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ВЗАИМОСВЯЗЬ ФЕНОТИПА И МЕТАБОЛИЗМА НЕЙТРОФИЛОВ КРОВИ У БОЛЬНЫХ РАКОМ ПОЧКИ

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Резюме. Целью исследования было изучение особенностей взаимосвязи между экспрессией рецепторов активации и адгезии нейтрофилов крови и внутриклеточной активностью ферментов у больных раком почки (РП). Больные (n = 72) с РП (T3N0M0, светлоклеточный тип) были обследованы до хирургического лечения на базе Красноярского краевого онкологического диспансера. Диагноз «РП» подтвержден гистологически. Фенотип нейтрофилов крови изучали методом проточной цитометрии. Уровни экспрессии поверхностных рецепторов нейтрофилов оценивали по средней интенсивности флуоресценции. Активность НАД- и НАДФ-зависимых дегидрогеназ в нейтрофилах крови измеряли биолюминесцентным методом. Установлено, что изменение фенотипа нейтрофилов крови у больных с РП проявляется на фоне торможения основных внутриклеточных метаболических процессов и во взаимосвязи с ними. Особенностями фенотипического состава нейтрофилов у больных с РП было уменьшение относительного количества клеток, экспрессирующих адгезивные (CD11b и CD62L) и функциональные (CD64 и HLA-DR) рецепторы. Нейтрофилы крови больных раком интенсивнее экспрессировали такие молекулы, как CD11b, CD16 и HLA-DR. Эти изменения в фенотипе нейтрофилов крови у больных с РП определялись на фоне выраженного уменьшения количества незрелых клеток. Метаболические особенности цитоплазматического компартмента нейтрофилов крови у больных с РП характеризовались снижением активности глюкозо-6-фосфатдегидрогеназы (ключевого и инициализирующего фермента пентозофосфатного цикла) и NADH-зависимой реакцией лактатдегидрогеназы (НАДН-ЛДГ, анаэробный гликолиз). Митохондриальный метаболизм у нейтрофилов больных с РП характеризовался разнонаправленными изменениями активности НАД-(НАДН-ГДГ) и НАДФ-зависимых глутаматдегидрогеназ (снижение активности НАД-зависимых и повышение активности НАДФ-зависимых) и снижением активности НАДН-зависимая реакция малатдегидрогеназы. Установленные особенности в активности митохондриальных ферментов характеризуют нарушение НАД-зависимых процессов, что может привести к снижению интенсивности аэробных энергетических процессов. С помощью корреляционного анализа было обнаружено, что особенности взаимосвязей у больных РП определялись отрицательным влиянием активности НАДН-ГДГ и НАДН-ЛДГ на уровни экспрессии рецепторов активации и адгезии нейтрофилов. Только ак-

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тивность глутатионредуктазы в нейтрофилах больных РП положительно коррелировала с экспрессией молекул CD23 и HLA-DR. Повышение активности энергетических процессов (в том числе процессов взаимодействия цикла трикарбоновых кислот с реакциями обмена аминокислот) в нейтрофилах крови больных раком почки может стимулировать уровни экспрессии рецепторов активации и адгезии и повышать противоопухолевую активность нейтрофилов.

Ключевые слова: нейтрофилы, фенотип, антигены, метаболизм, активность ферментов, рак почки

