Checkpoint proteins in patients with precancer and cervical cancer

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26 The efficiency of immuno-oncological drugs in treatment cervical cancer is not high. The research
27 of new immunological targets in cervical cancer is actual.

28 **The aim** was to study the local level of checkpoint proteins of immune cycle in patients with
29 cervical cancer of different stages (sCD25, 4-1BB, B7.2, TGF-b1, CTLA-4, PD-L1, PD-1, Tim-3,
30 LAG-3, Galectin-9, sCD27, PD-L2).

31 **Materials and research methods.** The objects of study were the patients with precancer (n=13)
32 and cervical cancer (n=49). The control group consisted of 13 gynecologically healthy women.
33 The material of study was cervical epithelium obtained by cervical brush. The research method
34 was flow cytometry. Test parameters: sCD25, 4-1BB, B7.2, TGF-b1, CTLA-4, PD-L1, PD-1,
35 Tim-3, LAG-3, Galectin-9, sCD27, PD-L2. Statistical analysis of the data was carried out using
36 nonparametric statistics using the Mann – Whitney U-test.

37 **The results of the study.**

38 The levels of sCD25, B7.2, PDL1, Tim-3, sCD27, PD-L2 were increased in the comparison
39 groups relation to the control ($p < 0.05$). The differential criterion for invasive cervical cancer may
40 be an increase in the level of LAG-3 in the cervical epithelium by 43% ($p < 0.05$). The levels of
41 PD-L1, PD-1, sCD27 and PD-L2 were increased opposite the levels LAG-3 and Galectin-9 were
42 decreased in patients with metastatic cervical cancer ($p < 0.05$).

43 **Key words:** cervical cancer, checkpoint proteins of immune cycle, immunotherapy, antitumor
44 immune response

45

46 INTRODUCTION

47 The efficiency of immunotherapy of patients with metastatic cervical cancer (MCC) is not high
48 [1], known difficulties of objective assessment of PD-L1 expression as a predictor of tumor
49 response to immunotherapy [2] are background causes for continuous search for pathogenetically
50 substantiated additional immunological targets of cervical cancer (CC) cells and tumor
51 microenvironment.

52 **EXPERIMENTAL PART**

53 **Aim of the research:** to assess levels of certain immune checkpoint proteins in CC patients
54 (sCD25, 4-1BB, B7.2, TGF- β 1, CTLA-4, PD-L1, PD-1, Tim-3, LAG-3, Galectin-9, sCD27, PD-
55 L2) according to the spread of the neoplastic process.

56 **Materials and methods**

57 We performed a non-randomized controlled prospective study among patients with precancerous
58 conditions and CC. The studied groups similar in age and associated diseases (average age was
59 39 ± 9.8 years old) were:

60 I clinical group - patients with precancerous cervical diseases - cervical intraepithelial neoplasia
61 III degree (n=13).

62 II group - initial non-treated patients with squamous CC of I-IV stages (n=49), of whom patients
63 with non-invasive (NI) n=8, invasive local (IL) n=18, local spread (n=19), metastatic CC n=4.

64 The control group included 13 gynecologically healthy volunteers, who were informed with the
65 design of the research, and who gave informed consent to participate in it (average age was
66 30.0 ± 4.4). The research was carried out in compliance with the principles of the WMA
67 Declaration of Helsinki, 1964, 2013 R.E), and upon approval of the Local committee on ethics of
68 the Chita State Medical Academy, Russian Federation (protocol 44 dated 09.11.2012).

69 The material of study was cervical epithelium, collected by standard method by taking with
70 cytobrush. The method of flow cytometry FC500 (Beckman Coulter, USA) with the use of
71 reagents included in the panel HU Immune Checkpoint Panel 1 - S/P (10-plex) w/FP was used to
72 identify parametrs: sCD25, 4-1BB, B7.2, TGF- β 1, CTLA-4, PD-L1, PD-1, Tim-3, LAG-3,
73 Galectin-9, sCD27, PD-L2.

74 Statistical processing of data was computer-based with application of BIOSTAT software by
75 nonparametric statistics methods using the Mann – Whitney U-test. The differences were
76 considered statistically significant at $p < 0.05$.

