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REPROGRAMMING OF GLUCOSE METABOLISM IN HUMAN BREAST CANCER CELLS AFTER CO-CULTIVATION WITH *BIFIDOBACTERIUM ANIMALIS*

Background. The ability to reorganize metabolic processes is one of the key properties of malignant cells necessary to ensure high energy needs, survival, proliferation, metastasis, and resistance to anticancer drugs. Lactic acid bacteria, in particular *Bifidobacteria*, are important elements of the tumor microenvironment in breast cancer (BC) and, as active lactate producers, can influence the metabolic phenotype of malignant cells. **Aim.** To study the effect of *B. animalis* on some components of glucose metabolism pathways and the expression of proteins associated with this process in human BC cells of different molecular subtypes. **Materials and Methods.** The study was performed on human BC cells of the T-47D, MCF-7 (luminal subtype), and MDA-MB-231 (basal subtype) lines and live culture of *Bifidobacterium animalis subsp. lactis (B. animalis*). A colorimetric enzymatic technique, flow cytometry, immunocytochemical analysis, and cell viability trypan blue exclusion assay were used in the study. **Results.** Co-cultivation of BC cells with *B. animalis* resulted in a significant ($p < 0.05$) increase in the glucose consumption rate by 1.2—4.7 times, lactate production by 15—115%, and LDH activity by 15—160% in BC cells compared to control cells. The most pronounced changes were observed in BC cells of the luminal subtype where they were accompanied by an increase in the expression of the GLUT1 glucose transporter by 30—80% compared to control cells. Also, after co-cultivation with *B. animalis,* we detected an increased expression of the STAT6 transcription factor in BC cells of all three lines. **Conclusions.** Co-cultivation of BC cells with *B. animalis* is accompanied by an increase in glycolysis. *B. animalis* affected not only the biochemical components of the glucose metabolism pathway but also the expression levels of STAT6, GLUT1, and insulin receptor.

Keywords: breast cancer, *Bifidobacterium*, glucose, lactate, STAT6, GLUT1.

Breast cancer (BC) is the most commonly diagnosed malignancy among women worldwide and the cause of high mortality rates, despite continuous improvement in treatment strategies [1]. This problem has become particularly important in recent years in young women, primarily with signs of metabolic syndrome and insulin resistance [2, 3]. The problem is complicated by tumor heterogeneity, which is reflected in variations in the morphology, transcriptional profiles, metastatic potential, and metabolism of malignant cells [4]. The diversity of carbon substrates fueling malignant cells suggests metabolic heterogeneity, even in tumors with the same clinical diagnosis [5]. One of the

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fundamental features of malignant cells' aggressiveness is their ability to undergo metabolic reprogramming in the context of the pathological process development, which contributes to their survival and adaptation to therapeutic agents [6, 7]. The greatest interest of researchers is focused on the features of glucose metabolism pathways since glycolysis ensures anabolic and energy needs of malignant cells, favoring glucose fermentation over mitochondrial oxidative phosphorylation [8].

During glycolysis, which requires an increased glucose consumption, monosaccharides are transported into BC cells by the members of the GLUT glucose transporters' family. As a result, tumor cells produce and secrete glucose metabolites, including lactate and growth factors, which affect various signaling pathways [9] and provide the metabolic plasticity of BC cells [8]. The metabolic features of malignant cells are regulated by cellular, extracellular, and soluble components of the tumor microenvironment (TME) [10]. Presently, the tumor microbiome, which has an extremely complex relationship with the tumor, is considered especially important [11].