IMMUNOPHENOTYPE AND METABOLISM ARE LINKED IN PERIPHERAL BLOOD NEUTROPHILS FROM PATIENTS WITH KIDNEY CANCER

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Abstract. The aim of the present study was to analyze the relationships between expression of activation and adhesion receptors on peripheral blood neutrophils, and intracellular activity of some neutrophil enzymes in patients with kidney cancer (KC). Patients and methods: the KC patients (n = 72) (T3N0M0, clear-cell type) were examined prior to surgical treatment at the Krasnoyarsk Regional Oncology Center. The diagnosis was verified histologically for all KC patients. The phenotype of blood neutrophils was studied using flow cytometry. The surface receptor expression levels of the neutrophils were evaluated by mean fluorescence intensity. NAD and NADP-dependent dehydrogenases activities in purified peripheral blood neutrophils were measured by bioluminescent method. Results: we have found that the phenotypic alterations in circulating KC patients' neutrophils appeared along with inhibition of main intracellular metabolic processes and were closely linked with them. The features of the phenotypic imbalance in the neutrophils from KC patients were associated with a decrease in blood cells expressing adhesive (CD11b and CD62L) and functional (CD64 and HLA-DR) receptors. Moreover, the patient's neutrophils expressed CD11b, CD16 and HLA-DR on their cell surface more intensively, than neutrophilic leukocytes from control group. These phenotypic changes in KC patients' blood neutrophils occurred in parallel with pronounced decrease in immature cells numbers. The metabolic changes of neutrophil cytoplasmic compartment in KC patients were determined by a decrease in Glu6PDH activity (a key and initializing enzyme of the pentose phosphate cycle) and NADH-LDH (anaerobic glycolysis). Mitochondrial metabolism in neutrophils of KC patients was characterized by multidirectional changes in the activity of NAD- and NADP-dependent glutamate dehydrogenases (decreased activity of NAD-dependent and increased activity of NADP-dependent) and a decrease in NADH-MDH activity. The established features in mitochondrial enzymes activities suggest some disturbances of NAD-dependent processes that could lead to down-regulation of aerobic energy processes. We guess that the decreased activity of plastic and energy processes in blood neutrophils of KC patients could affect the receptor expression levels. By means of correlation analysis, we have found that the relationships in KC patients were determined by negative effects of NADH-GDH and NADH-LDH activities upon expression of activation and adhesion receptors in blood neutrophils. Of these enzymes, only glutathione reductase activity in neutrophils from KC patients was positively linked with the CD23 and HLA-DR expression. Thus, an increase in activity of energy processes (e.g., coupling the tricarboxylic acid cycle to amino acid metabolism) in blood neutrophils from the patients with kidney cancer could stimulate expression levels of activation and adhesion receptors and potentially increase antitumor activity of neutrophils.

Keywords: neutrophils, phenotype, antigens, metabolism, enzyme activity, kidney cancer

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Introduction

Neutrophils are one of the most abundant type of innate immune cells that take part in various immuno-inflammatory processes. It is well-known that neutrophils participates not only in responses of the immune system to infection but also play an important role in antitumor immunity. Several studies have shown that the neutrophils frequency increases during tumor progression and cancer-associated chronic inflammation and, overall, can rise up 90% within total leucocytes population on terminal stages [20, 28, 37]. An increased neutrophil-to-lymphocyte ratio was a prognostic indicator of unfavorable prognosis in patients with different cancers [21, 22]. However, there were several papers showing that in patients with cancer peripheral blood neutrophils frequency could be down-regulated if compared with healthy controls [6, 10]. Furthermore, neutrophils are often recruited to the tumor microenvironment where they could constitute a significant part of tumor-infiltrating cells [9, 34, 36].

It has been established that the functional activity of neutrophils (such as phagocytes, ROS production ect.) from peripheral blood of cancer patients could be altered. For instance, most recent paper by Zeindler J. et al. (2019) have shown that neutrophilic granulocytes positive for myeloperoxidase carried out active phagocytosis of breast cancer cells [37]. Moreover, the favorable prognosis was strongly associated with high intracellular activity of this enzyme in phagocytic cells. Next, the mechanism of trogoptosis – antibody-dependent tumor cell killing by neutrophils – was defined [19]. During phagocytosis, neutrophils react to cancer cells releasing several types of oxidants to kill the cancer cells. Additionally, it has been shown that neutrophils basic level of primary reactive oxygen species production in patients with kidney cancer (KC) was increased [15, 26, 28]. While stimulated neutrophils from the same patients group exhibited the reduced ability of superoxide radical synthesis.