78 RESULTS

79 The results of intergroup analysis are given in Table 1.

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83

84 Table 1. The local level of immune checkpoint proteins in patients with the damage of uterine

85 cervix Me (Q1; Q3), pg/ml

characteristics	control (n=13)	precancer (n=13)	cervical cancer (n=49)
sCD25	2.83 [2.25; 5.16]	14.13* [5.29; 16.5]	8.59* [2.98; 14.6]
4-1BB	13.11 [7.03; 27.3]	22.11 [11.2; 98.7]	19.0 [7.62; 60.9]
B7.2	9.08 [7.8; 0.3]	27.94*# [15.7; 45.12]	13.2* [8.12; 29.8]
TGF-b1	5.85 [3.53; 18.3]	13.29 [6.45; 69.0]	19.8 [3.31; 58.9]
CTLA-4	2.86 [1.95; 17.2]	11.65 [8.37; 15.8]	8.5 [1.91; 32.2]
PD-L1	5.27 [1.53; 12.0]	33.18*# [6.71; 63.18]	14.1* [2.68; 31.6]
PD-1	17.99 [1.92; 60.1]	47.40 [6.34; 176.5]	26.9 [2.80; 92.83]
Tim-3	14.67 [11.6; 24.3]	47.40* [23.9; 243.7]	47.1* [16.3; 132.8]
LAG-3	26.15 [12.05; 39.3]	590.7 [24.2; 140.4]	36.1 [20.1; 78.4]
Galectin-9	1646.8 [937.2; 3087.1]	1329.9 [1060.3; 4258.4]	1852.5 [1347.8; 3186.8]
sCD27	7.17 [3.78; 24.9]	23.9*# [15.2; 48.0]	29.2* [17.5; 35.7]
PD-L2	11.7 [4.04; 18.3]	33.4# [15.3; 52.8]	29.2* [14.9; 42.5]

86 highlighted the statistical significance

87 * the statistical significance of parameters between the clinical groups and control (p<0,05)

88 # the statistical significance of parameters between precancer and cancer (p<0,05)

89

90 In the patients of the I clinical group the levels of sCD25, B7.2, PDL1, Tim-3, sCD27 were 5; 3;

91 6,3; 3,2 and 3,3 times higher than the corresponding control values (p<0,05). In the II clinical

92 group an increase of control values of sCD25, B7.2, PDL1, Tim-3, sCD27, PD-L2 by 3; 1,5; 2,7;

93 3,2; 4 and 2.5 times accordingly was identified (p<0,05). During intergroup analysis increased

94 level of indicators B7.2, PD-L1, PD-L2 (by 2,1; 2,4; 1,1 times accordingly ($p < 0,05$)) with
95 decreased sCD27 by 1.2 times ($p < 0,05$) was identified in the I group.

96 The results of subgroup analysis of CC patients according to the spread of the neoplastic process
97 are given in Table 2.

98

99 Table 2. Level of immune checkpoint proteins in cervical mucus of CC patients in accordance

100 with the spread of the neoplastic process Me (Q1; Q3), pg/ml

characteristics	control group (n=13)	noninvasive cancer (n=8)	invasive local cancer (n=18)	local spread cancer (n=19)	metastatic cancer (n=4)
sCD25	2.83 [2.25; 5.16]	6.85 [2.76; 13.3]	11.2* [4.57; 15.3]	13.8* [3.0; 12.2]	9.8 [2.29; 9.8]
4-1BB	13.11 [7.03; 27.3]	27.3 [15.1; 46.1]	56.0* [11.3; 81.6]	15.5# [7.57; 19.9]	169.9 [103.4; 266.8]
B7.2	9.08 [7.8; 0.3]	13.6 [8.57; 16.1]	22.6 [8.28; 55.0]	16.4 [8.08; 24.1]	23.5 [18.1; 33.7]
TGF- β 1	5.85 [3.53; 18.3]	14.7 [3.44; 34.5]	44.0 [12.5; 64.8]	18.6# [3.20; 27.1]	33.1 [28.9; 58.8]
CTLA-4	2.86 [1.95; 17.2]	[13.65 [3.53; 36.3]	17.8 [5.52; 37.7]	21.4# [1.79; 18.4]	2.27* [2.27; 2.93]
PD-L1	5.27 [1.53; 12.0]	15.0 [3.44; 28.9]	22.5* [5.25; 37.2]	25.6# [2.37; 23.4]	48.6^ [26.8; 72.1]
PD-1	17.99 [1.92; 60.1]	58.3 [19.8; 105.5]	73.0 [9.87; 109.1]	34.7# [1.85; 28.2]	193.9^ [142.0; 332.0]
Tim-3	14.7 [11.6; 24.3]	75.6 [19.8; 140.1]	74.6* [37.6; 147.8]	59.2# [14.4; 59.2]	52.8* [44.6; 65.5]
LAG-3	26.15 [12.05; 39.3]	34.3^v [21.1; 58.6]	58.9 [27.5; 95.7]	541.2# [11.3; 42.8]	377.3^ [377.3; 739.5]
Galectin-9	1646.8 [937.2; 3087.1]	1724.0 [1347.8; 1818.8]	2003.0 [1153.7; 3148.6]	1997.1 [1428.6; 3673.9]	1313.7^ [1129.8; 1413.9]
sCD27	7.17 [3.78; 24.9]	18.6* [11.9; 24.9]	20.4* [17.2; 26.5]	31.6*# [17.5; 52.5]	97.6*^ [66.8; 128.4]
PD-L2	11.7 [4.04; 18.3]	15.9 [11.3; 36.0]	28.9* [19.8; 36.6]	29.2* [17.5; 57.5]	66.9*^ [58.5; 75.4]

101 highlighted the statistical significance ($p < 0,05$)

102 * the statistical significance of parameters between the clinical groups and control

103 ^v - the statistical significance of parameters between noninvasive cancer and invasive local cancer

104 # - the statistical significance of parameters between invasive local cancer and local spread cancer

105 ^ - the statistical significance of parameters between local spread cancer and metastatic cancer

106

When we analyzed the data depending on the spread of the neoplastic process we did not identify any significant differences between the levels of B72, TGF- β 1, PD-1, Galectin-9, LAG-3 as compared to the control values ($p>0,05$).