Among the main representatives of the breast microbiota, one could mention lactic acid bacteria (LAB), in particular, bifidobacteria. The interaction of *Bifidobacterium* with cell cycle regulatory proteins causes inhibition of cancer cell proliferation via the activation of pro-caspases and up-regulation of pro-apoptotic BAX proteins [12]. The anti-inflammatory activity of bifidobacteria, in particular, *B. animalis subsp. lactis* BB-12 has also been demonstrated [13]. There are several published studies on the potential ability of *Bifidobacterium* and lactobacilli to interfere with the lactate cycle, increasing its local and systemic bioavailability and thus influencing tumor progression, as well as the efficacy of chemotherapy [14, 15]. Such assumptions were based on the ability of LAB to produce lactate, which can enhance the Warburg effect in malignant cells [16]. It is well known that elevated lactate levels stimulate tumor cell proliferation, epithelial-mesenchymal transition, and metastasis [17]. Therefore, numerous data on the influence of bifidobacteria on the biology of malignant cells emphasize the need for an assessment of changes in glucose metabolism in BC cells due to interaction with lactic acid-producing members of the human microbiome. However, when investigating the influence of any factors on the glucose metabolism in tumor cells, one should account that the heterogeneous and flexible metabolic phenotypes of tumor cells are due to the combined influence of the cell origin, differentiation, redox potential, and somatic mutations [5]. Therefore, in our study, we have chosen BC cells with different receptor statuses, metabolic profiles, and degrees of malignancy. The study of the glucose consumption rate (GCR) and lactate production rate (LPR) deserves special attention, since these factors can affect the activity of glycolysis and enzymes of the pentose phosphate pathway (PPP), as well as the expression of regulatory proteins associated with the glucose metabolism in BC cells co-cultured with *Bifidobacterium animalis* (*B. animalis*).

Materials and Methods

Cells. Human BC cells of the T-47D, MCF-7 (luminal subtype), and MDA-MB-231 (basal subtype) cell lines were obtained from the Bank of Cell Lines from Human and Animal Tissues of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine (Registration Certificate, series AN No. 41 dated 19.02.2009).

Cell culture. BC cells were cultured in DMEM medium containing 4 mmol/L L-glutamine (BioWest, France), 10% fetal bovine serum (FBS) (BioWest, France), and 1x penicillin-streptomycin (BioWest, France). Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. The medium replacing and cell passaging were performed according to standard procedures [18]. Cells were passaged after they reached 80—90% confluency using a versene solution (Vetline Agroscience, Ukraine) and trypsin — EDTA 1X in PBS (BioWest, France). Cells in the exponential growth phase were used in the experiments.

Bacteria. Live lyophilized cells of *Bifidobacterium animalis subsp. lactis* BB-12 isolated from a probiotic produced by Lek Pharmaceuticals, Ljubljana, Slovenia, were used in the study. Bacterial cells were diluted in DMEM without antibiotics.

Co-cultivation of BC cells and bifidobacteria. BC cells were seeded in 60 mm-diameter Petri dishes at 1.5×10^5 cells per dish. In parallel, cells at the same density were seeded in dishes with coverslips (Epredia, Netherlands) for an immunocytochemical analysis. The cells were incubated at 37 °C in a humidified atmosphere supplied with 5% CO_2. After 10 h, a suspension of live *B. animalis* was in-

troduced at the final ratio of eukaryotic-to-bacterial cells of 1/100 and 1/400. BC cells cultured without *B. animalis* were used as eukaryotic cell controls. *B. animalis* cultured in the medium under the same conditions without cancer cells were used as bacterial controls. BC cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere for 24 and 48 h after the introduction of bacteria. Then the medium was collected for further measurement of glucose and lactate concentrations. The number of live BC cells in each dish was determined by a trypan blue assay (Applichem, Germany). The cells were lysed in RIPA buffer [19] supplemented with a protease inhibitor cocktail (Sigma-Aldrich, USA) and used to measure the activities of intracellular lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PD).