It can be assumed that neutrophils functional activity alterations during tumor progression could be associated with their phenotype changes. It was shown that peripheral blood neutrophils from patients with epithelial ovarian cancer showed reduced levels of

Англоязычный список ферментов

Glucose-6-phosphate dehydrogenase (Glu6PDH)	Глюкозо-6-фосфатдегидрогеназа (Г6ФДГ)
Glycerol-3-phosphate dehydrogenase (Gly3PDH)	Глицерол-3-фосфатдегидрогеназа (Г3ФДГ)
Lactate dehydrogenase, NAD-dependent reaction (LDH)	Лактатдегидрогеназа, НАД-зависимая реакция (ЛДГ)
NADP-dependent malate dehydrogenase decarboxylated (NADP-MDH)	НАДФ-зависимая декарбоксилирующая малатдегидрогеназа (НАДФМДГ)
NADP-dependent glutamate dehydrogenase (NADP-GluDH)	НАДФ-зависимая глутаматдегидрогеназа (НАДФГДГ)
NADP-dependent isocitrate dehydrogenase (NADP-ICDH)	НАДФ-зависимая изоцитратдегидрогеназа (НАДФИЦДГ)
Malate dehydrogenase, NAD-dependent reaction (MDH)	Малатдегидрогеназа, НАД-зависимая реакция (МДГ)
NAD-dependent glutamate dehydrogenase (NAD-GluDH)	НАД-зависимая глутаматдегидрогеназа (НАДГДГ)
NAD-dependent isocitrate dehydrogenase (NAD-ICDH)	НАД-зависимая изоцитратдегидрогеназа (НАДИЦДГ)
NADH-dependent reaction of lactate dehydrogenase (NADH-LDH)	НАДН-зависимая реакция лактатдегидрогеназы (НАДН-ЛДГ)
NADH-dependent reaction of malate dehydrogenase (NADH-MDH)	НАДН-зависимая реакция малатдегидрогеназы (НАДН-МДГ)
Glutathione reductase (GR)	Глутатионредуктаза (ГР)
NADH-dependent reaction of glutamate dehydrogenase (NADH-GluDH)	НАДН-зависимая реакция глутаматдегидрогеназы (НАДН-ГДГ)
NADPH-dependent reaction of glutamate dehydrogenase (NADPH-GluDH)	НАДФН-зависимая реакция глутаматдегидрогеназы (НАДФН-ГДГ)

L-selectin expression while MAC-1 (macrophage-1 antigen, CR-3, CD11b/CD18) expression was increased [2]. Mac-1 has been proven to be a key adhesion molecule that promoted cancer progression and mediated tumor cells adhesion to blood vessel endothelial cell. Next, $CD66b^+$-neutrophil frequency was reduced in the invasive front from patients with lip squamous cell carcinoma [4]. CD66b (CGM6, NCA-95) is a glycosylphosphatidylinositol (GPI)-linked protein that induces the activation of neutrophilic granulocytes and plays an important part in cell-to-cell adhesion.

Eventually, peripheral blood neutrophils have both pro-tumor and antitumor activities. The antitumor activity of neutrophils is realized due to their mechanisms of cytotoxicity and their ability to stimulate different innate and adaptive immunity cells [3, 4, 11]. The following pro-tumor functions of neutrophils were distinguished: stimulation of angiogenesis, tumor cell dissemination, as well as immune suppression [3, 11, 36]. A change in the orientation of neutrophil function in relation to tumor cells was defined as reprogramming. Moreover, various methods for changing the functional activity of neutrophils were currently being developed [11]. Metabolic reprogramming is one of the most actively developing method for regulating immune system cells functional activity *in vitro* [1, 33]. Changes in cell metabolism through a system of transcription factors affect the differentiation and functional activation of cells. It is known that the activity of metabolic processes in circulating neutrophils were altered in patients with cancer [23, 29]. It can be suggested that neutrophils intracellular metabolism imbalance in cancer patients can also be linked with neutrophils phenotype variations.

The aim of the study was to analyze the correspondence between peripheral blood neutrophil activation and adhesion receptors expression and activity of neutrophils intracellular enzymes in patients with KC.

We have studied the activity of NAD(P)-dependent dehydrogenases as indicators characterizing the state of the intracellular metabolic processes in the neutrophils. This type of the enzymes plays an important part in intracellular metabolism. Firstly, the main electron carriers in cells are pyridine nucleotides and hence the dehydrogenases are actively involved in bioenergy processes. Secondly, the NAD(P)-dependent dehydrogenases determine adaptive changes in intracellular metabolism by participating in the directed coordination of conjugated metabolic fluxes [14, 24, 25].