The level of sCD25 was increased in patients with invasive local and local spreadCC by 4 and 5 times accordingly as compared to the control value ($p< 0.05$).

Changes in the values of 4-1BB and PD-L1 were identified only in the subgroup of patients with invasive local CC: the increase was by 4.3 times as compared with the control value for each of them ($p< 0,05$).

It was established that only the patients with metastatic CC had 20% decreased level of CTLA4 ($p< 0,05$).

The value of Tim-3 in the cervical smear of patients with invasive local and metastatic CC increased the control value by 5 and 3.6 times accordingly ($p< 0,05$).

The levels of sCD27 и PD-L2 increased in all subgroups of CC patients with the maximum levels being observed in patients with metastatic CC.

Subgroup analysis of indicators of patients with invasive local and local spreadCC showed decreased level of 4-1BB, TGF- β 1, PD-1 and Tim-3 (by 3.77 ($p<0.05$), 2.4 ($p<0.05$); 2.1 ($p<0.05$) и 1.3 ($p<0.05$) times accordingly) with increased levels of CTLA4, PD-L1, LAG-3, (by 1.2 ($p<0.05$); 1.1 ($p<0.05$), 9 ($p<0.001$); 1.5 and 1 times ($p<0.001$) accordingly).

In the group of patients with metastatic CC as compared with local spreadCC significant differences of PD-L1, PD-1, LAG-3 and Galectin-9 were identified, whereas the value of the first two was increased by 1.9 ($p<0.05$) and 5.6 ($p<0.05$) times accordingly and the value of the last ones was decreased by 1.4 ($p<0.05$) and 1.5 times ($p<0.05$) accordingly.

DISCUSSION

sCD25 is one of the regulators of immune reactions in autoimmune, oncological diseases and critical conditions of intensive care patients [3, 4]. sCD25 is produced under the influence of a number of ferments (elastase, MMP-9) during shedding, which leads to inactivation of immune

cells, in particular to functional deficiency of NK-cells, and to suppression of anti-tumor activity of T-cells which intensifies tumor progression [3]. Pathogenetic mechanisms of sCD25 interaction with immune cells are being studied. It should be noted that we have not found any data on the study of sCD25 levels in CC patients. However, our data conforms with the results of other research of other neoplasia [5]. High serum level of sCD25 is an indicator of worse survival rates and unresponsiveness to immunotherapy of patients with melanoma. Sh. Abdelfattah et al. suggested that identification of sCD25 in blood serum should be used as a marker of hepatocellular carcinoma taking into account the detected relationship between its quantity and disease stage.

Receptor 4-1BB is a member of TNF receptor family, expressed on CD8, CD4- T-cells, B-lymphocytes, regulatory T-lymphocytes, NK cells, dendritic cells, mast cells, etc. Its physiological role is to regulate activity of the above mentioned cells by activating a cascade of signaling events (JNK, ERK, β -catenin and AKT) [6]. 4-1BB promotes survival of T-cells, participates in the formation of immunological memory by activating antiapoptotic genes Bcl-2, Bcl-xl, и Bfl-1, induces proliferation of T-cells and enhances their effector function. Activation of 4-1BB leads to aging of dendritic cells, enhances their survival and production of cytokines IL-6, IL-12 и IL-27 [7]. Dual capacity of 4-1BB to stimulate reaction of effector T-cells on pathogens with simultaneous limitation of autoimmune reactions made this receptor an alluring target for tumor immunotherapy. There were published results of research studying 4-1BB agonists in the therapy of some tumors [8]. T. Bartkowiak et al. showed in vitro efficiency of administration of intranasal peptide vaccine HPV E6/E7, which contained α GalCer as an adjuvant in combination with systemic administration of agonistic antibodies to treat HPV-driven tumor [9]. We have not found any literature showing data on the study of 4-1BB levels in CC patients. Our data on the maximum level of 4-1BB in patients with local spreadCC suggests tense activation of one of the elements of antitumor immune response.