Assessment of glucose consumption rate and lactate production rate. Concentrations of glucose and lactate in a DMEM medium were determined after 24 and 48 h of incubation of cells with *B. animalis*. Glucose concentration was determined by an enzymatic colorimetric assay of glucose oxidase, and lactate concentration was determined by an enzymatic assay of lactate oxidase using diagnostic kits (Glucose (Ox) Liquid, MedTest Dx, Pointe Scientific, USA and Lactate, Greiner Diagnostic GmbH, Germany) according to the manufacturer's instructions. The indicators of energy metabolism in BC cells, GCR and LPR, were calculated using the following formulas [20]:

$$GCR = 2 \times \frac{\left(C_{gl}\left(t_i\right) - C_{gl}\left(t_{i+1}\right)\right) \times V}{N\left(t_i\right) + N\left(t_{i+1}\right)}$$

$$LPR = 2 \times \frac{\left(C_{L}\left(t_{i+1}\right) - C_{L}\left(t_i\right)\right) \times V}{N\left(t_i\right) + N\left(t_{i+1}\right)}$$

where the concentrations of glucose and lactate on two consecutive days (t_i and t_{i+1}) of cell growth are marked as $C_{gl}(t_{i+1})$, $C_L(t_i)$, $C_L(t_{i+1})$; $N(t_i)$, and $N(t_{i+1})$ are the counts of BC cells on the corresponding consecutive days of growth; V is the volume of the incubation medium per well equal to 7×10^{-3} L. GCR and LPR values were presented in 10^{-9} mmol/cell/day. The results were assessed using an ABL 800 FLEX automatic biochemical analyzer (Radiometer Medical, Denmark).

Measurement of intracellular LDH and G6PD activities. Enzyme activities were measured after 24 and 48 h of incubation of cells with *B. animalis* in lysates of BC cells. The resulting lysates were centrifuged in a high-speed refrigerated centrifuge (Sigma, USA) at 13,000 rpm and 4 °C for 15 min. The collected supernatant was used to analyze the enzyme activities. A kinetic method using L-lactate from a Lactate Dehydrogenase Set (Pointe Scientific, USA) and glucose-6-phosphate from a Glucose-6-Phosphate Dehydrogenase Set (MedTest Dx, Pointe Scientific, USA) was used. Both analyses were performed according to the manufacturer's instructions. The enzyme activities were calculated based on the number of cells in each sample.

Immunocytochemical (ICC) analysis. After incubation with *B. animalis* for 48 h, coverslips with BC cells were fixed in a 1:1 solution of methanol (Merck, Germany) and acetone (Khimrezerv, Ukraine) for 2 h at –20 °C and then washed three times with cold PBS (BioWest, France). The ICC reaction was performed using a Master Polymer Plus Detection System (Peroxidase) kit (Vitro SA, Spain) according to the manufacturer's instructions. The coverslips were covered with GLUT-1 (Vitro SA, Spain) and STAT-6 (Vitro SA, Spain) primary antibodies and incubated for 1 h at room temperature. Then the coverslips were stained with hematoxylin and eosin solution (Sigma, USA) and fixed in the Faramount Aqueous Mounting Medium (Thermo Fisher Scientific, USA). The resulting preparations were analyzed by counting cells with positive ICC reaction (brown), taking into account the color intensity using an Axiostar Plus light microscope (Carl Zeiss, Germany) at ×400 magnification, and photographed with a digital camera (Canon PowerShot G5, UK) at ×1000 magnification. At least three random fields of view were analyzed. Expression was assessed using the H-Score [21].

Quantification of insulin receptor expression levels in BC cells by flow cytometry. After incubation with *B. animalis,* the BC cells were removed from the substrate, counted, and diluted in PBS. FITC-insulin (Sigma, USA) was added to 0.5×10^6 cells to a final concentration of 3 μg/mL, and the cells were incubated at 27 °C for 1.5 h in the dark on a mini-shaker platform (Biosan, Latvia) at 200 rpm. After incubation, the cells were fixed with a 1% paraformaldehyde solution (Sigma-Aldrich, USA) for 20 min. The number of FITC-labeled cells and the fluorescence intensity were determined on a DxFlex flow cytometer (Beckman Coulter, USA).

