

Characterization of Ca^{2+} , Mg^{2+} -ATPase of blood lymphocytes in women with ovarian cancer

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Ionized Ca^{2+} is crucial for regulation of practically all intracellular processes, including tumor growth, cell proliferation, apoptosis, etc. The plasma membrane Ca^{2+} , Mg^{2+} -ATPase plays an important role in maintaining intracellular Ca^{2+} homeostasis. The function of this enzyme is to reduce the Ca^{2+} concentration in the cytosol, namely its transport against a concentration gradient in the extracellular medium. We have investigated the activity of plasma membrane Ca^{2+} , Mg^{2+} -ATPase of lymphocytes of practically (clinically) healthy women of different age groups and also patients with ovarian cancer stage III and IV. It was found that the enzyme activity in women of the older age group was not significantly reduced in relation to the activity of the younger age group. Thus, the value of the maximum rate of ATP hydrolysis by plasma membrane Ca^{2+} , Mg^{2+} -ATPase of blood lymphocytes in practically healthy women under the conditions of physiological norm was 1.1 times higher than under of pre-nosological state. In patients with ovarian cancer (stages III and IV), plasma membrane Ca^{2+} , Mg^{2+} -ATPase activity of blood lymphocytes significantly differed from the physiological norm and decreased by 1.6 and 1.8 times, compared with the physiological norm. The decrease of the plasma membrane Ca^{2+} , Mg^{2+} -ATPase activity of blood lymphocytes in patients with ovarian cancer indicates an increase of Ca^{2+} in the cytosol of lymphocytes. Determination of affinity constants showed that these values were in the submillimolar range of concentration, corresponding to the physiological concentration in the cell cytoplasm (0.5–5.0 mM). In healthy persons, under the condition of physiological norm, the affinity constant of plasma membrane Ca^{2+} , Mg^{2+} -ATPase to the ATP was 0.16 ± 0.02 mM and at pre-nosological state – 0.19 ± 0.02 mM. The affinity constant of plasma membrane Ca^{2+} , Mg^{2+} -ATPase of lymphocytes to ATP in patients with ovarian cancer (stage III) was 0.32 ± 0.03 mM and with ovarian cancer (stage IV) 0.35 ± 0.03 mM. That is, the affinity constant of plasma membrane Ca^{2+} , Mg^{2+} -ATPase of lymphocytes to ATP in patients with ovarian cancer was 2.0–2.1 times higher than this value for the blood lymphocytes in the control group (physiological norm). The kinetic analysis of Ca^{2+} -activated, Mg^{2+} -dependent hydrolysis of ATP in blood lymphocytes in women showed that the decrease in the activity of Ca^{2+} , Mg^{2+} -ATPase was due to a decrease in the affinity of the enzyme to the substrate (KATP increases 2-fold).

Keywords: plasma membrane; Ca^{2+} -ions; ATPase activity; ATP hydrolysis rate; affinity constant; Ca^{2+} -pump; lymphocytes

Introduction

It is known that ovarian cancer (OC) occupies a leading place among the causes of mortality from malignant formations (Howlader et al., 2013). Specifically, according to the International Agency for Research of Cancer, more than 165,000 newly diagnosed cases of ovarian cancer are reported annually in the world. It is the cause of death of more than 100,000 women (Howlader et al., 2013).

Ovarian cancer refers to severe pathology of the female reproductive system (Buys et al., 2011; Paryzhak et al., 2014). This pathology manifests itself especially through its high ability to proliferate and metastasize, which determines the clinical course of the disease (Paryzhak et al., 2014; Vovchuk, 2014). It is widely studied with the aim of both improving the methods of diagnosis and detection of the tumor process in the early stages, as well as optimizing the treatment based on modern ideas about its pathogenesis. Probably, the violation of proliferative processes and the development of OC is preceded by a pre-nosological state that is clinically asymptomatic. Data from the literature show that the greater the age of a woman, the greater the probability of developing ovarian cancer (Markman et al., 2004; Lukianova et al., 2006; Vovchuk, 2014; Yakubets et al., 2016).