Ligand B7.2 is expressed on antigen presenting cells (APCs) and can interact with T-lymphocytes receptor CD28, inducing their activation and triggering immune response, or with activated T-lymphocytes receptor CTLA4 blocking immune reactions. In the absence of costimulation of CD28 T-cells affected by the antigen become anergic. Due to insufficient expression B7-2 cells are unable to transmit co-stimulatory signals required for efficient activation of T-cells, which potentiates tumor progression [10]. Pathogenetic participation of B7 family proteins in carcinogenesis has been proven for pulmonary cancer, hemato-oncological diseases, stomach cancer, ovarian cancer, clear-cell carcinoma [11]. We are the first to study local level of B7.2 in patients with cervical disease. High level of B7.2 in both comparison groups is a reflection of activation of T-cell antitumor immune response. It's interesting to note the discovered fact of decreased level of B7.2 in CC patients by 2.1 times ($p < 0,05$) as compared to the corresponding indicators of patients with precancerous cervical conditions, which can indicate reduced efficiency of immune reactions during cervical carcinogenesis.

TGF- β 1 (Transforming growth factor beta) acts as one of the key regulators of the development and progression of tumor. It influences all important stages of cancer progression including migration and invasion. Paradoxical effect of TGF- β in the neoplastic process has been established. At the initial stages of tumor development, it gives antineoplastic action, but during its progression its effect becomes co-carcinogenic. This TGF- β effect is explained by changing activity of micro-RNA which control TGF- β activity, TGF- β gene polymorphism, condition of endogenous proteins [12]. Pathogenetic role of TGF- β was established in carcinogenesis of colorectal cancer, urogenital cancers, breast cancer. In our research we have not received any significant differences in the value of TGF- β among the studied groups [12].

CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) is a negative immune regulator which suppresses both antiviral and antitumor immune response. CTLA4 integrates with co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) expressed on the surface of antigen presenting cells (APCs) and suppress T-lymphocyte function. The data on the research of CTLA4 expression in

184 CC patients is scarce. Meta-analysis of 11 international studies among 3899 cases of CC showed
 185 significant connection between the development of tumor in carriers of polymorphism rs5742909
 186 gene CTLA4 among Asians [13]. Abnormal expression of CTLA-4 in T-lymphocytes isolated
 187 from peripheral blood of metastatic CC patients is known, which is related to genetic defects in
 188 the gene that codes a corresponding protein, as well as with a low level of its antagonist with
 189 immune stimulating action - CD28, which is associated with reciprocal interaction [14]. Increased
 190 expression of CTLA-4 on circulating T-lymphocytes reflects exhaustion of T-cells due to
 191 continuous antigen stimulation, and simultaneous identification of CD28 expression in CD8+
 192 subset of T-cells is a marker that this process can be reversed [15]. CTLA-4 can as well be
 193 expressed on the surface of T-regulatory cells and enhance its immunosuppressive action in case
 194 of distributed CC [16]. The data we received on the extremely low CTLA4 level in metastatic CC
 195 patients coincide with the conclusions of researchers headed by A. Gutiérrez-Hoya (2019), who
 196 established low expression of CTLA4 by cells of experimental CC-Hela cell line [17].

197 **PD-1** (Programmed cell death-1 protein) is a transmembrane protein which is expressed by T-
 198 lymphocytes. One of its ligands is **PD-L1**, which is expressed on the surface of tumor cells of
 199 cervix, APCs, and T-lymphocytes. During interaction between PD-1 and PD-L1 suppression of
 200 antitumor and antiviral immune response is observed. Information on PD-1 and PD-L1 expression
 201 is numerous and contradictory. Generally, 46-61% of initial CC express PD-1 and 22-96% - PD-
 202 L1 [18]. There is an assumption on the influence of HPV contamination on the expression of PD-
 203 L1 and PD-1 [19]. Analysis of PD-1 and CTLA4 expression in 33 various morphological types of
 204 tumor has been published. PD-1 and CTLA4 expression varies in different types of tumor and is
 205 not characteristic for squamous CC. High level of PD-1 expression in tumor samples of CC
 206 patients correlated with the best disease-free survival ($p = 0.047$) [20]. Such conclusions were
 207 received by R Karim [21]. R. Grochot and al. reported the absence of prognostic levels of PD-L1
 208 and PD-1 expression in CC patients [22]. Y. Meng et al. published information on detected
 209 correlation between PD-L1 expression in CC patients with clinical signs of disease progression

210 and unfavorable morphological prognosis factors: metastatic lesions of lymph nodes and lymph
211 vessel invasion [23]. Y. Liu et al reported unfavorable prognosis role of high levels of PD-L1 and
212 PD-1 in patients with recurrent and metastatic CC which corresponds to worse disease free and
213 general survival of patients [18].

214 In order to give prognosis on the course of neoplastic process not only the level of immune
215 response inhibitors expression is important, but also the number of immune cells located in the
216 locus of cancer and at the periphery. All this can be assessed by a prognostic immune scoring
217 system called Immunoscore. H. Chen et al. were the first to apply Immunoscore to CC patients
218 and established that a favorable prognostic factor for CC patients was a positive PD-L1 expression
219 on tumor cells surrounded by a large number of tumor-infiltrating T-lymphocytes [24].