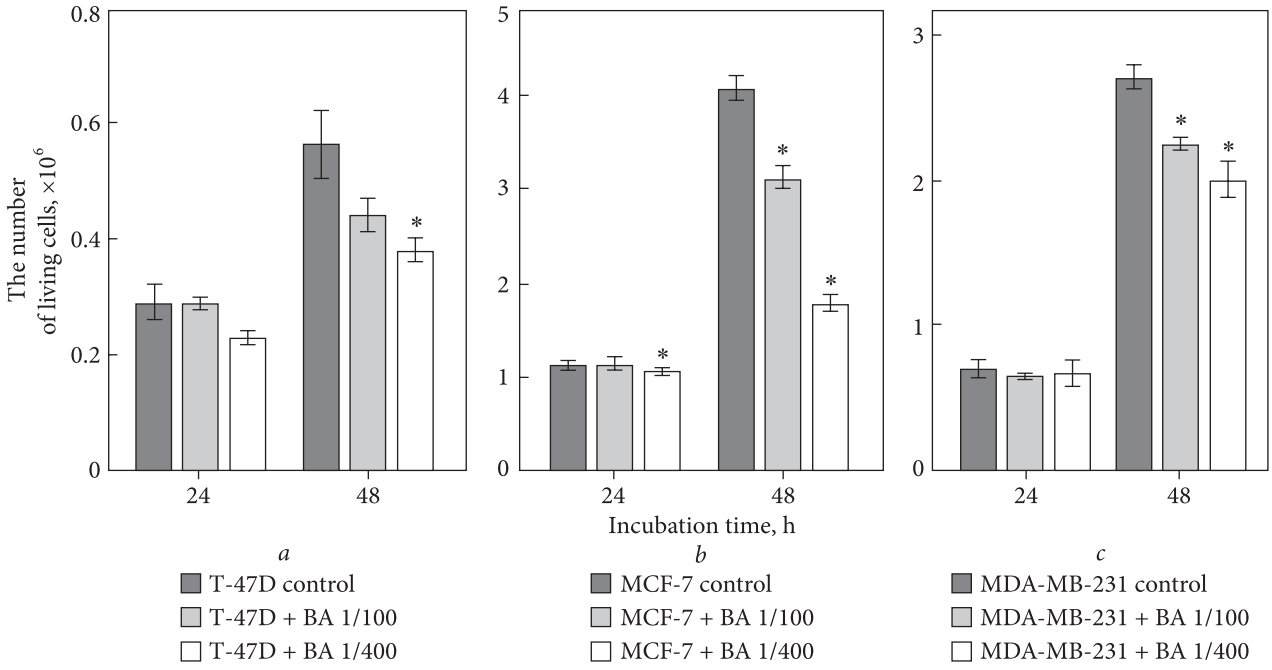

Fig. 1. Viability of T-47D (*a*), MCF-7 (*b*), and MDA-MB-231 (*c*) BC cells after co-cultivation with *B. animalis*. The number of viable cells was determined using the trypan blue exclusion test. *$p < 0.05$ compared to the control cells

Statistical processing of results. All experiments were performed in triplicate. The mean value of the studied parameters (M), standard deviation (SD), and Student's *t*-test were calculated using GraphGad Prism 8.0.1 software. Differences were considered statistically significant at $p < 0.05$.

Results

BC cells actively metabolize glucose due to enhanced proliferative activity and, accordingly, an increased need for ATP. The altered metabolism of tumor cells ensures continuous production of energy and metabolites to support rapid proliferation and cell survival [22]. Therefore, when studying the metabolic plasticity of BC cells after their co-cultivation with *B. animalis*, it was important to consider the effect of bifidobacteria on the viability of malignant cells.

After 24 h of incubation of BC cells with *B. animalis*, no changes in the survival of eukaryotic cells were observed in most of the studied groups compared to the control. Only after 48 h of co-cultivation, a significant decrease in the number of live BC cells of all three lines compared to the control was noted (Fig. 1). A decrease in the number of live T-47D cells by 33%, MCF-7 cells by 23—56%, and MDA-MB-231 cells by 16—25% was recorded.

The metabolic phenotype of the control and *B. animalis*-co-cultivated BC cells was determined by analyzing GCR and LPR. We revealed that triple-negative MDA-MB-231 cells preferred the glycolytic pathway of glucose metabolism, as evidenced by a higher GCR and LPR than in cells of the luminal subtype (Fig. 2, *c*, Fig. 3, *c*).

