DOI 10.1007/s10517-023-05972-2
Bulletin of Experimental Biology and Medicine, Vol. 176, No. 1, November, 2023 ONCOLOGY

Soluble B7-H3 in Colorectal Cancer

O. V. Kovaleva, A. N. Gratchev, N. Yu. Sokolov, V. V. Maslennikov, Yu. B. Kuzmin, E. S. Gershtein, A. A. Alferov, Z. Z. Mamedli, I. S. Stilidi, and N. E. Kushlinskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 176, No. 7, pp. 101-105, July, 2023
Original article submitted April 13, 2023

We present the results of comparative ELISA of the concentration of soluble form of immunity checkpoint B7-H3 (sB7-H3) in the serum of patients with colorectal cancer (CRC) at different stages before treatment and healthy control donors. The analysis revealed a statistically significant difference between the median levels of sB7-H3 in the blood serum of CRC patients (19.66 ng/ml) and healthy donors (16.76 ng/ml) (p=0.0025). ROC analysis showed 62.9% sensitivity and 56.7% specificity for CRC patients (cut-off 17.62 ng/ml; p=0.0028). An association of sB7-H3 levels with tumor progression was revealed. We demonstrated that sB7-H3 levels were significantly lower in patients with regional metastases than in patients without metastases (p=0.039) and that sB7-H3 concentration tends to decrease at the late stages of the disease. Thus, high serum level of sB7-H3 in CRC patients can be a favorable prognostic factor in future.

Key Words: *colorectal cancer; sB7-H3; blood serum; prognosis; tumor progression*

B7-H3, also known as CD276, is an immunoregulatory molecule with costimulatory/coinhibitory effects [1]. It was first cloned in 2001 from a human dendritic cell (DC) cDNA library [2]. The B7-H3 protein exists as a transmembrane or soluble isoform. Membrane-bound B7-H3 is a type I transmembrane protein comprising 316 amino acids and having a molecular weight of ~45-66 kDa [3]. It consists of an extracellular domain, a transmembrane domain, and a short intracellular domain. Soluble B7-H3 (sB7-H3) can be formed by cleavage from the cell surface by MMP or by alternative splicing. sB7-H3 can be detected in human sera [4] and in extracellular vesicles [5].

In most normal human tissues, B7-H3 is widely expressed at the mRNA level, while B7-H3 protein is relatively rare detected, suggesting a tight post-transcriptional regulation mechanism [5]. In tumor cells, B7-H3 is highly expressed at both mRNA and protein levels. Its expression can be regulated by various microRNAs such as *miR-199a*, *miR-128*, and *miR-187* [6]. In addition, BRD4, ILT-4, and ELK1 regulate B7-H3 expression via the PI3K/AKT/mTOR signaling pathway and modulate its content at the transcriptional or epigenetic level [7].

Tissue expression of B7-H3 has been widely studied in various types of malignancies, including breast, lung, ovarian, gastric, and other cancers. Its expression level often correlates with unfavorable prognosis and higher recurrence rate [8]. For some types of tumors, an increase in sB7-H3 content during their invasion and metastasis has also been described. Numerous studies on both model systems and clinical samples show that B7-H3 is a promising target for the development of new immunotherapeutic agents.

This paper focuses on the study of sB7-H3 levels in serum samples from colorectal cancer (CRC) patients by ELISA.

MATERIALS AND METHODS

The study included 71 patients aged 27-83 years (median age 60 years) treated at the N. N. Blokhin National Medical Research Center for Oncology with

N. N. Blokhin National Medical Research Center of Oncology, Ministry of Health of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** ovkovaleva@gmail.com. O. V. Kovaleva

a verified diagnosis of CRC. All procedures comply with the standards of the Ethical Committee of the N. N. Blokhin National Medical Research Center for Oncology and the Declaration of Helsinki. Informed voluntary consent was obtained from each participant included in the study. Clinical diagnosis in all patients was confirmed by data of morphological examination of the tumor according to the WHO Classification of Tumours: Digestive System Tumours (2019). In all patients, adenocarcinoma of the colon was detected. The control group consisted of healthy donors (20 women and 10 men) aged 23-71 years (median age 47 years).

The concentration of sB7-H3 protein was measured in the serum obtained according to the standard procedure before specific treatment using the Human B7-H3 Quantikine ELISA Kit (cat.# DB7H30, R&D) according to the manufacturer's instructions. Measurements were performed on a BEP 2000 Advance automated immunoassay (Siemens Healthcare Diagnostics). The content of the marker was expressed in ng/ml blood plasma.