Therefore, an important direction in physiological, biochemical and other biomedical investigations is the elucidation of mechanisms regulating the functioning of the cell both in practically healthy individuals and those with pathological conditions (Radchenko, 2004; Gzhegotsky et al., 2008). However, the limits of the majority of physiological processes in practically healthy individuals are quite broad and can conditionally correspond to both the physiological norm and the pre-nosological state (Radchenko, 2004; Gzhegotsky et al., 2008; Yakubets et al., 2016). It is believed that the state of the physiological norm is characterized by the balance of the work of many regulatory and functional systems of the body, while in the pre-nosological state, the mobilization of functional resources and the tension of regulatory systems is necessary (Gzhegotsky et al., 2008). In this regard, the recognition of intermediate, that is, prenosological states preceding nosological-definite forms of diseases is a highly topical issue. Often the term "practically (clinically) healthy" corresponds to clinically asymptomatic conditions at the border of norm and pathology, which may require preventive correction (Radchenko, 2004; Gzhegotsky et al., 2008).

Currently, there are no clear criteria to differentiate between the state of the physiological norm and the pre-nosological state, which makes it difficult to use them in medical and biological research and in

clinics. To identify the earliest preclinical stages of pathological processes, study of cellular regulatory systems and the search for new biochemical and other markers is carried out. In this aspect, the role of Ca^{2+} ions as a universal intracellular messenger in the regulation of cellular functions is indisputable (Feske, 2007; Monteith et al., 2007; Bergner et al., 2008; Monteith et al., 2012; Pinto et al., 2015; Dang et al., 2016; Padanyi et al., 2016; Peters et al., 2016; Monteith et al., 2017).

Specifically, Ca^{2+} is one of the major determinants of invasiveness and metastatic potential of transformed cells (Feske, 2007; Monteith et al., 2007; Paryzhak et al., 2014). It regulates the transcription of genes, metabolism, proliferation, apoptosis, etc. Malignant growth is accompanied by increased proliferation and decreased apoptosis. Therefore, in the study of tumor growth, it is particularly important to study the Ca^{2+} homeostasis. The increase in Ca^{2+} concentration in the cytoplasm is the result of its transport from the extracellular medium and release from the intracellular stores. Two main structures involved in maintaining and controlling intracellular Ca^{2+} homeostasis are plasma membrane $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase and $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase of the endoplasmic reticulum (Monteith et al., 2007; Bergner et al., 2008; Monteith et al., 2012; Padanyi et al., 2016).

On the other hand, it is known that T-lymphocytes play a central role in the antitumor protection of the body. They carry out antitumor protection by destroying cancer cells, as well as synthesizing substances that activate other cells in the immune system. Some T-lymphocytes kill cancer cells (T-killers). Others cells help the latter kill cancer cells (T-helpers). It is believed that the presence or absence of certain groups of T-lymphocytes, is associated with important differences in the prediction of the development of ovarian cancer in patients (Gavalas et al., 2010; Knutson et al., 2015; Krishnan et al., 2017).

Also, peripheral blood lymphocytes can serve as an adequate model for studying pre-nosological conditions and the development of ovarian cancer and objectively reflect changes of the genetic and metabolic homeostasis of an organism (Davtian et al., 2001; Krishnan et al., 2017). That is, they can be test systems for study of regulatory mechanisms of the cell, in particular Ca^{2+} -transporting systems for ovarian cancer. Thus, in spite of existing research, which is devoted to pre-nosological states, different age aspects, the functioning of blood lymphocytes of different age groups and so on, the role of a number of regulatory systems, in particular, ATP-hydrolase in both practically healthy individuals and in neoplastic transformations of organs and tissues, still remains unclear.

The purpose of present work was to determine the activity and characterize the kinetic properties of plasma membrane $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPases of blood lymphocytes in practically healthy women of different age groups and in women with ovarian cancer.