The resulting values of GCR and LPR (Fig. 2, Fig. 3) reflected the total metabolic activity of eukaryotic and bacterial cells in the condition of their co-cultivation since these parameters were determined in the culture medium. However, an analysis of the metabolic profile of *B. animalis* indicated that GCR and LPR were significantly lower than in the cancer cells (Fig. 2, *d*, Fig. 3, *d*). Indeed, the number of bacterial bodies was greater than that of BC cells. However, calculations of the utilized glucose amount indicated that *B. animalis* consumed no more than 13% of the total amount of utilized glucose in the BC + *B. animalis* groups and did not cause depletion of glucose, the main source of energy in the medium.

As shown in Fig. 2, in the T-47D + *B. animalis* group, GCR significantly increased by 1.4—3.1 times, in MCF-7 + *B. animalis* cells by 1.6—4.7 times, and in MDA-MB-231 + *B. animalis* cells by 1.2—2.0 times compared to the control.

The rate of glucose uptake in eukaryotic cells is regulated by insulin, a unique hormone that acts at the membrane level through insulin receptors (IR).

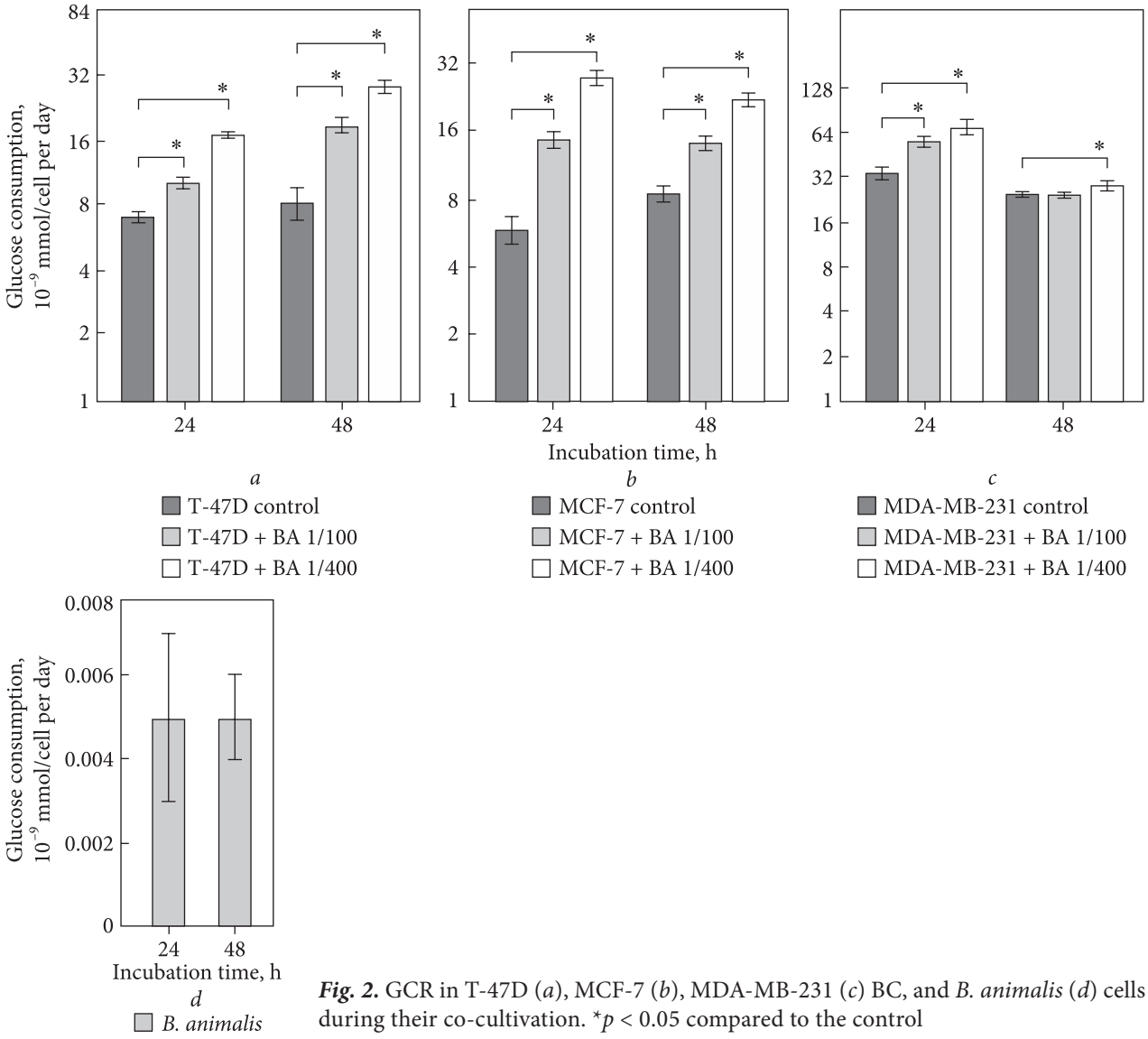

Fig. 2. GCR in T-47D (*a*), MCF-7 (*b*), MDA-MB-231 (*c*) BC, and *B. animalis* (*d*) cells during their co-cultivation. *$p < 0.05$ compared to the control

