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Soluble Ligand of the Immune Checkpoint Receptor (sPD-L1) in Blood Serum of Patients with Renal Cell Carcinoma

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The content of the soluble ligand of the immune checkpoint receptor (sPD-L1) was determined in the blood serum of 106 patients with renal cell carcinoma and 11 patients with benign kidney tumors by direct ELISA (Human sPD-L1 Platinum ELISA; Affimetrix, eBioscience). The control group included 19 healthy men and 18 women. Serum level of sPD-L1 significantly surpassed the control values in both patients with primary renal cancer ($p<0.0001$) and in patients examined during disease progression ($p<0.05$). In patients with benign kidney tumors, the level of this marker was significantly higher than in the control ($p<0.05$), but lower than in patients with renal cell carcinoma. The sPD-L1 level significantly increased with disease stage ($p<0.001$); it was higher in the presence of metastases in regional lymph nodes irrespective of their number (N1 or N2) than in the absence of metastases (N0); it was also increased in patients with distant metastases (M1) and patients with grade III-IV tumors in comparison with grade III-IV tumors ($p<0.05$). The highest sPD-L1 levels were recorded in patients with tumor size corresponding to T2 and T3 and decreased in patients with T4 tumors. Thus, sPD-L1 level in patients with renal cell carcinoma correlated with tumor grade and metastasizing and can be considered as a promising marker in monitoring of the effect of anti-PD1/PD-L1 therapy.

Key Words: *immune checkpoint proteins; sPD-L1; renal cell carcinoma; blood serum*

Resistance to the body immune response is an important property of malignant tumors determining their growth and progression. Among different ways of escaping antitumor immunity, an important role is played by modification of the so-called immunity checkpoint signaling pathway (PD-1/PD-L) that under physiological conditions controls the severity and duration of the immune response, prevents autoimmune aggression and damage own tissues [1]. The main components of this signaling pathway are programmed cell death protein PD-1, membrane receptor-1 of the CD28/CTLA-4 family of T-cell regulators expressed on their surface, and two its ligands PD-L1 and PD-L2. PD-L1 known also as CD274 or B7 homologue-1 (B7-H1) is most important. Normally, PD-L1 is expressed primarily on antigen-presenting cells, on dendritic and macrophage-like cells of peripheral organs, as well as on cells of the placenta, pancreatic islets, and retina. Nevertheless, the corresponding mRNA has been revealed in a wider spectrum of tissues, and induced PD-L1 expression can be observed in T and B lymphocytes, natural killers, macrophages, mesenchymal stem cells, and epithelial cells. The PD-1/PD-L1 pathway stimulates apoptosis of antigen-specific T cells in the lymph nodes and simultaneously suppresses apoptosis of regulatory suppressor T cells (Treg).

Activation of the PD-1/PD-L1 pathway in the tumor enables its escape from the immune response via

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the development of tolerance to T cells, activation of apoptosis followed by exhaustion of the pool of effector T cell, and enhancement of the immunosuppressive function of Treg. Specific suppression of activity of the PD-1/PD-L1 pathway with immune checkpoint inhibitors is considered as a most promising field in antitumor immunotherapy. In this context, monoclonal antibodies suppressing the interaction of PD-1 with its ligands are actively used in cancer treatment [7], including renal cell carcinoma, one of the most immunesensitive tumors [1,10].

In addition to extensive investigation of the possible role of PD-1 and/or PD-L1 expression on tumor cells or tumor-infiltrating lymphocytes as immunotherapy effectiveness predictors [1], these proteins are considered as molecular markers of the overall prognosis of the disease course and survival in cancer patients. Unfavorable effect of PD-1 pathway on the clinical course of non-small-cell lung cancer [11], melanoma [2], breast cancer [14], renal cell carcinoma [9], and osteosarcoma [8] has been demonstrated. In a number of large randomized studies, evaluation of the association of PD-L1 expression with effectiveness of anti-PD-1 therapy by immunohistochemical methods (IHC) yielded ambiguous results that depended on both the preparation and the disease type. In addition, standardization of IHC-detection of PD-1 and PD-L1 expression is associated with a number of difficulties related to sample preparation technique, application of antibodies with different affinity, specificity and ability to bind to different PD-L1 epitopes, and criteria used in interpretation of the results [13]. A problem in IHC detection is that PD-L1 is expressed not only by tumor cells, but also by immune cells infiltrating it, and at this research stage it is not known which expression pattern has the greatest clinical significance. Another problem is the presence of non-membrane PD-L1 forms that can determine false positive results, though their role in tumor pathogenesis is not quite clear yet.