Materials and methods

Patients. The research was carried out on blood lymphocytes isolated from practically healthy women and patients with neoplastic changes in the ovary. The total number of practically (clinically) healthy women, representative by age (mean age 53.8 ± 5.4 years) was 44 persons. This group was formed from volunteers from among the employees of Danylo Halytsky Lviv National Medical University and also of the employees of the Lviv State Regional Oncology Treatment and Diagnostic Center. In turn, a group of practically healthy women was conditionally divided into two subgroups: FN (26 people, 20–40 years old, physiological norm) and PS (18 people, 41–60 years old, pre-nosological state). Such a conditional division was based on the fact that with increasing age of women, the probability of ovarian cancer increases and the concentration of the tumor marker CA-125 increases in the blood (Yakubets et al., 2016).

The group of women with neoplastic ovarian changes was 32 women aged 24–75 years (average age 55.4 ± 5.3 years) who were receiving inpatient treatment at the Lviv Regional State Oncology Treatment and Diagnostic Center in the period 2013–2017 and passed the complete clinical and laboratory diagnostic. The study included patients with an established diagnosis of ovarian cancer without the presence of concomitant diseases at the start of the study.

The research group was divided into two subgroups, depending on the stage of development of ovarian cancer: OC 1 – the patients with III stage of ovarian cancer, a tumor is distributed on one or two ovaries and gives metastases on the peritoneum beyond the pelvis (or metastases in retroperitoneal lymph nodes) ($n = 22$); OC 2 – the patients with IV stage of ovarian cancer, a tumor is extended on one or two ovaries with distant metastases ($n = 10$).

Appropriate diagnoses were established on the basis of a wide range of general-clinical, laboratory, special oncology, instrumental research methods. In addition, for the differentiation of practically healthy women and diagnosis of ovarian cancer, the level of the tumor marker of glycoprotein CA-125 in blood serum was determined (Paryzhak et al., 2014; Yakubets et al., 2016). All patients with ovarian cancer and practically healthy persons were well informed about the purpose, tasks and term of the study and provided written informed consent to participate in conducting research on blood samples. All patients and healthy donors gave written informed consent to participate in research (Ethical Committee Approval, protocol No 4, April 18, 2016).

Cell preparation. Blood sampling by means of venipuncture was carried out from the elbow vein in the morning hours under conditions of physiological rest, on an empty stomach, in a quantity of 20 ml in test tubes, and stabilized with heparin (final dilution 1 : 100). Whole blood diluted in the ratio 1 : 1 by physiological solution was layered in a density gradient of the ficol triambrast ($\rho = 1.08 \text{ g/cm}^3$) and centrifuged for 20 min at 500 g. The removed interphase rings of mononuclear cells were washed twice within 10 min with a physiological solution (Boyum, 1968; Pidkovka et al., 2002). After the last centrifugation, a small amount of saline solution was added to the precipitate, resuspended and using a trypan blue, the count of the number of live and dead cells in the Goryaev cell (Mishell, 1980) was measured. The integrity and viability of blood lymphocytes in all researches was not less than 95%.

For permeabilization of blood lymphocyte membranes and disclosure of enzyme latent activity, saponin was added to the suspension. This technique is based on work previously performed on lymphocytes. Blood lymphocytes were incubated for 10 min at moderate shaking in a solution containing saponin at a concentration of 0.2% (optimal concentration) (Pidkovka et al., 2002; Vorobets et al., 2006; Fafula et al., 2011).

Assay of $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase activity. $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase activity of blood lymphocytes was determined by registering the process of ATP hydrolysis by accumulation of inorganic phosphatate (P_i). The determination of the total $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase activity of blood lymphocytes was carried out at 37 °C in an incubation medium (volume – 1 ml) of the following composition (mM): 150 KCl, 0.05 CaCl_2 , 5 MgCl_2 , 5 ATP, 1 NaNO_3 (mitochondrial ATPase inhibitor); 1 ouabain (inhibitor Na^+,K^+ -ATPase) (Pidkovka et al., 2002; Fafula et al., 2011), 20 HEPES-Tris buffer (pH = 7.4). For division the total $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase activity into components: thapsigargin-insensitive plasma membrane $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase and thapsigargin-sensitive $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase membranes of the endoplasmic reticulum (EPR) the inhibitor an $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase EPR-thapsigargin (0.1 μM) was added to the standard Ca^{2+} and Mg^{2+} -containing incubation medium.