Materials and methods

Study participants

Patients (n = 72, age – 45-62 years old) with KC (T3N0M0, clear cell type) were examined prior to surgical treatment in Krasnoyarsk Regional Oncology Center. The diagnosis of KC of all patients was verified histologically. Peripheral blood samples from 51 healthy volunteers with the same age range were used as a control group. All studies were performed with the informed consent of the patients and in accordance with the Helsinki Declaration of the World Association "Ethical principles of scientific medical research involving human" as amended in 2013 and "Rules of clinical practice in the Russian Federation" approved by the Order of the Ministry of Health of Russia of 19.06.2003 (No. 266).

Flow cytometry

The phenotype of blood neutrophils were studied by direct immunofluorescence using monoclonal antibodies (all from Beckman Coulter, USA) labeled with FITC (fluorescein isothiocyanate), PE (phycoerythrin), ECD (phycoerythrin-Texas Red-X), PC5 (phycoerythrin-cyanin 5) и PC7 (phycoerythrin-cyanin 7), AF700 (alexa fluor 700) and AF750 (alexa fluor 750): CD11b, CD62L, CD16, CD23, CD28, CD64 и HLA-DR. The distribution of antibodies in fluorescence channels was carried out in accordance with the principles of panel formation for the multicolor cytofluorimetric investigations [13]. The surface receptor expression levels were evaluated by mean fluorescence intensity (MFI). Sample preparation was performed according to the standard procedure [17]. Erythrocyte lysis was carried out by a no-wash technology using the reagent VersaLyse (Beckman Coulter, USA). The analysis of stained cells was performed on a flow cytometer Navios (Beckman Coulter, USA) of the Center of collective usage Krasnoyarsk Regional Center of Research Equipment of Federal Research Center "Krasnoyarsk Science Center SB RAS". Not less than 50 000 neutrophils were analyzed per each sample.

Bioluminescent analysis

Neutrophils were isolated from whole heparinized blood by a standard method in the density gradient of ficoll-urografin. The cells were destroyed by osmotic lysis with addition of 2 mM dithiotreitol. Activities of the following enzymes were measured by the bioluminescent method: glucose-6-phosphate dehydrogenase (Glu6PDH), glycerol-3-phosphate dehydrogenase (Gly3PDH), NAD- and NADH-dependent reactions of lactate dehydrogenase (LDH and NADH-LDH, respectively), NADP-dependent malate dehydrogenase decarboxylated (NADP-MDH), NADP and NADPH-dependent reactions of glutamate dehydrogenase (NADP-GluDH and NADPH-GluDH, respectively), NADP-dependent isocitrate dehydrogenase (NADP-ICDH), NAD- and NADH-dependent reactions of malate dehydrogenase (MDH and NADH-MDH, respectively), NAD- and NADH-dependent reactions of glutamate dehydrogenase (NAD-GluDH and NADH-GluDH, respectively), NAD-dependent isocitrate dehydrogenase (NAD-ICDH) and glutathione reductase (GR). The bioluminescent study was carried out on NAD(P):FMNoxidoreductase-luciferase from

Photobacterium leiognathi (Institute of Biophysics, Siberian Division of Russian Academy of Sciences, Krasnoyarsk) [25]. Activities of NAD(P)-dependent dehydrogenases in the neutrophils was determined on the 36-channel biochemiluminescence analyzer BLM-3607 (MedBioTech Ltd, Russia) and were expressed in enzymatic units (1 U = 1 μmol/min).

Statistical analysis

Statistical description was performed by counting the median (Me) and the inter-quarter span in the form of 25 and 75 percentiles ($Q_{0.25}$-$Q_{0.75}$). Significance of differences between indicators was assessed by nonparametric criterion Mann–Whitney U test. The Spearman rank correlation coefficients were calculated to characterize the strength of the relationship between the expression of receptors on the neutrophil membrane and the activity of their enzymes. Statistical analysis was performed in an application package Statistica 6.1 (StatSoft Inc.).

