залози та знайти клінічно значущі зміни в пухлинах і кореляції з генами, пов'язаними з раком передміхурової залози, та іншими імуноасоційованими генами. **Методи.** Рівні ВЕ різних ізоформ *PDCD1* і *PDL1* були проаналізовані в 35 зразках тканин раку передміхурової залози з різним ступенем за Глісоном (СГ) і при різних стадіях, парних зразках умовно нормальної тканини передміхурової залози (УНТ) і 20 аденом передміхурової залози шляхом кількісного ПЛР аналізу. **Результати.** Ми оцінили рівні ВЕ для 5 варіантів довгих і коротких ізоформ *PDCD1* і *PDL1* у зразках раку передміхурової залози та неракових тканинах. Ми виявили значне зниження рівнів ВЕ довгих ізоформ *PDL1* при раку з СГ > 7 по-

рівняно з групою аденоми. Слід зазначити, що ВЕ коротких ізоформ *PDCD1* і *PDL1* мають позитивні кореляції з віком. Патерни ВЕ ізоформ *PDCD1* і *PDL1* продемонстрували значні кореляції з експресією 31 гена, пов'язаного з з пухлино-асоційованими макрофагами, фібробластами та маркерами імунних клітин у групі раку передміхурової залози. **Висновки.** Вивчені гени беруть участь у прогресуванні раку передміхурової залози, пов'язаному із запаленням. Рівні ВЕ показали високу дисперсію у всіх групах зразків тканини передміхурової залози. Ми пропонуємо оцінювати рівні ВЕ довгих ізоформ генів *PDCD1* і *PDL1* перед призначенням методів імунотерапії, щоб проаналізувати передбачувану чутливість пухлин до такого лікування. Для підтвердження представлених тут результатів необхідні подальші дослідження на більшій когорті пацієнтів.

Ключові слова: пухлини передміхурової залози, відносна експресія генів, *PDCD1*, *PDL1*, довгі та короткі ізоформи, фармакологічні маркери.

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