Plasma membrane $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase was calculated as the difference between total $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase activity and $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase activity in the presence of thapsigargin. $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase activity was expressed in $\mu\text{moles of P for 1 min per 1 mg of protein}$.

The kinetic parameters characterizing the Ca^{2+} -activated Mg^{2+} -dependent ATP-hydrolysis – the affinity constant (K_{ATP}) and the maximum rate of ATP hydrolysis determined by ATP (V_{ATP}) were calculated by the Lineweaver-Burk plot. The resulting concentration dependences of the rate of ATP-hydrolysis reaction on the substrates of reaction were plotted in the coordinates: $\{1/V; 1/[S]\}$, where S is the substrate concentration and V is the rate of enzymatic hydrolysis of ATP at a given concentration of the substrate.

Results

It is known that $\text{Ca}^{2+},\text{Mg}^{2+}$ -ATPase transport Ca^{2+} ions across the membrane against their electrochemical gradient. This process is conju-

gated with ATP hydrolysis. $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase of blood lymphocytes has been demonstrated by researchers earlier (Pidkovka et al., 2002; Vorobets et al., 2006; Fafula et al., 2011). The physiological role of this enzymatic system in the regulation of Ca^{2+} -homeostasis of the cell is determined by its high affinity to the substrate of transporting, which is Ca^{2+} (Fafula et al., 2011; Padanyi et al., 2016).

Disturbances of the activity of Ca^{2+} -dependent ATP-hydrolysis systems indicate structural and functional changes in biological membranes in the development of pathological processes. Changes in the activity of these systems of the cell lead to the redistribution of ions between the cytoplasm and the extra-cellular medium, changes in cell membrane potential. With growth of tumor, disturbances of the functional activity of the membrane-bound enzymatic systems acquires a general (systemic) character (Monteith et al., 2012; Pinto et al., 2015; Padanyi et al., 2016; Peters et al., 2016).

As a result of the performed studies, it was found that plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity of lymphocytes in practically healthy women aged 20–40 years (FN) was $2.97 \pm 0.26 \mu\text{mol P}_i/\text{min}\cdot\text{mg}$ of protein (Fig. 1).

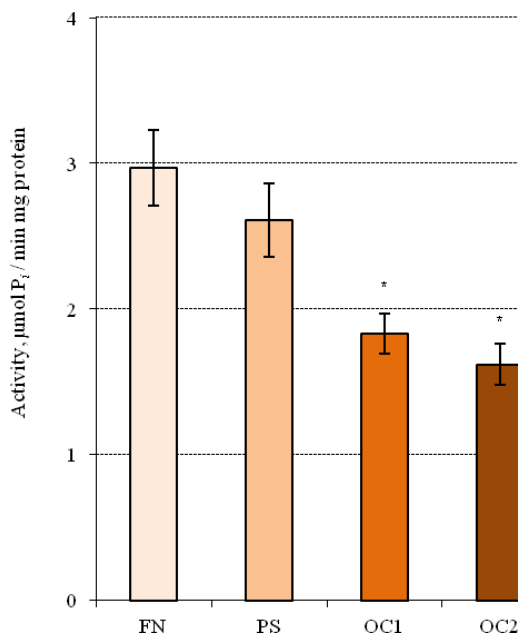


Fig. 1. Plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity of blood lymphocytes of women at the physiological norm (FN), pre-nosological state (PS), in patients with ovarian cancer III (OC1) and IV (OC2) stages: $X \pm m$, $n = 8-12$; * – $P < 0.05$ compared to physiological norm

In practically healthy women 40–60 years old (PS) this value was $2.61 \pm 0.25 \mu\text{mol P}_i/\text{min}\cdot\text{mg}$ of protein. In patients with OC (Stages III and IV), plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity of blood lymphocytes significantly differed from the physiological norm and was 1.83 ± 0.14 and $1.62 \pm 0.14 \mu\text{mol P}_i/\text{min}\cdot\text{mg}$ of protein. Enzyme activity decreased by 1.6 and 1.8 times, respectively ($P < 0.05$), compared with the physiological norm.