Statistical analysis of the results was performed using GraphPad Prism 9 (GraphPad Software). Mann–Whitney and Kruskal–Wallis tests were used to determine statistically significant differences in the independent groups. Analysis of the informativity of diagnostic method by evaluating its sensitivity and specificity was performed by plotting ROC curves and calculating the area under them (AUC). Analysis of overall survival was performed by plotting survival curves according to the Kaplan–Meier method. To analyze long-term results of treatment (overall survival rate), the patients were divided into 2 comparison groups depending on the sB7-H3 content in blood plasma above or below the median. Comparison of the statistical significance of differences was performed using the log-rank criterion. The differences were considered statistically significant at $p<0.05$.

RESULTS

At the first stage of the study, we evaluated the level of sB7-H3 in the control group and in patients with CRC. In addition, we analyzed the information value of the diagnostic method by evaluating its sensitivity and specificity by ROC curves and AUC (Fig. 1).

The analysis revealed a statistically significant difference between the median levels of sB7-H3 in the serum of CRC patients and healthy donors. The median sB7-H3 content was 16.76 (11.08-20.58) ng/ml in the control group and 19.66 (16.08-25.04) ng/ml in the group of patients with CRC ($p=0.0025$). ROC analysis showed a sensitivity of 62.9% and specificity of 56.7% (cut-off 17.62 ng/ml; $p=0.0028$) for CRC patients.

Analyzed of serum sB7-H3 levels with consideration for the main clinical and morphological characteristics of the disease showed that sB7-H3 content is associated with tumor progression (Table 1). The content of sB7-H3 in the group of patients with regional metastases was lower than in patients without metastases ($p=0.039$). A tendency to a decrease in this parameter at the late stages of the disease was also revealed ($p=0.069$).

Evaluation of prognostic significance of sB7-H3 showed that high serum levels of this protein in CRC patients can be a promising favorable prognostic factor ($p=0.05$; Fig. 2).

Published reports on the sB7-H3 protein are scanty. It was found that the content of this protein in patients with CRC was higher than in healthy donors [9], which is consistent with our findings. Several

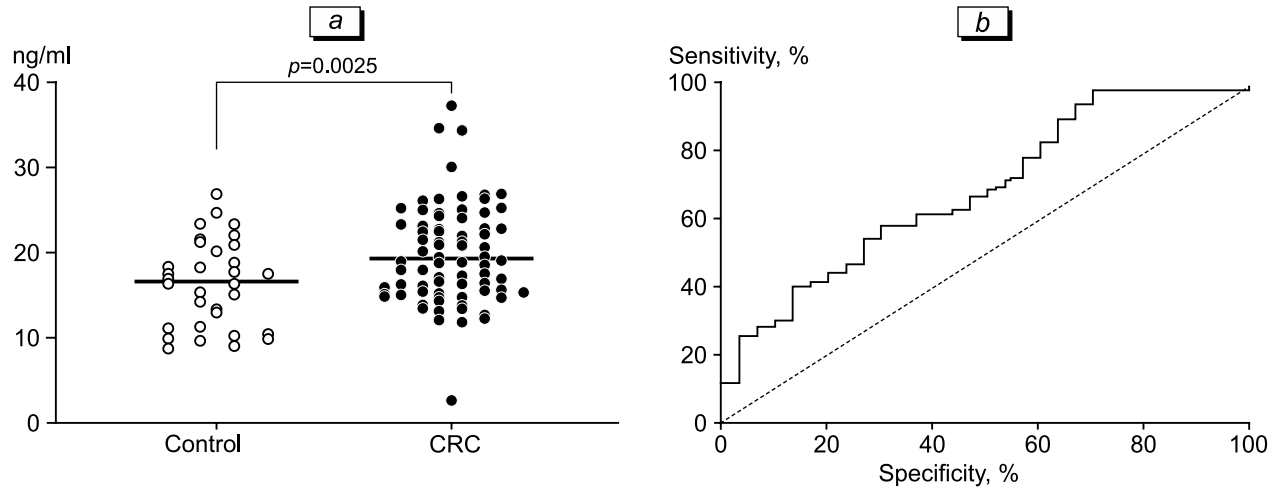

Fig. 1. Comparative analysis of sB7-H3 content in the serum of CRC patients and healthy donors (control) (*a*) and ROC analysis for sB7-H3 in CRC patients (*b*): AUC=0.689 (0.0028).