220 Our data on the local levels of PD-L1 and PD-1 among the studied groups conform with the
221 results of research by L. Mezache et al [19]. PD-L1 expression on tumor cells was less in case of
222 squamous CC (51%) as compared to CIN 1-2 (95%). However, the level of PD-L1 expression on
223 T-lymphocytes was the maximum in CC patients (80% versus 61%). The maximum values of PD-
224 L1 and PD-1 in metastatic CC patients that we discovered is a mechanism for inhibiting antitumor
225 immune response.

226 **PD-L2** is a second ligand for PD-1 receptor. It is expressed on APCs, and fibroblasts. PD-L2 as
227 well as PD-L1 expression in normal tissues is limited. However, during carcinogenesis activation
228 of PD-L2 / PD-1 path leads to the suppression of antitumor immune response which intensifies
229 cancer growth [25]. Participation of PD-L2 / PD-1 in carcinogenesis is scarcely studied [27]. The
230 role of PD-L2 is known in intensifying cancer progression (by activating Rhoa-ROCK-LIMK2,
231 PI3K / AKT / mTOR paths), formation of tumor chemoresistance. Information on possible
232 prognostic role of PD-L2 expression levels for some oncological diseases has been published [26].

233 **Tim-3** (T-cell immunoglobulin-3) is a protein, which is expressed on T-lymphocytes, and tumor
234 cells, interacts with ligand – Galectin 9 and inhibits antitumor immune response. Tim-3 is seen as
235 a negative regulator molecule important for the creation of immune tolerance in tumor

microenvironment. Tim-3 and ligand PD-1 expression in T-cells leads to exhaustion of T-cell element and tumor growth [27]. Tim-3 activates IL-6-STAT3 path, which promotes metastasis of various tumors. The results of Y. Cao et al. conform with our data on the high level of Tim-3 in precancerous and cancerous lesions of cervix. The authors established correlative relationship between high level of Tim-3 and the level of neoplastic process, worse general survival, and metastatic potential of tumor [28]. The research of L. Zhang et al. established epigenetic regulation of Tim-3 expression in CC under the influence of E6 and E7 HPV proteins [29, 30].

LAG-3 (lymphocyte-activation gene 3) is another protein which belongs to immune response inhibitors. LAG-3 is expressed on cell membranes TIL, activated CD4+ и CD8+ T-cells, and regulatory T-cells, NK, B-lymphocytes, dendritic cells. LAG-3 is connected with the main major histocompatibility complex-II on APCs, thus downregulating antitumor immune response. In the literature we found a reference to only one research which indicated LAG-3 overexpression in HPV-infected CC cells, which conforms with our data [31].

Galectin 9 (Gal-9) belongs to the family of beta-galactoside-binding proteins. Its pathophysiological role in the carcinogenesis is complex. It is known that Gal-9 exhibits proapoptotic effect by activating NK and p38-MAPK paths, Caspase 3, -8, - 9, which was demonstrated on myeloma cells. Gal-9 inhibits invasion and metastasis of myeloma tumor cells and hepatocarcinoma by influencing the epithelial–mesenchymal transition. The receptor for Gal-9 on immune cells is Tim-3. Their interaction leads to reduction of anti-tumor activity of immune cells [33]. Sporadic publications report low expression of Gal-9 on CC tumor cells [33]. With increase in the level of dysplastic changes in the cervical epithelial cells the level of Gal-9 expression decreases, which conforms to the significantly low level of Gal-9 in metastatic CC patients, that we established.

sCD27 is a soluble form of transmembrane protein CD27 (T-lymphocyte receptor), that serves as a co-stimulator of immune reactions. Its ligand is CD70 expressed on immune cells. Their interaction promotes stimulation of immune response. Pathogenetic role of sCD27 has been

established for the development of autoimmune (systemic lupus erythematosus), viral (HIV infection) and some oncological diseases (Hodgkin lymphoma, prostate cancer, lung cancer, breast cancer, colon cancer, glioblastoma, etc.) [34, 35, 36]. We have not found any literature showing data on the study of sCD27 in CC patients. The information about increased amount of sCD27 in the cervical mucus of patients with precancerous and oncological cervical diseases indicate activation of cell-bound immunity. However, when we cross-check this data with clinical information, we can conclude that sCD27 activation is not sufficient to inhibit progression of CC. It may be connected with blockage of sCD27 ligand expressed not on T-lymphocytes but on different cells which interferes with the activation of antitumor immune response.

CONCLUSIONS

1. It was found that patients with precancerous diseases and CC patients have increased levels of sCD25, B7.2, PDL1, Tim-3, sCD27, PD-L2 in the cervical mucus as compared with control value ($p < 0.05$). Significant increase in the levels of B7.2, PD-L1 and PD-L2 was found in the patients with precancerous diseases by 2.1; 2.2, and 1.1 times respectively relative to the comparison groups ($p < 0.05$).

2. Differential criterion of invasive CC can be 43% increased level of LAG-3 in cervical mucus ($p < 0.05$) compared to the corresponding indicator in patients with non-invasive CC.