The decrease of the plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity of blood lymphocytes in patients with OC indicates the increase in $[\text{Ca}^{2+}]_i$ in the cytosol of lymphocytes. $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase and Na^+, K^+ -ATPase use the energy of ATP hydrolysis to transport ions against their electrochemical gradient. Therefore, changes in the ATP concentration in the incubation medium will affect the rate of ATP hydrolysis.

The dependence of $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity on the substrate concentration (ATP) in the incubation medium was determined by the affinity constant to the substrate (KATP). It was calculated by determining the plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity in the incubation medium which contained the substrate in the concentration range from 1 to 5 mM (with a constant concentration of Ca^{2+} ions – 0.05 mM and Mg^{2+} ions – 5 mM).

It was shown that an increase in the ATP concentration in an incu-

bation medium in the range from 1.0 to 4.0 mM leads to a gradual increase in plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity of blood lymphocytes of practically healthy persons reaching a plateau (Fig. 2).

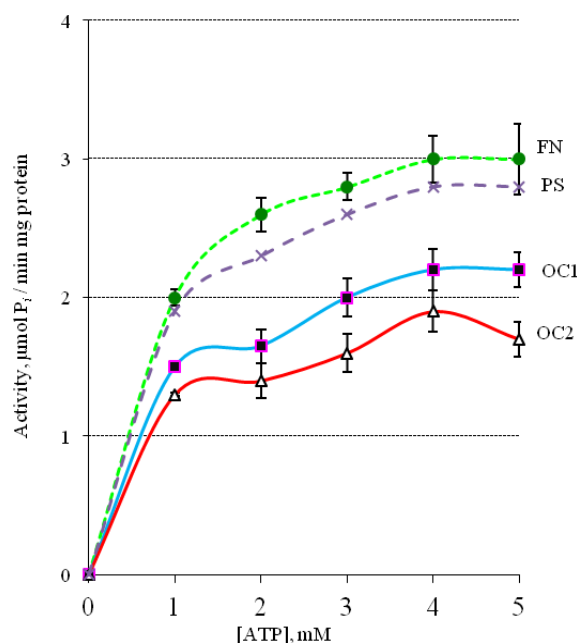


Fig. 2. Dependence of plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity of blood lymphocytes of women at the physiological norm (FN), pre-nosological state (PS), patients with ovarian cancer III (OC1) and IV (OC2) stages on the ATP concentration in the incubation medium ($x \pm m$, $n = 8-12$)

The maximum values of the hydrolase activity of plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase of the blood lymphocytes in healthy subjects and in patients with OC were noted at 4 mM ATP in the incubation medium. The study of the concentration dependence of $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity on ATP shows that the activity of plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPases in patients with OC was decreased in comparison with control groups throughout the range of studied concentrations of the substrate.

For clarification of possible mechanisms of changes in plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity, the main kinetic parameters of ATP hydrolysis in immunocompetent cells in healthy persons and patients with OC were determined (Fig. 3). The dependence curves ($1/V$; $1/[\text{ATP}]$) differ by angle of inclination for physiological norm and patients with ovarian cancer. Curves ($1/V$; $1/[\text{S}]$) at the normal physiological state and at pathology cross the X and Y axes at different points. This dependence corresponds to a mixed type of inhibition of the enzyme.

To determine the main kinetic parameters of ATP hydrolysis with the participation of plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase of blood lymphocytes in patients with OC and the elucidation of the possible mechanism of enzymatic activity change, the curves of concentration dependences were linearized in the Lineweaver-Burk plot.

It was established that the values of the maximum rate of ATP hydrolysis by plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase of blood lymphocytes of practically healthy women in the conditions of FN was 3.02 ± 0.26 and in the conditions of PS $2.76 \pm 0.22 \mu\text{mol P}_i/\text{min}\cdot\text{mg}$ of protein (Table 1). The maximum rate of ATP hydrolysis by plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase of lymphocytes in patients with OC of III stage was 1.95 ± 0.20 and IV stage $1.77 \pm 0.15 \mu\text{mol P}_i/\text{min}\cdot\text{mg}$ of protein. It can be seen that the maximum rate of ATP hydrolysis by $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase of blood lymphocytes in patients with OC of both stages and controls group was different and this difference was statistically significant ($P < 0.05$).