TABLE 1. sB7-H3 Content in Serum of Patients with CRC Depending on Clinical and Morphological Characteristics of the Disease (Me (Q1-Q3))

Characteristic		Level of B7-H3, ng/ml
Age	≤60 years	18.19 (15.2-23.17)
	>60 years	20.93 (16.79-26.35)
Sex	male	19.79 (15.3-26.48)
	female	19.53 (16.38-23.08)
Stage	I-II	22.14 (17.26-25.23)
	III-IV	17.7 (15.34-23.96)
Grade	G1	19.86 (16.14-26.99)
	G2-G3	19.53 (15.88-23.48)
Tumor size (T)	T1-T2	19.86 (16.63-24.47)
	T3-T4	19.14 (15.4-25.27)
Regional metastasis (N)	N0	21.71 (17.47-25.25)
	N1	17.21 (15.25-23.44)*
Distant metastasis (M)	M0	20.4 (16.55-24.44)
	M1	17.21 (15.36-26.35)
Localization	large intestine	22.93 (18.79-25.19)
	sigmoid colon	19.04 (15.40-26.77)
	rectum	18.42 (16.14-22.56)+
Large intestine portion	left	19.09 (15.61-24.6)
	right	23.2 (18.97-25.45)

Note. $p<0.05$ in comparison with *N0, +localization in the large intestine.

recent studies have focused on the tissue expression of B7-H3 in CRC. For example, B7-H3 expression was detected in 50.8% of a large cohort of primary CRC samples and was associated with advanced stage, decreased recurrence-free survival rate, and increased tumor infiltration with T cells [10]. It was also reported that increased B7-H3 expression was associated with tumor involvement of regional lymph nodes and low tumor differentiation [11]. However, it should be noted that these findings do not necessarily contradict our results, because our study specifically investigated the soluble form of this protein.

Our findings suggest that serum sB7-H3 content increases during the development of malignant colorectal tumors, but as the tumor progresses, its concentration begins to decrease, which ultimately becomes an unfavorable prognostic factor for this disease. It is possible that sB7-H3 plays a critical role during the initiation of tumor development, but its production decreases as the tumor progresses. Similar results confirming the favorable prognostic significance of the sB7-H3 were recently reported [12]: high blood content of this protein was a favorable prognostic factor in non-small cells lung cancer. However, for many other tumor types, *e.g.* ovarian cancer, unfavorable prognostic significance of high content of this protein in the blood has been shown [13]. Recent studies have demonstrated that the resident microbiota can affect B7-H3 expression. For example, *H. pylori* induces expression of this protein on gastric epithelial cells [14]. In the CRC model, a microbiota-dependent cross-interaction pathway between myeloid cells, T cells, and tumor cells has been recently demonstrated. Bacteria recognized by myeloid cells contribute to the release of calcineurin and NFAT-dependent IL-6, the latter in turn promotes the expression of B7-H3 by tumor cells and suppresses the T-cell anti-tumor immune response [15]. The interaction between B7-H3 and the microbiota is currently an understudied area, but it could potentially explain the opposing effects observed in CRC and other tumor types.

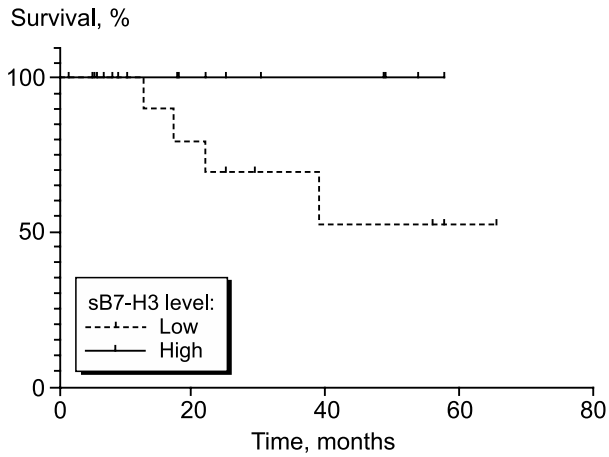

Fig. 2. Overall survival of CRC patients with serum sB7-H3 concentrations below and above the median (19.66 ng/ml).

The study was supported by the Russian Science Foundation (grant No. 22-15-00291, https://rscf.ru/project/22-15-00291).

Conflict of interest. Authors declare that they have no conflict of interest.

REFERENCES

1. Hofmeyer KA, Ray A, Zang X. The contrasting role of B7-H3. Proc. Natl. Acad. Sci. USA. 2008;105(30):10277-10278. doi: 10.1073/pnas.0805458105
2. Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, Liu D, Dong H, Sica GL, Zhu G, Tamada K, Chen L. B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. Nat. Immunol. 2001;2(3):269-274. doi: 10.1038/85339
3. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat. Rev. Immunol. 2008;8(6):467-477. doi: 10.1038/nri2326
4. Chen W, Liu P, Wang Y, Nie W, Li Z, Xu W, Li F, Zhou Z, Zhao M, Liu H. Characterization of a soluble B7-H3 (sB7-H3) spliced from the intron and analysis of sB7-

