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Soluble B7-H3 in Ovarian Cancer and Its Predictive Value

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The content of the soluble form of protein of the key point of immunity B7-H3 (sB7-H3) in the blood plasma of 75 patients with epithelial ovarian cancer before treatment was measured by ELISA. It is known that B7-H3 belongs to the immunoglobulin superfamily (B7 molecule family) and is involved in the regulation of the immune response mediated by T cells. The sB7-H3 concentration correlated with the clinical and morphological parameters of ovarian cancer. The content of sB7-H3 was higher at the later stages of the disease, in the presence of ascites, and in patients with poorly differentiated ovarian cancer. It was revealed that increased plasma content of sB7-H3 in patients with epithelial ovarian cancer is associated with unfavorable prognosis of the disease. Therefore, sB7-H3 can be used as a prognostic marker in ovarian cancer patients.

Key Words: *ovarian cancer; sB7-H3; survival*

Recent scientific research has identified several promising immune checkpoints, such as VISTA, B7-H3, LAG3, and others, as targets that can be used to develop new effective immunotherapy for the treatment of ovarian cancer. B7-H3, a key representative of the B7 molecule superfamily, also called CD276, is a type I transmembrane glycoprotein encoded by a gene on human chromosome 15 [4]. Protein B7-H3 encoded by this gene is involved in the regulation of the immune response mediated by T cells. However, the role of B7-H3 in modulating the immune response is controversial. The scientific community has published a large number of works that report both the activating and inhibitory properties of the B7-H3 protein. Thus, recent studies have shown that B7-H3 plays a co-inhibitory role towards T cells, which contributes to the tumor immune escape [8,12]. Moreover, B7-H3 was found to be a critical promoter of proliferation, migration, invasion, epithelial-mesenchymal transition, and drug resistance [6].

However, the stimulating effect of B7-H3 on the T-cell response and IFNγ production was discovered first [1]. Then, it was found that B7-H3 can both stimulate T cells *in vitro* [7,14] and inhibit their functions [5]. Other researchers revealed no correlation between the expression of B7-H3 and functional activity of T cells [9]. It was hypothesized that the immunomodulatory properties of B7-H3 depend on the presence of other signals. Soluble form of this protein (sB7-H3) can be detected in blood plasma and is involved in the progression of colorectal cancer, pancreatic cancer, and some other types of cancer [10,13].

Our aim was to analyze the content of the soluble form of B7-H3 (sB7-H3) in the blood plasma of patients with epithelial ovarian cancer and its association with the clinical and morphological characteristics and prognosis of the disease.

MATERIALS AND METHODS

The study included 75 women at the age of 29 to 81 years (mean age 54.6 years) with verified epithelial ovarian cancer, patients of the N. N. Blokhin National

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Medical Research Center of Oncology. Blood plasma samples were obtained from patients with three main histological types of the tumors: serous, mucinous, and endometrioid ovarian cancer (Table 1). All tumors were examined histologically and characterized in accordance with the International Classification of Tumors of the Female Reproductive System (WHO, 2013). All patients signed informed consent to participate in the study.

The concentration of sB7-H3 protein was determined by ELISA in blood plasma obtained according to the standard method using EDTA before the start of specific treatment. Human B7-H3 Quantikine ELISA Kit (Cat # DB7H30, R&D) was used according to the manufacturer's instructions. Measurements were performed on an automatic BEP 2000 Advance ELISA analyzer (Siemens Healthcare Diagnostics). The content of the marker was expressed in ng/ml plasma.

To analyze delayed results of treatment (overall survival), the patients were divided into 2 comparison groups: with sB7-H3content above or below the median concentration.

The results were statistically analyzed using GraphPad Prizm 9.0. The significance of differences in the observed frequencies of signs in the studied groups was determined using the χ^2. Survival analysis was performed by constructing survival curves using the Kaplan—Meier method for the total surveyed group irrespective of the histological characteristics of the tumor and stage of the disease. Significance of differences was evaluated using the log rank test. The differences were considered statistically significant at $p<0.05$.