Investigation of soluble PD-1 (sPD-1) forms and its ligand (sPD-L1) that have been also detected recently in the peripheral blood of cancer patients would be useful for solution of at least some of these problems [15]. The exact origin of sPD-1 and sPD-L1 remains unclear. Similar to soluble forms of other membrane proteins, they can be produced as a result of two processes: hydrolytic cleavage of the extracellular domain or alternative mRNA splicing of native membrane form. There are few publications on this topic, most of them are summarized in the fundamental review [15], in the meta-analytical study [4], and in some other publications [6,12], but this field is actively developing.

The aim of this study was to compare the serum sPD-L1 level in practically healthy subjects and in patients with renal cancer and benign tumors, as well as to analyze the relationship of this marker and the main clinical and morphological features of renal cell carcinoma.

MATERIALS AND METHODS

We examined 106 patients with renal cell carcinoma (98 primary patients and 8 patients during disease progression), 66 men and 40 women (aged 33-81 years, median 59 years), and 11 patients with benign kidney tumors, 3 men and 8 women (aged 29-84 years, median 52 years). The control group included 19 conventionally healthy men and 18 women aged from 22 to 82 years (median 49 years). In all cases, the clinical diagnosis of the primary kidney tumor was confirmed by histological examination. The tumor differentiation degree was investigated in cases of clear-cell and papillary renal cancer.

Blood serum samples were collected by the standard method prior to the beginning of specific treatment. Serum sPD-L1 concentration was measured by direct ELISA (Human sPD-L1 Platinum ELISA, Affimetrix, eBioscience) according to manufacturer's instructions. Measurements were carried out on BEP 2000 Advance automated immunoassay analyzer (Siemens Healthcare Diagnostics). sPD-L1 concentration was expressed in pg/ml serum.

The results were processed using Statistica 7.0. The data were compared and interrelationships were analyzed using non-parametric Mann—Whitney test, Kruskal—Wallis test, and median test. The differences were significant at $p<0.05$.

RESULTS

Serum level of sPD-L1 was significantly elevated relative to the control in both primary patients with renal carcinoma ($p<0.0001$) and in patients with tumor progression ($p<0.05$) (Table 1). Marker concentration in patients with benign kidney neoplasms was also significantly higher than in the control ($p<0.05$), but lower than in patients with renal cell carcinoma, however, this difference was statistically insignificant.

There were no significant differences between serum sPD-L1 levels in primary patients with renal cell carcinoma and patients with tumor progression. Therefore, further analysis of clinical and morphological correlations was performed in the total population of 106 patients.

No significant correlation of sPD-L1 level with patient's age was found either in patients or in the control group, although the increase in sPD-L1 concentration with age was reported previously [3]. Among patients with renal cancer, the serum marker level in

TABLE 1. sPD-L1 Concentration (pg/ml) in Blood Serum of Patients with Renal Tumors

Group	N	Range	Median	25-75%
Patients with primary renal cancer	98	0-464	27.8**	12.2-38.7
Patients with renal cancer during disease progression	8	0-88.1	35.2*	12.2-48.6
Patients with benign kidney tumors	11	0.5-67.3	19.3*	14.6-42.2
Control	37	0-41.8	13.0	0.9-19.3

Note. *p<0.05, **p<0.0001 in comparison with the control.

men was by 2 times higher than that in women (medians 32.1 and 17.9 pg/ml, respectively; p<0.05), in the control group such a pattern was not detected.

Analysis of the relationship between serum sPD-L1 level and the main parameters renal cancer expansion (Table 2) showed that the marker level significantly increased with increasing the disease stage (p<0.001, Kruskal—Wallis test): at stage I (median 17.8 pg/ml) it practically did not differ from indicators of patients with benign neoplasms and the control group (Table 1), and at stages III-IV it surpassed the median in the above groups by 2 and 3 times, respectively.

The relationship of marker concentration with tumor dimensions and primary tumor expansion (T index) was more complicated: the highest sPD-L1 levels were recorded in T2 (tumor diameter>7 cm located within the kidney) and T3 (tumor spreads to the main veins or invades the adrenal gland or adjacent tissues, but not the renal fascia), but in T4 tumors (tumor spreads beyond the Gerota's fascia), sPD-L1 level decreased.

In the presence of metastases in regional lymph nodesirrespective of their number (N1 or N2), the sPD-L1 level was significantly higher than in patients without regional metastases (N0). Marker concentration was also elevated in the presence of distant metastases (M+).