Determination of affinity constants showed that these values were in the submillimolar range of concentration, corresponding to the physiological concentration in the cytoplasm of cells (0.5–5.0 mM). In healthy persons, under the condition of FN, the affinity constant of plasma membrane $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase to the ATP was 0.16 ± 0.02

mM, and at PS – 0.19 ± 0.02 mM. The affinity constant of plasma membrane Ca^{2+} , Mg^{2+} -ATPase of lymphocytes to ATP in patients with OC (stage III) was 0.32 ± 0.03 mM and at OC (stage IV) 0.35 ± 0.03 mM.

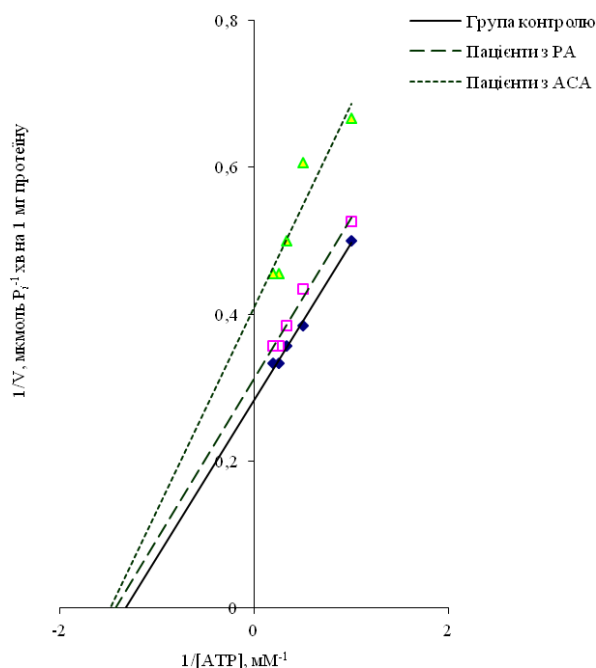


Fig. 3. Linearization of concentration curves in Lineweaver-Burk plot, here V is ATP-hydrolase activity of plasma membrane Ca^{2+} , Mg^{2+} -ATPase of women at the physiological norm (FN), patients with ovarian cancer III (OC1) and IV (OC2) stages (n = 8–12; r > 0,85)

Table 1

Kinetic parameters of Ca^{2+} -activated, Mg^{2+} -dependent ATP hydrolysis in blood lymphocytes of women at physiological norm (FN), pre-nosological state (PS), patients with ovarian cancer III (OC1) and IV (OC2) stage defined by ATP ($x \pm m$, n = 8–12)

Kinetic parameters	Control group		Patients with OC	
	FN	PS	III stage (OC1)	IV stage (OC2)
V_{ATP} , $\mu\text{mole P}_i / \text{min}$ 1 mg protein	3.02 ± 0.26	2.76 ± 0.22	$1.95 \pm 0.20^*$	$1.77 \pm 0.15^*$
K_{ATP} , mM	0.16 ± 0.02	0.19 ± 0.02	$0.32 \pm 0.03^*$	$0.35 \pm 0.03^*$

That is, the value of the affinity constant to ATP for plasma membrane Ca^{2+} , Mg^{2+} -ATPase of lymphocytes in patients with OC was 2.0–2.1 times higher than this value for the blood lymphocytes in the group of physiological norm. It can be concluded that the inhibition of enzyme activity occurs both by reducing the maximum rate of ATP hydrolysis (V_{max} was decreased) and by reducing the affinity of plasma membrane Ca^{2+} , Mg^{2+} -ATPase to the substrate (the affinity constant to ATP was increased).