This transmembrane protein with tyrosine kinase activity mediates both metabolic and mitogenic effects in malignant cells, especially when overexpressed, which is also characteristic of BC cells [23].

The binding of FITC-insulin with IR on the surface of BC cells was measured after their co-cultivation with *B. animalis*. In the T-47D cells co-cultured with *B. animalis,* we observed a significant decrease in IR expression by 30% vs control, while in MCF-7 and MDA-MB-231 cells cultured with *B. animalis*, on the contrary, an increase in IR expression by 24.3% and 17.6%, respectively. (Table 1). Possibly, the detected changes in IR expression may be caused by the activation of glucose

Table 1. **Changes in IR expression in *B. animalis*-co-cultured BC cells**

Cells	Cell control		+ *B. animalis* 1:400	
	Number of IR$^+$ cells, % (M ± SD)	Level of IR expression, c.u. (M ± SD)	Number of IR$^+$ cells, % (M ± SD)	Level of IR expression, c.u. (M ± SD)
T-47D	38.1 ± 1.1	21667.0 ± 1878.1	28.0 ± 1.6*	14285.0 ± 648.3*
MCF-7	65.4 ± 2.3	8528.2 ± 270.7	81.8 ± 1.1*	10524.8 ± 180.0*
MDA-MB-231	54.0 ± 2.9	5998.8 ± 343.7	48.0 ± 2.2	7052.2 ± 51.4*

Note: * $p < 0.05$, compared to the control cells.

