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Abstract**Introduction and purpose**

Understanding the movement of immune cells in prostate cancer is the best solution for development antitumor therapy.

In our study, we will evaluate level mRNA CD16A (*FCGR3A*) and mRNA CD16B (*FCGR3B*) in patients diagnosing benign hyperplasia and patients diagnosing prostate cancer (Pc).

Materials and methods In the study, we analyzed 240 samples of mRNA, 49 was the blood of healthy donors, 37 was the blood of prostate cancer patients and 62 tumors of prostate, 37 were blood of hyperplasia and 55 was tissue of hyperplasia, all patients treated in the Hospital 33 (Nizhny Novgorod, Russia). The relative level of mRNA in peripheral blood and tumors was determined by the method of reverse transcription-polymerase chain reaction in real time.

Results In the peripheral blood of patients with prostate cancer and patients with hyperplasia, the level of mRNA *FCGR3A* and *FCGR3B* was statistically significantly lower than in healthy individuals. The normalization of the CD16 level in the blood of healthy donors was higher

The relative level of mRNA *FCGR3A*, *FCGR3B* was the highest in patients with Prostate antigen specific (PSA) from 10Ng/ml to 20Ng/ml. The higher level mRNA *FCGR3A* and *FCGR3B* was for patients with higher testosterone ≥ 8 mmol/L. also a higher level of *FCGR3A*, *FCGR3B* was found in patients diagnosed with an adenopathy, a higher size prostate and a higher Gleason Scores.

The results of Classification based on the degree of differentiation shows that the level of mRNA *FCGR3A* and *FCGR3B* in patients with medium differentiation was higher and statistically significant than in patient with lower differentiation.

Conclusion. The Changes in the mRNA level of genes encoding CD16A (*FCGR3A*) and CD16B (*FCGR3A*) was detected in blood and tumor samples. The results indicate the potential use of these indicators as monitoring immunological markers in hyperplasia and prostate cancer.

Keywords: CD16, prostate cancer, Prostate volume, PSA.

Introduction

In humans, the immunoglobulin G receptor is encoded by two genes *FCGR3A* and *FCGR3B*. *FCGR3A* (CD16A) is characteristic of the membranes of monocytes, tissue macrophages, $\gamma\delta$ T-lymphocytes and dendritic cells, as well as natural killer, while the *FCGR3B* is a molecular marker of neutrophils, *FCGR3B* is also expressed in basophils, and eosinophils after induction with interferon $\gamma(1)$

Natural killer are activated by CD16A when they bind to cells that have already been opsonized with antibodies, they induce degranulation which liberate their lytic components, as well as the production of various cytokines and chemokines(2) also the neutrophils play an important role in the inflammatory response. In the consequence suppress the proliferation of T-cells through reversible arrest of the cell cycle, rather than induction of apoptosis (3)

Evaluation of the expression of membrane molecules CD16 is used to determine the population composition of peripheral blood cells and their functional state in various diseases, including oncological diseases.

The purpose of the study was to evaluate the level of mRNA of gene encoding CD16A (FCGR3A) and CD16B (FCGR3B) in peripheral blood and PC tumors.

Materials and methods

In the study, we analyzed 240 mRNA samples, including 49 blood from healthy donors, 37 blood from prostate cancer patients and 62 tissue prostate tumors, 37 blood from patients with hyperplasia and 55 tissue from patients with hyperplasia, all patients signed consent to participate in the study

All test samples were mixed with an equal volume of a preservative solution (4M guanidine thiocyanate, 100mM sodium citrate, and 0.1% Triton X-100) and stored at -20 ° C until use. Isolation of nucleic acids was carried out by the method proposed by Chomczynski P. and Sacchi N. [4]. Complementary DNA was synthesized using M-MLV reverse transcriptase (CJSC Sileks, Russia) according to the manufacturer's recommendation. Determination of the relative levels of FOXP3 mRNA was carried out using real-time polymerase chain reaction (PCR) using an amplifier CFX96 Touch™ (Bio-Rad, USA) under the following temperature conditions: 94 ° C - 10 min, 50 PCR cycles 94 ° C - 20 sec, 60 ° C - 20 sec, 72 ° C - 20 sec. For FOXP3 mRNA, the following conditions were used: 94 ° C for 10 min, 50 PCR cycles at 94 ° C for 30 sec, 55 ° C for 30 sec, 72 ° C for 30 sec. The reaction mixture contained 70mM Tris-HCl, pH 8.3, 16 mM (NH₄)₂SO₄, 2 mM MgCl₂, 2.5 units of HotTaq polymerase activity (ZAO Biolabmix, Russia), 0.8 mM deoxynucleoside triphosphates and 0, 4 pM of each of the oligonucleotides (CJSC Syntol, Russia), the sequence of which is presented in Table 1.