TABLE 1. Clinical and Morphological Characteristics of Patients

Parameter		Number of patients	
		n	%
Histology	mucinous	10	13
	serous	52	70
	endometrioid	13	17
Stage	I-II	39	52
	III-IV	36	48
Age	<60 years	52	69
	≥60 years	23	31
Ascites (A)	no	32	43
	yes	43	57
Grade (G)	G1/G2	39	52
	G3	36	48

RESULTS

The analysis showed the presence of significant correlations between the concentration of sB7-H3 in the blood plasma of patients with ovarian cancer and the main clinical and morphological characteristics of the disease (Table 2). Higher plasma levels of sB7-H3 were found in patients with advanced stages of the tumor process, with ascites, and with low-differentiated neoplasm. These findings suggest that the concentration of sB7-H3 in blood plasma of patients with ovarian cancer before treatment correlated with tumor progression and unfavorable clinical and morphological characteristics of the disease. Analysis of the prognostic significance of sB7-H3 levels in blood plasma showed that it is an unfavorable prognostic marker, but the data did not reach statistical significance ($p=0.075$) (Fig. 1).

TABLE 2. Association of sB7-H3 Content in Blood Plasma of Patients and Clinical and Morphological Characteristics of Ovarian Cancer

Characteristic		sB7-H3 level, number of patients		Significance
		high	low	
Histology	mucinous	8	2	$p=0.11$
	serous	23	29	
	endometrioid	7	6	
Stage	I-II	14	25	*$p=0.02$
	III-IV	23	13	
Age	< 60 years	24	28	$p=0.41$
	≥60 years	13	10	
Ascites (A)	no	15	26	*$p=0.02$
	yes	22	12	
Grade (G)	G1/G2	15	24	*$p=0.03$
	G3	23	13	

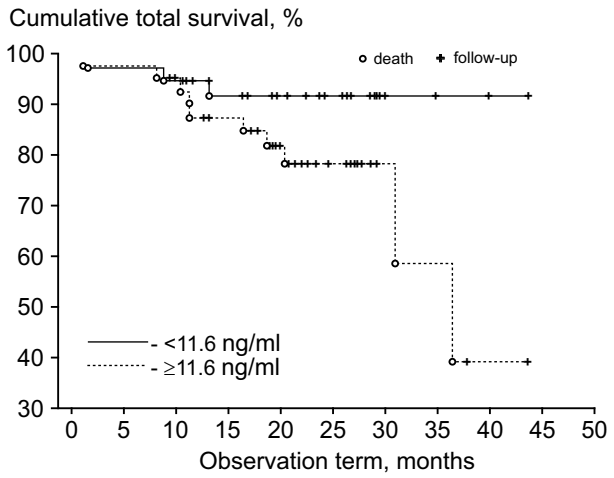

Fig. 1. Overall survival of patients with ovarian cancer with blood concentration of sB7-H3 below (<11.6 ng/ml) and above the median (≥11.6 ng/ml).

In evolutionary terms, ligand B7-H3 is one of the most conserved members of the B7 family that was found in various animal species, from teleost fish to mammals [11]. B7-H3 can bind to such known checkpoint proteins as CTLA-4, PD-1, and CD28. Various studies have demonstrated that B7-H3 is predominantly expressed by cells of various malignant tumors, but its weak expression was also detected in normal tissues. The highest levels of B7-H3 were found in the placenta, endometrium, gallbladder, urinary bladder, and prostate tissues. Interestingly, this marker is overexpressed in a wide range of tumor cells and is associated with disease progression and prognosis [3]. For ovarian cancer, increased expression of B7-H3 in the tumor correlates with the degree of malignancy of the neoplasm [15], which is consistent with our data.

Recently, a humanized monoclonal antibody (Fc-mAb IgG1) enoblituzumab (MGA271) against B7-H3 was developed and transferred to clinical trials [2]. Of the five clinical trials in progress with enoblituzumab, one has been completed, but definitive results are not yet available. All these data testify in favor of the relevance and prospects of the study of this protein for the development of new methods of immunotherapy.

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