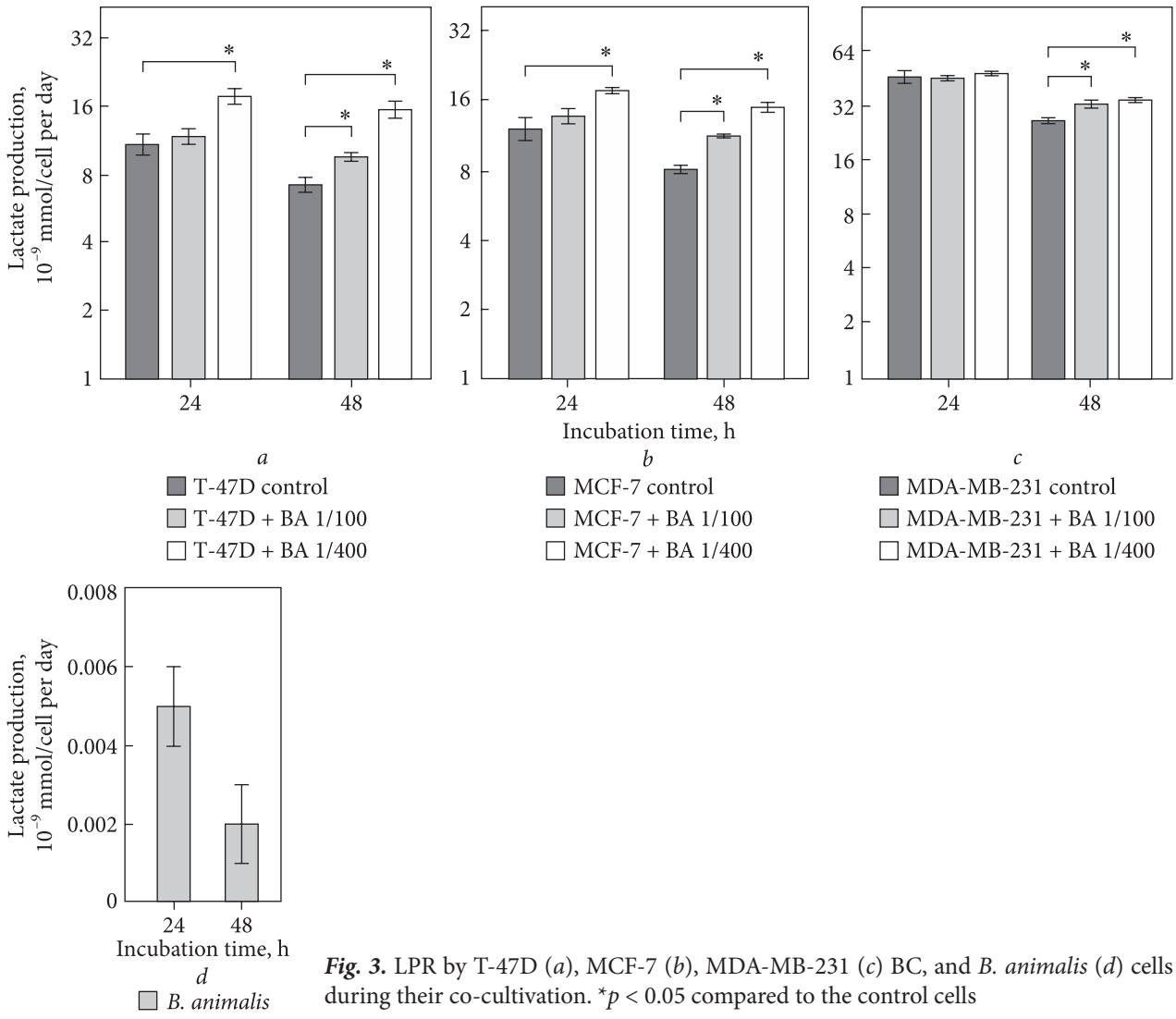

Fig. 3. LPR by T-47D (*a*), MCF-7 (*b*), MDA-MB-231 (*c*) BC, and *B. animalis* (*d*) cells during their co-cultivation. *$p < 0.05$ compared to the control cells

utilization via different IR-dependent and IR-independent signaling pathways in T-47D and MCF-7/MDA-MB-231 cells.

The end product of glycolysis is lactate, an important metabolite that regulates cellular redox homeostasis, energy substrate distribution, and intracellular signaling [24]. Therefore, LPR is an important marker of changes in the metabolic phenotype of BC cells under the influence of various factors.

Co-cultivation of T-47D and MCF-7 cells with *B. animalis* resulted in a significant increase in LPR by 30—95% and 14—115%, respectively, compared to the control cells (Fig. 3, *a*, *b*). Exposure of MDA-MB-231 cells to *B. animalis* was accompanied by an increase in LPR after 48 h of incubation by 20—34% compared to the control cells (Fig. 3, *c*).

Another group of important and informative indicators of the malignant cells' metabolic state is the intracellular activity of glycolytic enzymes, in particular LDH and G6PD. Therefore, we determined the activity of these enzymes in BC cells after their co-cultivation with *B. animalis* for 24 or 48 h.