3. Metastatic CC by contrast with local spread CC is characterized by increased levels of PD-L1, PD-1, sCD27 and PD-L2 by 1.9 ($p < 0.05$); 5.6 ($p < 0.05$); 3 ($p < 0.001$) and 2.3 times ($p < 0.001$), correspondingly with decreased values of LAG-3 and Galectin-9 by 1.4 ($p < 0.05$) and 1.5 times accordingly ($p < 0.05$).

4. Invasive local CC by contrast with local spread CC is characterized by decreased levels of 4-1BB, TGF- β 1, PD-1 and Tim-3 (by 3.77 ($p < 0.05$), 2.4 ($p < 0.05$); 2.1 ($p < 0.05$) и 1.3 ($p < 0.05$) times accordingly) with increased levels of CTLA4, PD-L1, LAG-3, sCD27 (by 1.2 ($p < 0.05$); 1.1 ($p < 0.05$), 9 times ($p < 0.001$); 1.6 times accordingly ($p < 0.001$)).

CONCLUSION

In the course of cervical carcinogenesis expression of immune proteins that have a key role in the balanced immune cell responses is interrupted on the local level. It is possible that the identified changes have a prognostic character in relation to the progression of the disease, and have a predictive value for an efficient immunotherapy of CC patients, which requires further research.

The authors declare that there is no conflict of interests.

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References

1. Khokhlova S.V. Immunotherapy of patients with cervical cancer. *Effektivnaya farmakoterapiya*. 2015; 15 (24): 20–26. (In Rus). <https://doi.org/10.33978/2307-3586-2019-15-24-20-26>. (In Rus).
2. Janus G.A., Ievleva A.G., Suspitsyn E.N., Tyurin V.I., Bizin I.V., Gorustovich O.A. [et al] Predictive response markers for immune response blocks. *Siberian journal of oncology*. 2020;19(4):123-131. (In Russ.) <https://doi.org/10.21294/1814-4861-2020-19-4-123-131>. (In Rus).
3. Brusko T., Wasserfall C., Hulme M. [et al.] Influence of Membrane CD25 Stability on T Lymphocyte Activity: Implications for Immunoregulation . *PLoS One*; 4 (11): e7980. <https://doi.org/10.1371/journal.pone.0007980>
4. von Bahr Greenwood T., Palmkvist-Kaijser K., Chiang S. [et al.] Elevated ferritin and soluble CD25 in critically ill patients are associated with parameters of (hyper) inflammation and lymphocyte cytotoxicity. *Minerva Anesthesiol*. 2019; 8 (12) : 1289-1298. <https://doi.org/10.23736/S0375-9393.19.13534-1>
5. Abdelfattah Sh., Haseeb A., Tawfik M. [et al.] Soluble CD25 as a predictor of hepatocellular carcinoma compared with alpha-fetoprotein. *Clin Exp Hepatol*. 2019; 5 (2) : 140–146. <https://doi.org/10.5114/ceh.2019.85165>

- 313 6. Kim J.O., Kim H.W., Baek K.M., Kang C.Y. NF-kappaB and AP-1 regulate activation-
314 dependent CD137 (4-1BB) expression in T cells. *FEBS Lett.* 2003; 541 (1): 163-170.
315 [https://doi.org/ 10.1016/s0014-5793\(03\)00326-0](https://doi.org/10.1016/s0014-5793(03)00326-0)
- 316 7. Dawicki W., Watts T.H. Expression and function of 4-1BB during CD4 versus CD8 T cell
317 responses in vivo. *Eur J Immunol.* 2004; 34 (3): P. 743-751. [https://doi.org/](https://doi.org/10.1002/eji.200324278)
318 [10.1002/eji.200324278](https://doi.org/10.1002/eji.200324278).
- 319 8. Bartkowiak T., Curran M. 4-1BB Agonists: Multi-Potent Potentiators of Tumor Immunity.
320 *Front Oncol.* 2015; 5 (117): e 2015. Available from: <https://doi.org/10.3389/fonc.2015.00117>
- 321 9. Bartkowiak T., Singh Sh., Yang G. [et al.] Unique potential of 4-1BB agonist antibody to
322 promote durable regression of HPV+ tumors when combined with an E6/E7 peptide vaccine . *Proc*
323 *Natl Acad Sci U S A.* 2015; 112 (38): 5290–5299. [https://doi.org/ 10.1073/pnas.1514418112](https://doi.org/10.1073/pnas.1514418112).
- 324 10. Seliger B., Marincola F., Ferrone S., Abken H. The complex role of B7 molecules in tumor
325 immunology. *Trends Mol Med.* 2008; 14 (12): 550–559.
326 <https://doi.org/10.1016/j.molmed.2008.09.010>.
- 327 11. Seliger B., Quandt D. The expression, function, and clinical relevance of B7 family
328 members in cancer. *Cancer Immunol Immunother.* 2012; 61 (8): 1327-1341.
329 <https://doi.org/10.1007/s00262-012-1293-6>.
- 330 12. Boguslawska J., Kryś P., Poletajew S., Pieklik-Witkowska A. TGF-β and microRNA
331 Interplay in Genitourinary Cancers. *Cells.* 2016; 8 (12): 1619.
332 <https://doi.org/10.3390/cells8121619> .
- 333 13. Chetveryakov A.V., Tsepelev V.L. Role of the T-lymphocyte membrane protein CTLA-4 in
334 regulating immunity and immunotherapy of tumor. *Zabajkal'skij medicinskij vestnik.* 2020; 4:
335 206-214. (In Rus). Available from: <http://zabmedvestnik.ru/index.php>.
- 336 14. Hu Sh., Pu D., Xia X. [et al.] CTLA-4 rs5742909 polymorphism and cervical cancer risk. A
337 meta-analysis / *Medicine (Baltimore).* 2020; 99 (11): e19433. Available from:
338 <https://pubmed.ncbi.nlm.nih.gov/32176070/> <https://doi.org/10.1097/MD.00000000000019433>.