Results

First, we found that the relative number of stab (band cells or immature neutrophils) neutrophils was reduced in the peripheral blood from KC patients (Table 1). The phenotypic composition of neutrophils in patients with KC was characterized by decreased levels of $CD11b^+$, $CD62L^+$, $CD64^+$, $HLA\text{-}DR^+$ and $CD64^+HLA\text{-}DR^+$ cells, but we found the increased relative number of $CD28^+$ neutrophils. Next, our findings demonstrated that peripheral blood neutrophils from cancer patients showed significant increase in CD11b, CD16, CD28 and HLA-DR MFI levels if compared with healthy control values (Table 2).

Additionally, using bioluminescent method it was found that the activities of such enzymes as Glu6PDH, NADP-MDH, NAD-GluDH, NADH-LDH, NADH-MDH and NADH-GluDH were reduced in KC patients blood neutrophils (Table 3). At the same time, the activities of Gly3PDH, NADP-GluDH and NADPH-GluDH in circulating neutrophils from KC patients were significantly higher than in samples from control group. The relationships between enzymes activities and cell-surface phenotypes were studied using correlation analysis. In the control group positive correlation was detected between NADH-GluDH activity and relative number of mature segmented neutrophils ($r = 0.68$, $p = 0.003$). In addition, the negative correlation between Glu6PDH activity and $CD62L^+$ neutrophil percentage ($r = -0.78$, $p = 0.039$) was revealed in the control group. In blood samples fron control group we also found the following significant association: NADP-MDH *versus* HLA-DR MFI levels ($r = 0.79$, $p = 0.033$) and NADH-GluDH *versus* CD11b MFI levels ($r = 0.90$, $p = 0.009$).

Finally, in KC patients we revealed statistically significant negative correlation between NADH-GluDH activities and relative numbers of segmented neutrophils ($r = -0.37$, $p = 0.017$). Within KC group correlations between NADH-LDH activity and $CD62L^+$ as well as $HLA\text{-}DR^+$ neutrophil frequencies were also identified ($r = -0.46$, $p = 0.008$ and $r = -0.36$, $p = 0.041$, respectively) were found. Moreover, HLA-DR expression on peripheral blood neutrophils from those patients was negatively correlated with NADP-MDH and NADP-ICDH activities ($r = -0.38$, $p = 0.029$ and $r = -0.39$, $p = 0.029$, respectively). Next, NAD-GluDH was the only one enzyme in KC patients' neutrophils whose activity was negatively interconnected with CD16 ($r = -0.49$, $p = 0.007$), CD23 ($r = -0.67$, $p = 0.017$) and HLA-DR ($r = -0.52$, $p = 0.002$) MFI values. While GR enzymatic activity showed positive relationships with CD23 and HLA-DR MFI levels ($r = 0.66$, $p = 0.020$ and $r = 0.37$, $p = 0.037$, respectively).

Discussion

Currently, we investigated frequency, maturation stages and phenotype alterations in peripheral blood neutrophils from kidney cancer patients. Here, we describe for the first time that the number of stab neutrophils in the peripheral blood of KC patients was significantly reduced if compared with healthy control. Moreover, the results of the Sumida K. et al. (2012) study showed that the number of tumor-infiltrating neutrophils increased primarily due to the CD11-positive stab cells [30]. Our study showed the reduced frequency of CD11b- and CD62L-positive neutrophils in blood samples from of KC patients. In is known that CD11b is a membrane glycoprotein, the member of integrin superfamily, that forms α-chain of CR3 receptor, and it functionally mediates cell adhesion, transmigration and chemotaxis [12]. While CD62L belongs to the family of L-selectins and together with P-selectin provides the initial contact of circulating leukocytes with endothelial cells, and these interaction is the first stage of cell extravasation [16]. We assume that the decrease in the number of blood neutrophils expressing adhesion receptors in KC patients could be closely linked with cell migration at tumor site. Functionally active neutrophils in tumor environment could be reprogrammed into so-called "polymorphonuclear neutrophil myeloid-derived suppressor cells" (or PMN-MDSCs) – f specific subset of myeloid cells able to inhibit antitumor immune response [9, 33]. It is also known that PMN-MDSCs could be detected within peripheral blood circulating immature neutrophils, and PMN-MDSCs frequency mostly decreased in cancer patients [33].