Most malignant kidney tumors (85%) had a histological structure of clear cell carcinoma, 7% had a chromophobic structure and 8% had a structure of papillary renal cell carcinoma (type 1 in 6 cases and type 2 in 3 cases). There were no significant differences in serum sPD-L1 levels in tumors with different histological structure (Table 3). It is worthy of note that relatively high marker concentrations were detected in case of papillary cancer type 2 and in grade III-IV tumors (G3-G4) compared to grade I-II tumors (G1-G2) (p<0.05). Abnormally high sPD-L1 level (464 pg/ml) was detected in only one patient with stage I (T1N0M0) of type 1 papillary cancer and low tumor grade (G1).

Our findings suggest that sPD-L1 level (soluble form of the key ligand of the controlled cell death protein PD-1) in the serum of patients with renal cell carcinoma is increased in comparison with the con-

TABLE 2. sPD-L1 Concentration (pg/ml) in Blood Serum of Patients with Renal Tumors Depending on Tumor Expansion Indices

Expansion index		N	Range	Median	25-75%
Stage	I	57	0-464	17.8	10.7-33.3
	II	12	0-81.5	27.4	14.7-40.6
	III	15	0-107	38.4*	4.9-69.3
	IV	22	4.9-88.1	42.6**	28.6-70.9
Tumor size (T)	T1	55	0-464	17.3	10.7-32.5
	T2	18	0-86.7	35.7^{+}	27.0-46.9
	T3	30	0-107	38.4^{+}	17.7-69.3
	T4	3	20.9-32.1	25.3	20.9-32.1
Metastases in lymph nodes (N)	N0	88	0-464	23.8	10.7-36.9
	N1	8	9.2-86.7	42.0^{x}	28.7-63.7
	N2	10	6.4-88.1	42.6^{x}	26.6-77.2
Distant metastases (M)	M0	95	0-464	25.9	10.7-38.4
	M1	11	12.2-88.1	40.5^{o}	26.9-65.7

Note. *p<0.05, **p<0.01 in comparison with stage I, ^{+}p<0.01 in comparison with T1, ^{x}p<0.03 in comparison with N0, ^{o}p<0.05 in comparison with M0 (Mann—Whitney test).

TABLE 3. sPD-L1 Concentration (pg/ml) in Blood Serum of Patients with Renal Cancer Depending on the Tumor Histology and Grade

Expansion index		N	Range	Median	25-75%
Histology	clear cell cancer	90	0-107	26.8	12.2-40.7
	chromophobe cancer	7	0.5-50	18.0	12.2-47.1
	papillar cancer type 1	6	0-464	27.7	12.2-33.6
	papillar cancer type 2	3	29.6-78.8	77.2	29.6-78.8
Grade	G1	8	0-464	23.8	4.9-79.3
	G2	60	0-107	24.7	10.7-36
	G1-G2	68	0-464	24.3*	10.7-36
	G3	17	0-88.1	32.1	19.5-69.3
	G4	14	0-87.9	35.5	9.3-51
	G3-G4	31	0-88.1	32.3	18.1-56.7

Note. *$p<0.05$ in comparison with G3-G4 (Mann—Whitney test).

trol; it increases with process expansion and in case of highly malignant tumors. These data are in line with the results of the only sPD-L1 (sB7-H1) study published until now that demonstrated poorer survival rate of patients with clear cell renal cell carcinoma and high sB7-H1 levels [5]. The increased serum sPD-L1 concentration and its association with tumor dissemination were also noted in patients with gastric, liver, non-small cell lung cancer, and some types of lymphomas [15]. The negative effect of high sPD-L1 level on patient survival rates has also been demonstrated for these diseases. Based on these data, it can be assumed that sPD-L1 circulating in the blood binds to PD1 on lymphocytes and contributes to tumor escape the from the immune response and disease progression, although existence of such a mechanism has not yet been proven.

At the same time, the data on head and neck squamous cell carcinoma are contradictory, and no significant increase in sPD-L1 level and its relationship with the main clinical and morphological disease factors has been found in cases of pancreatic and cervical carcinomas [15]. It should be noted that for the majority of localizations there have been published only single studies using different test systems. In particular, the study [5] of sB7-H1 in renal cancer used a custom-made system developed in the laboratory, whereas our study was performed using standardized ELISA reagent kits. Nevertheless, various data on sPD-L1 accumulated over the past few years indicate future outlook of further investigation of the role of this marker in tumors of various localizations. Analysis of its dynamics against the background of specific anti-PD-1/PD-L therapy is of particular interest.

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