- 339 15. Kosmaczewska A., Bocko D., Ciszak L. [et al.] Dysregulated Expression of Both the
340 Costimulatory CD28 and Inhibitory CTLA-4 Molecules in PB T Cells of Advanced Cervical
341 Cancer Patients Suggests Systemic Immunosuppression Related to Disease Progression. *Pathol*
342 *Oncol Res.* 2012; 18 (2): 479–489. [https://doi.org/ 10.1007/s12253-011-9471-y](https://doi.org/10.1007/s12253-011-9471-y).
- 343 16. Takahashi T., Tagami T., Yamazaki S. [et al.] Immunologic self-tolerance maintained by
344 CD25 + CD4+ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated
345 antigen 4. *J Exp Med.* 2000; 192 (2): 192:303–309. [https://doi.org/ 10.1084/jem.192.2.303](https://doi.org/10.1084/jem.192.2.303).
- 346 17. Gutiérrez-Hoya A., Zerecero-Carreón O., Valle-Mendiola A. [et al.] Cervical Cancer Cells
347 Express Markers Associated with Immunosurveillance. *J Immunol Res.* 2016; 2019^ e1242979.
348 Available from: <https://pubmed.ncbi.nlm.nih.gov/31198791/> [https://doi.org/](https://doi.org/10.1155/2019/1242979)
349 [10.1155/2019/1242979](https://doi.org/10.1155/2019/1242979).
- 350 18. Liu Y., Wu L., Tong R. [et al.] PD-1/PD-L1 Inhibitors in Cervical Cancer. *Front*
351 *Pharmacol.* 2016; 10 (65). Available from:
352 <https://pubmed.ncbi.nlm.nih.gov/30774597/>[https://doi.org/ 10.3389/fphar.2019.00065](https://doi.org/10.3389/fphar.2019.00065).
- 353 19. Mezache L., Paniccia B., Nyinawabera A., Nuovo G. Enhanced expression of PD L1 in
354 cervical intraepithelial neoplasia and cervical cancers. *Modern Pathology.* 2015; 28 (12): 1594–
355 1602. <https://doi.org/10.1038/modpathol.2015.108>.
- 356 20. Jian-Nan L., Xiang-Shuo K., Tao H. [et al.] Clinical Implications of Aberrant PD-1 and
357 CTLA4 Expression for Cancer Immunity and Prognosis: A Pan-Cancer Study. *Front Immunol.*
358 2020; 11 (11): 2048. Available from: <https://pubmed.ncbi.nlm.nih.gov/33072070>.
359 <https://doi.org/10.3389/fimmu.2020.02048>
- 360 21. Karim R., Jordanova E., Piersma S. [et al.] Tumor-expressed B7-H1 and B7-DC in relation
361 to PD-1+ T-cell infiltration and survival of patients with cervical carcinoma. *Clin Cancer Res.*
362 2009; 15 (20): 6341-6347. [https://doi.org/ 10.1158/1078-0432.CCR-09-1652](https://doi.org/10.1158/1078-0432.CCR-09-1652)