Table 1

Structure of oligonucleotides

Gene	Oligonucleotide	Primer structure (5' - 3')
UBC	F	GCACAGCTAGTTCGTCGCA
	R	TGCATTGTCAAGTGACGAT
	Z	CY5-ATTTGGGTCGCAGTTCCTTGTGGAT-BHQ-2
FCGR3A/or B	FAB	GCTCTGCTACTTCTAGTTTCA
FCGR3A	RA	CACTGTCCTTCTCGAGCACC
FCGR3B	RB	CACTGTCCTTCTCAAGCACG
FCGR3A/or B	FAB Z	ROX-CTGTGGTGTTCCTGGAGCCTCAATGGTA-BHQ-2

The mRNA levels were calculated using the formula $\Delta\Delta C_t$ taking into account the efficiency of the reaction [9]. Normalization of mRNA levels was carried out relative to the mRNA level of ubiquitin C (UBC).

Statistical processing of the results was performed using the Graph Pad Prism 8.4.3 software. To determine quantitative indicators, the median (Me), the lower quartile Q1 (25%), and the upper quartile Q3 (75%) were calculated. To compare three independent groups, we used the Anova One way test; to compare two independent groups, we used the Test-non parametric. Differences between groups were considered statistically significant at $p < 0.05$.

Also in our analysis, we propose a classification model for three parameters: PSA concentration, testosterone concentration, and prostate size. Model Based on the distribution relative level expression of CD16 (Table 2)

Results

Determination of the relative level of mRNA of the FCGR3A and FCGR3B genes in the peripheral blood of donors and patients with prostate cancer and hyperplasia showed that it was detected in all studied samples.

To get the most effective results and to avoid false-negative or false-positive results. We defined the minimum and the maximum relative level expression (RLE).

Our results show that the amount of RNA CD16 was higher and significant in healthy donors compared to adenom and cancer patients (**** $p < 0.0001$; *** $p < 0.001$). (Fig. 1)

In tumor, we identified that patients diagnosed with Pc had more CD16 than patients diagnosed with Adenoma (* $p = 0.03$). (Fig. 2)

Table 2

<i>The FCGR3A and FCGR3B mRNA level in peripheral blood and tumors of prostate cancer patients, Me (Q₁-Q₃)</i>				
<i>Category</i>		<i>Number of patients</i>	<i>mRNA levels FCGR3A</i>	<i>mRNA levels FCGR3B</i>
<i>Tissue</i>	<i>Adenom</i>	55	0,6353 (0,3127 - 2,014)	1,376 (0,5366 - 2,399)
	<i>Cancer</i>	62	1,499 (0,5993 - 3,352)	2,167 (0,7335 - 4,009)
<i>Blood</i>	<i>Adenom</i>	37	0,9235 (0,5978 - 3,113)	1,974 (0,4394 - 3,546)
	<i>Cancer</i>	37	1,812 (0,9947 - 3,554)	2,300 (0,8578 - 5,264)
	<i>Healthy</i>	49	4,759 (0,8456 - 13,61)	5,434 (0,7967 - 14,54)

Figure 1. The FCGR3A and FCGR3B mRNA level in peripheral blood: 1 –adenoma, 2 – patients with prostate cancer, 3 –healthy donors

**** $p < 0,000$; *** $P = 0,0002$

Figure 2. The FCGR3A and FCGR3B mRNA level in Tissue: 1 –adenoma, 2 – patients with prostate cancer.

* $p = 0,03$

Table 3

Parameter	category	Quantity	Relative level of mRNA in tissues	
			FCGR3A	FCGR3B
PSA	0-10Ng/ML.	85	0,9093 (0,4650 - 2,028)	1,280 (0,5366 - 2,170)
	10-20 Ng/ML.	10	1,765 (1,391 - 4,55)	4,281 (2,617 - 5,521)
	+20Ng/ML.	22	1,520 (0,4503 - 2,497)	2,151 (0,8105 - 3,863)
Testosterone	<8Mmol/L	50	1,250 (0,5097 - 1,985)	1,246 (0,3481 - 2,710)
	≥ 8Mmol/L	67	1,211 (0,3445 - 3,510)	1,762 (0,8560 - 4,306)
Tumor size	<35 Cm ³	40	1,283 (0,2275 - 1,880)	1,328 (0,3473 - 2,396)
	≤45 Cm ³	43	0,9093 (0,5012 - 2,497)	2,141 (0,6260 - 4,004)
	>45 Cm ³	34	1,800 (0,4712 - 4,314)	3,772 (1,696 - 6,975)
Gleason Scores	<7	30	1,318 (0,4063 - 2,580)	1,272 (0,2522 - 2,342)
	=7	22	1,563 (0,4199 - 3,425)	3,264 (1,107 - 4,012)
	>7	10	5,379 (4,287 - 6,530)	6,105 (4,293 - 8,253)
LYMPH NODES	Intact	95	1,039 (0,4831 - 2,533)	1,978 (0,5980 - 3,766)
	adenopathy	22	3,211 (1,808 - 4,550)	2,978 (1,547 - 5,049)
DEGREE OF DIFFERENTIATION	Low	25	1,563 (0,3286 - 2,570)	2,552 (0,6286 - 3,601)
	Moderate	37	3,173 (1,196 - 5,152)	3,000 (1,396 - 5,779)

The mRNA level of FCGR3A, FCGR3B was the highest in patients with PSA from 10 Ng/ml to 20Ng/ml. Comparison results between groups show that there is a statistical increase *** $p=0.0005$ on FCGR3A, and with patient PSA>20Ng/ml than a statistical decrease * $p=0.01$. The same result On FCGR3B, there was an increase of both more statistical significance and more important * * * * $p<0.0001$ between the first and second group, we also found a statistical decrease (* $p = 0.01$) in patients with PSA higher than 20 Ng / ml.