The phenotypic state of the blood neutrophils in cancer patients was also characterized by reduced number of cells expressing on their cell membranes such activation receptors as CD64 and HLA-DR. CD64 (FcγRI) is a high affinity IgG receptor which is the only receptor type capable of binding free antibodies [8]. Activated neutrophils during the expression of this receptor can retain a large number

TABLE 1. MORPHOLOGY AND PHENOTYPE OF BLOOD NEUTROPHILS IN PATIENTS WITH KC, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control (n = 51)	Patients with KC (n = 72)	p
Neutrophils, 10^9/l	3.25 (2.48-4.23)	3.18 (2.35-4.56)	
Stab neutrophils, %	2.1 (0.0-4.0)	0.0 (0.0-1.0)	< 0.001
Segmented neutrophils, %	55.1 (47.0-62.0)	58.0 (49.0-65.1)	
$CD11b^+$, %	99.9 (99.8-100.0)	99.8 (99.7-99.9)	0.032
$CD16^+$, %	92.4 (88.4-96.7)	90.1 (85.8-95.4)	
$CD23^+$, %	10.5 (6.6-15.5)	8.5 (4.7-17.1)	
$CD62L^+$, %	99.6 (98.8-99.8)	97.4 (90.4-99.3)	< 0.001
$CD64^+$, %	15.7 (5.5-92.1)	7.4 (4.1-15.5)	0.045
$HLA\text{-}DR^+$, %	98.2 (94.2-99.2)	89.8 (59.7-97.7)	< 0.001
$CD64^+HLA\text{-}DR^+$, %	8.4 (5.2-13.0)	5.7 (2.8-9.6)	0.048

TABLE 2. EXPRESSION LEVELS OF THE ANTIGENS ON BLOOD NEUTROPHILS IN PATIENTS WITH KC, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control (n = 51)	Patients with KC (n = 72)	p
$CD11b^+$	110.40 (19.33-214.09)	128.92 (71.96-211.46)	0.048
$CD16^+$	108.51 (79.80-177.05)	157.04 (93.21-230.07)	0.046
$CD23^+$	6.39 (5.00-7.78)	6.23 (4.86-7.30)	
$CD62L^+$	5.61 (2.79-7.54)	5.57 (3.54-7.96)	
$CD64^+$	2.23 (1.61-3.45)	2.17 (1.71-7.68)	
$HLA\text{-}DR^+$	1.66 (1.47-1.85)	1.85 (1.63-2.76)	0.005

TABLE 3. ACTIVITY OF NAD- AND NADP-DEPENDENT DEHYDROGENASES IN BLOOD NEUTROPHILS OF THE PATIENTS WITH KC, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control n = 51	Patients with KC n = 72	p
Glu6PDH	1.94 (0.63-9.93)	0.35 (0.02-1.28)	< 0.001
Gly3PDH	0.12 (0.01-0.36)	1.02 (0.11-3.25)	< 0.001
LDH	7.11 (1.62-28.91)	7.45 (1.55-26.34)	
NADP-MDH	1.54 (0.52-2.20)	0.21 (0.01-1.35)	0.014
NADP-GluDH	1.58 (0.15-6.08)	5.44 (2.95-15.99)	0.048
NADP-ICDH	3.66 (1.33-10.30)	2.31 (1.41-10.89)	
MDH	12.96 (2.10-54.00)	9.33 (1.08-53.38)	
NAD-GluDH	1.26 (0.31-5.23)	0.45 (0.01-2.68)	0.038
NAD-ICDH	0.18 (0.01-1.24)	0.14 (0.01-1.71)	
NADH-LDH	61.25 (10.01-80.07)	15.98 (2.31-30.46)	0.009
NADH-MDH	50.13 (10.82-106.11)	25.67 (3.08-44.03)	0.024
GR	1.06 (0.11-8.22)	5.44 (1.05-19.36)	
NADH-GluDH	15.85 (1.61-46.04)	6.86 (0.80-19.39)	0.037
NADPH-GluDH	0.12 (0.01-2.70)	21.90 (4.68-74.08)	< 0.001