- 363 22. Grochot R., Brollo J., Neto F. [et al.] Expression of PD-L1 in cervical carcinoma and its
364 impact on survival associated with T-cell infiltration and FoxP3 expression. *Cancer Manag Res.*
365 2019; 11 : 4597-4605. [https://doi.org/ 10.2147/CMAR.S194597](https://doi.org/10.2147/CMAR.S194597)
- 366 23. Meng Y., Liang H., Hu J. [et al.] PD-L1 Expression Correlates With Tumor Infiltrating
367 Lymphocytes And Response To Neoadjuvant Chemotherapy In Cervical Cancer. *J Cancer.* 2018;
368 9 (16): 2938-2945. [https://doi.org/ 0.7150/jca.22532](https://doi.org/0.7150/jca.22532)
- 369 24. Chen H., Xia B., Zheng T., Lou G. Immunoscore system combining CD8 and PD-1/PD-L1:
370 A novel approach that predicts the clinical outcomes for cervical cancer // *Int J Biol Markers.* Vol.
371 35. №1. 2020. P. 65-73. [https://doi.org/ 10.1177/1724600819888771](https://doi.org/10.1177/1724600819888771) Zak K.M., Grudnik P.,
372 Magiera K. [et al.] Structural Biology of the Immune Checkpoint Receptor PD-1 and Its Ligands
373 PD-L1/PD-L2. *Structure.* 2017; 25 (8): 1163-1174. [https://doi.org/ 10.1016/j.str.2017.06.011](https://doi.org/10.1016/j.str.2017.06.011).
- 374 25. Marinelli O., Annibali D., Aguzzi C. [et al.] The Controversial Role of PD-1 and Its
375 Ligands in Gynecological Malignancies. *Front Oncol.* 2016; 9: e1073. Available from:
376 <https://pubmed.ncbi.nlm.nih.gov/31681606>. [https://doi.org/ 10.3389/fonc.2019.01073](https://doi.org/10.3389/fonc.2019.01073).
- 377 26. Marinelli O., Annibali D., Morelli M. [et al.] Biological Function of PD-L2 and Correlation
378 With Overall Survival in Type II Endometrial Cancer. *Front Oncol.* 2020; 10. 538064. Available
379 from: [https://doi.org/ 10.3389/fonc.2020.538064](https://doi.org/10.3389/fonc.2020.538064)
- 380 27. Yan J., Zhang Y., Zhang J. [et al.] Tim-3 Expression Defines Regulatory T Cells in Human
381 Tumors. *PLoS One.* 2013; 8 (3): e58006. Available from:
382 <https://pubmed.ncbi.nlm.nih.gov/33194598>. [https://doi.org/ 10.1371/journal.pone.0058006](https://doi.org/10.1371/journal.pone.0058006).
- 383 28. Cao Y., Zhou X., Huang X. [et al.] Tim-3 Expression in Cervical Cancer Promotes Tumor
384 Metastasis. *PLoS One.* 2013; 8 (1): e53834. Available from:
385 <https://pubmed.ncbi.nlm.nih.gov/23335978>. [https://doi.org/ 10.1371/journal.pone.0053834](https://doi.org/10.1371/journal.pone.0053834).
- 386 29. Zhang L., Tian S., Pei M. [et al.] Crosstalk between histone modification and DNA
387 methylation orchestrates the epigenetic regulation of the costimulatory factors, Tim-3 and

- 388 galectin-9, in cervical cancer . *Oncol Rep.* 2019; 42 (6) : 2655–2669. <https://doi.org/10.3892/or.2019.7388>.
- 390 30. Zhang L., Tian S., Zhao M. [et al.] SUV39H1-DNMT3A-mediated epigenetic regulation of
391 Tim-3 and galectin-9 in the cervical cancer. *Cancer Cell Int.* 2020; 20 (325). Available from:
392 <https://doi.org/10.1186/s12935-020-01380-y>.
- 393 31. Panda A., Rosenfeld J., Singer A. [et al.] Genomic and immunologic correlates of LAG-3
394 expression in cancer. *Oncoimmunology.* 2020; 9 (1): e1756116. Available from:
395 <https://pubmed.ncbi.nlm.nih.gov/32923111>. <https://doi.org/10.1080/2162402X.2020.1756116>.
- 396 32. Wang L., Zhao Y., Wang Y., Wu X. The Role of Galectins in Cervical Cancer Biology and
397 Progression. *Biomed Res Int.* 2018; 2018: e2175927. Available from:
398 <https://pubmed.ncbi.nlm.nih.gov/29854732>. <https://doi.org/10.1155/2018/2175927>.
- 399 33. Liang M., Ueno M., Oomizu S. [et al.] Galectin-9 expression links to malignant potential of
400 cervical squamous cell carcinoma. *J Cancer Res Clin Oncol.* 2008; 134 (8): 899-907.
401 <https://doi.org/10.1007/s00432-008-0352-z>.
- 402 34. Starzer A., Berghoff A. New emerging targets in cancer immunotherapy: CD27 (TNFRSF7).
403 *ESMO Open.* 2019; 4 (3): e000629. Available from: <https://pubmed.ncbi.nlm.nih.gov/32152062>.
404 <https://doi.org/10.1136/esmoopen-2019-000629>.
- 405 35. Bullock T. Stimulating CD27 to quantitatively and qualitatively shape adaptive immunity to
406 cancer. *Curr Opin Immunol.* 2017; 45: 82–88. <https://doi.org/10.1016/j.coi.2017.02.001>.
- 407 36. Burchill M., Tamburini B., Kedl R. T cells compete by cleaving cell surface CD27 and
408 blocking access to CD70 bearing APCs. *Eur J Immunol.* 2015; 45 (11): 3140–3149.
409 <https://doi.org/10.1002/eji.201545749>.