(RLE) FCGR3A, and FCGR3B in patients with a concentration of testosterone ≥ 8mmol / L was higher and statically significant than in patients with testosterone concentration (<8mmol / L) (* $p = 0.01$.) Fig 4. Also we identify very important increase on FCGR3A, FCGR3B in patients diagnosing adenopathy (** $p = 0.005$) on FCGR3A and (* $p = 0.04$) FCGR3B. (Fig7).The growth in RLE FCGR3A, and FCGR3B was statistically significant and progressively with the growth of prostate size and Gleeson scor (* $p = 0.03$, ** $p = 0.003$, **** $p < 0.0001$), (Fig 5.6). More the cancer is aggressive, more the size of prostate is higher, and more the RLE CD16 in tumor is higher. Classification of the results according to the degree of differentiation shows that patients with a moderate degree of differentiation had a higher level of CD16 (** $p = 0.005$; * $p = 0.04$).

Our results show also that the level of FCGR3B is always higher than the level of FCGR3A in peripheral blood (Blood of Adenoma, Blood of prostate cancer, healthy donors) and in tumors tissues (Table. 2)

Figure 3. The FCGR3A and FCGR3B mRNA level according to the concentration of PSA
1- PSA 0-10 Ng/ml, 2-PSA 10-20 Ng/ml, 3- PSA +20 Ng/ml.
**** $p < 0,0001$; *** $p = 0,0005$; * $p = 0,01$

Figure 4. The FCGR3A and FCGR3B mRNA level according to the concentration of testosterone
1- Testosterone < 8 Mmol/L, 2- Testosterone ≥ 8 Mmol/L
* $p = 0,01$

Figure 5. The FCGR3A and FCGR3B mRNA level according to the Volume of prostate
1- Volume ≤ 35 cm³, 2- Volume ≤ 45 cm³, 3- Volume > 45 cm³
**** $p < 0,0001$; * $p = 0,03$; ** $p = 0,003$

Figure 6. The FCGR3A and FCGR3B mRNA level according to the Gleason score
1- Gleason score <7. 2- Gleason score =7. 3- Gleason score >7
**** $p < 0,0001$; *** $p = 0,0004$; ** $p = 0,003$; * $p = 0,01$

Figure 7. The FCGR3A and FCGR3B mRNA level according to the situation of lymph nodes
1- Lymph nodes intact. 2- Presence of adenopathy
** $p = 0,005$; * $p = 0,04$

Figure 8. The FCGR3A and FCGR3B mRNA level according to the situation of lymph nodes
1-Low degree. 2-Medium degree
** $p = 0,005$; * $p = 0,04$

Discussion

The development of prostate cancer is accompanied by multiple disorders in the state of the immune system, in particular the number of cells expressing CD16A and CD16B. according to the literature, there is a tendency to an increase in the number of natural killer, which are one of the main carriers of CD16A, on the other side a decrease in their functional activity is recorded in the peripheral blood(5). In addition, there is an increase in the number of CD16B + neutrophils in the peripheral blood (6). According to our results, we found a decrease in the expression of both genes CD16 in the blood of patients. RLE CD16 increases with concentration of PSA, as well as the size of tumors and the Gleason Scores, and also the degree of differentiation, the same results were obtained by Yiyang Wang et al on the number of neutrophils with PSA concentration, and the Gleason Scores(7) decreased RLE CD16 in patients with PSA concentration. +20Ng/ml is not yet clear, the only hypothesis that can be said is that tumors change to PSA independently, which gives a decrease in factors synthesizing cell tumors, and as a result, a decrease in level CD16 (8), a decrease concentration in testosterone was statistically significant with a decrease in CD16 levels. our results were confirmed by Janet L. Markman, et al. Scientists found that lower testosterone concentrations attenuated the antitumor function of neutrophils(12)

The development of tumor cells, invasion and the existence of metastases are guaranteed by neutrophils and lymphocytes that penetrate into the tumor microenvironment. scientifics think that neutrophils play “role double agent” , some of them found that Neutrophils promote tumor-related inflammation, thereby contributing to tumor cell proliferation, invasion, and tumor angiogenesis. Others found that neutrophils enhance the reactivity of CD8⁺ T cells and activate T cell receptors, contributing to the death of tumor cells (9-11)

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