It is known that the extrusion of Ca^{2+} from the cell through the plasma membrane is carried out by two main mechanisms: Ca^{2+} , Mg^{2+} -ATPase and Na^+ / Ca^{2+} -exchanger. However, most researchers demonstrate that under carcinogenesis, the main mechanism is the Ca^{2+} , Mg^{2+} -ATPase, which supports the concentration of cytosolic Ca^{2+} at a level of ~ 100 nM (Monteith et al., 2012; Monteith et al., 2017). In order to achieve precise Ca^{2+} control over several processes in the same cell, it is paramount that the Ca^{2+} -homeostasis is strictly controlled in time and space. The amplitude-temporal and spatial aspects of the Ca^{2+} -signal should be precisely regulated to achieve specific results, such as, for example, the cell cycle regulation, apoptosis or the cell proliferation (Monteith, 2007). Plasma membrane Ca^{2+} , Mg^{2+} -ATPase is not only itself involved in controlling the intracellular Ca^{2+} -concentration, but also controls the formation of inositol-1,4,5-triphosphate and, accordingly, a decrease of Ca^{2+} -efflux from the endoplasmic reticulum (Padanyi et al., 2016). Changes of the expression of plasma membrane

Ca^{2+} , Mg^{2+} -ATPase in the process of tumor growth, resulting in unbalanced homeostasis in tumor cells were shown (Padanyi et al., 2016). Our data concerning the decrease of Ca^{2+} , Mg^{2+} -ATPase activity agree that in cases of ovarian cancer there is an increase in the Ca^{2+} concentration in cytosol and even hypercalcemia (Pinto et al., 2015; Padanyi et al., 2016; Peters et al., 2016). The increase of the Ca^{2+} -concentration is due primarily to the fact that extrusion of Ca^{2+} from the cell decreases due to a decreased Ca^{2+} , Mg^{2+} -ATPase activity. The increase in Ca^{2+} concentration by inhibiting the ATPases activity in ovarian cancer also induces apoptosis (Monteith et al., 2007; Monteith et al., 2017).

The data on the expression of endoplasmic reticulum Ca^{2+} , Mg^{2+} -ATPase in cells of patients with colon carcinoma and breast cancer (Papp et al., 2012) also indicate about a decrease in the Ca^{2+} efflux from cytosol in tumor growth. It was shown that the expression of this enzyme was reduced and the search for therapeutic drugs was directed at their effect by reducing the Ca^{2+} concentration in cytosol.

By transferring Ca ions to the extracellular medium across the plasma membrane, the Ca^{2+} -pump reduces the cytosolic Ca^{2+} level and thus helps control over cells activity.

Ca^{2+} -pumps can be not only the biomarkers, they can also serve as anticancer therapeutic targets (Monteith et al., 2007). One of the important features of the Ca^{2+} -pump in comparison with other existing or potential therapeutic targets in cases of cancer is the presence of pharmacological activators and inhibitors of Ca^{2+} -pumps. From the chemogenic point of view, many Ca^{2+} -pumps are potential pharmacological targets, and from the biological point of view, they are modulators of Ca^{2+} -signals that can affect to tumorigenic regulatory pathways. By modulating the activity of Ca^{2+} -channels and pumps that are expressed in cancer cells, their activators or inhibitors can purposefully change Ca^{2+} homeostasis in cancer cells (Monteith et al., 2012; Monteith et al., 2017).

Conclusion

It was found that Ca^{2+} , Mg^{2+} -ATPase activity of blood lymphocytes in women of different age groups was not significantly different. However, in women of the older age group, this activity had a tendency to decrease. With ovarian cancer, the Ca^{2+} , Mg^{2+} -ATPase activity of blood lymphocytes was significantly decreased in relation to the control group, but there was no significant difference in the enzyme activity between the 3rd and 4th stages of ovarian cancer. The kinetic analysis of Ca^{2+} -activated, Mg^{2+} -dependent ATP hydrolysis in plasma membrane of blood lymphocytes of women showed that decrease in Ca^{2+} , Mg^{2+} -ATPase activity was due to a decrease in affinity to the substrate (KATP increases 2-fold).

The work is a part of the research work "Investigation of functional and metabolic reserves of stress-limiting systems of organism by extreme conditions in order to identify effective ways of their correction" (state registration number 0116U004510) and "Development and introduction of immuno-biochemical methods of early diagnostics of the development of pathological processes in the body" (Grant of the President of Ukraine No. 1039/2014-pri, September 24, 2014).

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