of IgG molecules on their surface and realize the function of the trogoptosis [19]. HLA-DR is major histocompatibility complex class II molecule that is responsible for antigen presentation to Th cells [18]. Reduced levels of the blood neutrophils expressing activation markers could also be linked with cell migration alterations occurred in KC patients. It should be also noted that circulating neutrophils from KC patients were characterized by up regulated low affinity IgG-receptor (CD16), CD11b and HLA-DR expression.

Intracellular metabolism largely determines functional activity of neutrophils in various immunopathological conditions [24, 27]. Tumor-infiltrating cell reprogramming could be realized through alterations in cell metabolic processes [35]. Our study found that the imbalance in peripheral blood neutrophils phenotype found in KC patients was accompanied by altered activity of intracellular metabolic processes. For example, Glu6PDH is the key and initializing enzyme of the pentose phosphate cycle [14, 32]. The activity of this enzyme in blood neutrophils in KC patients was reduced, that fact pointed to down-regulation of several reactions in macromolecular synthesis. The most likely cause could be the insufficient intensity of the initial glycolysis reactions. While the increase in Gly3PDH activity in patients was apparently a compensatory reaction of intracellular metabolism. This enzyme transfers lipid catabolism products to redox reactions of the glycolysis [14, 26]. However, the low activity of the anaerobic LDH reaction in neutrophils from KC patients could indicate the insufficiency of anaerobic glycolysis. It should be noted that a low activity of the glycolysis (the main energy process for granulocytes) was associated with a lack of functional reactions of neutrophils [14, 26, 31].

Malic-enzyme is involved in xenobiotic catabolism reactions, and it also is a key enzyme in lipid anabolism reactions [26]. A decrease in the enzyme activity in patients' neutrophils could affect the sensitivity of cells to regulatory and functional signals as well as their resistance to toxic effects.

Mitochondrial respiration is not the main energy process of neutrophils, but mitochondrial metabolism integrates all the basic metabolic reactions together and, thus, being the key mechanisms of functional regulation [1, 5]. The activity of the two studied enzymes of the tricarboxylic acid cycle – NAD-ICDH and MDH – in blood neutrophils of KC patients corresponded to the control values. Accordingly, we could assume that the similar state of substrate flow rate along the tricarboxylic acid cycle was provided by mechanisms of additional substrate administration (with insufficient glycolysis activity). Next, in neutrophils from KC patients we found multidirectional changes in functional activity of NAD and NADP-dependent glutamate dehydrogenases – the activity of NAD-dependent glutamate dehydrogenase (both reactions) was decreased while the activity of NADP-dependent glutamate dehydrogenase (both reactions) was increased. The similar changes in the activity of glutamate dehydrogenases determined a decrease in NAD-dependent and an increase in NADP-dependent substrate metabolism between the tricarboxylic acid cycle and amino acid metabolism reactions in neutrophils of KC patients. We assume that the increase in NADP-dependent substrate metabolism could support the intensity of substrate flow along the tricarboxylic acid cycle. However, the decrease in NAD-dependent substrate metabolism could reduce the intensity of aerobic respiration of mitochondria in the neutrophils of KC patients as evidenced by a decrease in the activity of NADH-MDH. This enzyme is the key in the reactions of the malate-aspartate shunt that determines the effectiveness of the respiratory chain of mitochondria [14, 26].