H3 in the sera of patients with hepatocellular carcinoma. PLoS One. 2013;8(10):e76965. doi: 10.1371/journal.pone.0076965
5. Flem-Karlsen K, Tekle C, Andersson Y, Flatmark K, Fodstad Ø, Nunes-Xavier CE. Immunoregulatory protein B7-H3 promotes growth and decreases sensitivity to therapy in metastatic melanoma cells. Pigment Cell Melanoma Res. 2017;30(5):467-476. doi: 10.1111/pcmr.12599
6. Nygren MK, Tekle C, Ingebrigtsen VA, Mäkelä R, Krohn M, Aure MR, Nunes-Xavier CE, Perälä M, Tramm T, Alsner J, Overgaard J, Nesland JM, Borgen E, Børresen-Dale AL, Fodstad Ø, Sahlberg KK, Leivonen SK. Identifying microRNAs regulating B7-H3 in breast cancer: the clinical impact of microRNA-29c. Br. J. Cancer. 2014;110(8):2072-2080. doi: 10.1038/bjc.2014.113
7. Zhang P, Yu S, Li H, Liu C, Li J, Lin W, Gao A, Wang L, Gao W, Sun Y. ILT4 drives B7-H3 expression via PI3K/AKT/mTOR signalling and ILT4/B7-H3 co-expression correlates with poor prognosis in non-small cell lung cancer. FEBS Lett. 2015;589(17):2248-2256. doi: 10.1016/j.febslet.2015.06.037
8. Zhou WT, Jin WL. B7-H3/CD276: An Emerging Cancer Immunotherapy. Front. Immunol. 2021;12:701006. doi: 10.3389/fimmu.2021.701006
9. Sun J, Chen LJ, Zhang GB, Jiang JT, Zhu M, Tan Y, Wang HT, Lu BF, Zhang XG. Clinical significance and regulation of the costimulatory molecule B7-H3 in human colorectal carcinoma. Cancer Immunol. Immunother. 2010;59(8):1163-1171. doi: 10.1007/s00262-010-0841-1
10. Lu Z, Zhao ZX, Cheng P, Huang F, Guan X, Zhang MG, Chen HP, Liu Z, Jiang Z, Zheng ZX, Zou SM, Wang XS. B7-H3 immune checkpoint expression is a poor prognostic factor in colorectal carcinoma. Mod. Pathol. 2020;33(11):2330-2340. doi: 10.1038/s41379-020-0587-z
11. Zhang W, Acuna-Villaorduna A, Kuan K, Gupta S, Hu S, Ohaegbulam K, Albanese J, Kaumaya M, Levy R, Hwang RR, Zang X, Lin J, Liu Q, Maitra R, Goel S. B7-H3 and PD-L1 Expression Are Prognostic Biomarkers in a Multi-racial Cohort of Patients with Colorectal Cancer. Clin. Colorectal. Cancer. 2021;20(2):161-169. doi: 10.1016/j.clcc.2021.02.002
12. Genova C, Tasso R, Rosa A, Rossi G, Reverberi D, Fontana V, Marconi S, Croce M, Dal Bello MG, Dellepiane C, Tagliamento M, Ciferri MC, Zullo L, Fedeli A, Alama A, Cortese K, Gentili C, Cella E, Anselmi G, Mora M, Barletta G, Rijavec E, Grossi F, Pronzato P, Coco S. Prognostic Role of Soluble and Extracellular Vesicle-Associated PD-L1, B7-H3 and B7-H4 in Non-Small Cell Lung Cancer Patients Treated with Immune Checkpoint Inhibitors. Cells. 2023;12(6):832. doi: 10.3390/cells12060832
13. Kovaleva OV, Belova TP, Korotkova EA, Kushlinskii DN, Gratchev AN, Petrikova NA, Kudlay DA, Kushlinskii NE. Soluble B7-H3 in Ovarian Cancer and Its Predictive Value. Bull. Exp. Biol. Med. 2021;171(4):472-474. doi: 10.1007/s10517-021-05253-w
14. Lina TT, Gonzalez J, Pinchuk IV, Beswick EJ, Reyes VE. Helicobacter pylori elicits B7H3 expression on gastric epithelial cells: Implications in local T cell regulation and subset development during infection. Clin. Oncol. Res. 2019;2(5):10.31487/j.cor.2019.05.05. doi: 10.31487/j.cor.2019.05.05
15. Peuker K, Strigli A, Tauriello DVF, Hendricks A, von Schönfels W, Burmeister G, Brosch M, Herrmann A, Krüger S, Nitsche J, Južnić L, Geissler MM, Hiergeist A, Gessner A, Wirbel J, Ponnudurai RP, Tunger A, Wehner R, Stange DE, Weitz J, Aust DE, Baretton GB, Schmitz M, Röcken C, Hampe J, Hinz S, Zeller G, Chavakis T, Schafmayer C, Batlle E, Zeissig S. Microbiota-dependent activation of the myeloid calcineurin-NFAT pathway inhibits B7H3- and B7H4-dependent anti-tumor immunity in colorectal cancer. Immunity. 2022;55(4):701-717.e7. doi: 10.1016/j.immuni.2022.03.008