Correlation analysis between the neutrophil phenotype (including receptor expression) and enzyme activities revealed metabolic reactions that were associated with activation and adhesion receptors expression. The inversion of the correlation in KC patients between mature neutrophils content and NADH-GluDH activity was found: in control group the correlation was positive, while in KC patients – negative. This inversion characterized a decrease in tricarboxylic acid cycle substrates outflow with an increase in the number of mature neutrophils in patients. Therefore, it could be a compensatory reaction aimed to the maintenance of aerobic respiration in neutrophils. The interconnections of the intracellular enzymes activity with the neutrophil phenotype were mainly determined by the role of metabolic processes in different aspects of cells functional activation. In particular, the number of $CD62L^+$ neutrophils in control group was negatively correlated with Glu6PDH activity while in KC patients – with NADH-LDH. Accordingly, the migration activity of blood neutrophils in control group was largely determined by the activity of plastic metabolism and in KC patients – by the intensity of substrate flow in anaerobic glycolysis. In addition, $HLA\text{-}DR^+$ neutrophils frequency in KC patients was negatively associated with NADH-LDH activity that characterized the disruption of anaerobic energy during functional activation of cells.

Correlations between enzyme activity and cell-surface receptor expression levels were more pronounced in both groups. Moreover, this complex of relationships in the control group was represented only by positive relationships while the KC patients revealed mostly negative correlations. The inversion of the relationship between the expression of the HLA-DR and NADP-MDH activity has been established: the positive relationship in control group and negative correlation in cancer patients. Accordingly, HLA-DR expression was accompanied by lipid anabolism activation in neutrophils from control group while it manifested itself when inhibiting these reactions in cancer. In addition, the expression of this antigen was

accompanied by a decrease in anaerobic glycolysis intensity (low NADH-LDH activity) as well as in mitochondrial compartment metabolism (low activity of NADP-ICDH and NAD-GluDH) of KC patients neutrophils. In general, expression of activation (CD16 and CD23) and adhesion (CD62L) antigens by KC patients neutrophils was mainly accompanied by the inhibition of NAD-dependent influx of substrates onto the tricarboxylic acid cycle *via* NADGDG. Glutathione reductase was the only enzyme whose activity was positively correlated with the expression of CD23 and HLA-DR by KC patients neutrophils. This enzyme is part of the glutathione-dependent antioxidant system, and its activity increases with the intensification of cell peroxide processes [7, 26]. Therefore, the level of CD23 and HLA-DR expression on the neutrophil membrane of KC patients changed according to the intensity of intracellular antioxidant processes.

Conclusion

Alterations in blood neutrophils phenotype received from patients with kidney cancer were manifested against the background of inhibition of the main intracellular metabolic processes. The features of the phenotypic imbalance of KC patients' neutrophils were the associated with the diminution in peripheral blood of cells expressing adhesive (CD11b and CD62L) and functional (CD64 and HLA-DR) receptors. Moreover, circulating patient' neutrophils expressed such molecules as CD11b, CD16 and HLA-DR more intensively. These phenotype changes in KC patients blood neutrophils were carried out against a background of a pronounced decrease in immature cells number. Moreover, myeloid-derived suppressor cells with the function of inhibiting antitumor immunity could be defined as immature polymorphonuclear neutrophils. The metabolic features of the cytoplasmic compartment of KC patients blood neutrophils were determined by a decrease in Glu6PDH activity (a key and initializing enzyme of the pentose phosphate cycle) and NADH-LDH (anaerobic glycolysis). Mitochondrial metabolism in neutrophils of KC patients was characterized by multidirectional changes in the activity of NAD- and NADP-dependent glutamate dehydrogenases (decreased activity of NAD-dependent and increased activity of NADP-dependent) and a decrease in NADH-MDH activity. The established features in mitochondrial enzymes activities characterized the violation of NAD-dependent processes which could led to down-regulation of aerobic energy processes. We believe that a decrease in the activity of plastic and energy processes of KC patients blood neutrophils could affect receptor expression levels. Using correlation analysis that features of the relationships in KC patients were determined, we found the negative effect of NADH-GDH and NADH-LDH activity on activation and adhesion neutrophil' receptors expression. Only glutathione reductase activity in KC patients' neutrophils was positively linked with the CD23 and HLA-DR expression. Thus, an increase in the activity of energy processes (including the processes of interaction of the tricarboxylic acid cycle with amino acid exchange reactions) in the blood neutrophils from patients with kidney cancer could stimulate expression levels of activation and adhesion receptors and, thereby, increase the antitumor activity of neutrophils.

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