Co-cultivation of T-47D and MCF-7 cells with *B. animalis* for 24 h resulted in a significant increase in their LDH activity by 111.5—160.0% and 12.2—15.7%, respectively, compared to the control cells. After 48 h of co-cultivation, in T-47D cells, LDH activity increased by 26.2—66.3% and in MCF-7 cells by 18.7—59.5% ($p < 0.05$) (Fig. 4, *a*, *b*). The elevated intracellular LDH activity was also observed in MDA-MB-231 cells, however, it increased only after 48 h of co-cultivation with *B. animalis* by 18.2—40.9% ($p < 0.05$) compared to the control cells (Fig. 4, *c*).

The activity of the main PPP enzyme G6FD dynamically changed in all studied cells after their co-cultivation with *B. animalis* for 24—48 h. Thus, in T-47D cells co-cultivated with *B. anima-*

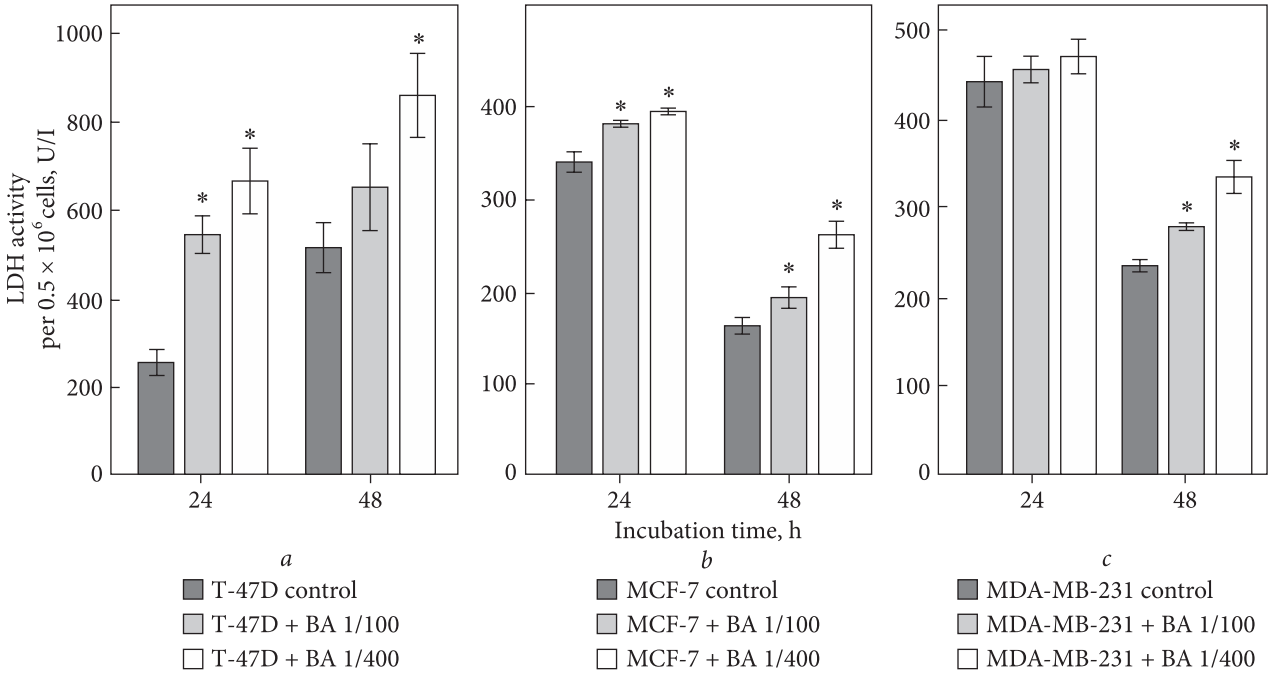

Fig. 4. LDH activity in T-47D (*a*), MCF-7 (*b*), and MDA-MB-231 (*c*) cells after their co-cultivation with *B. animalis*. * $p < 0.05$ compared to the control cells

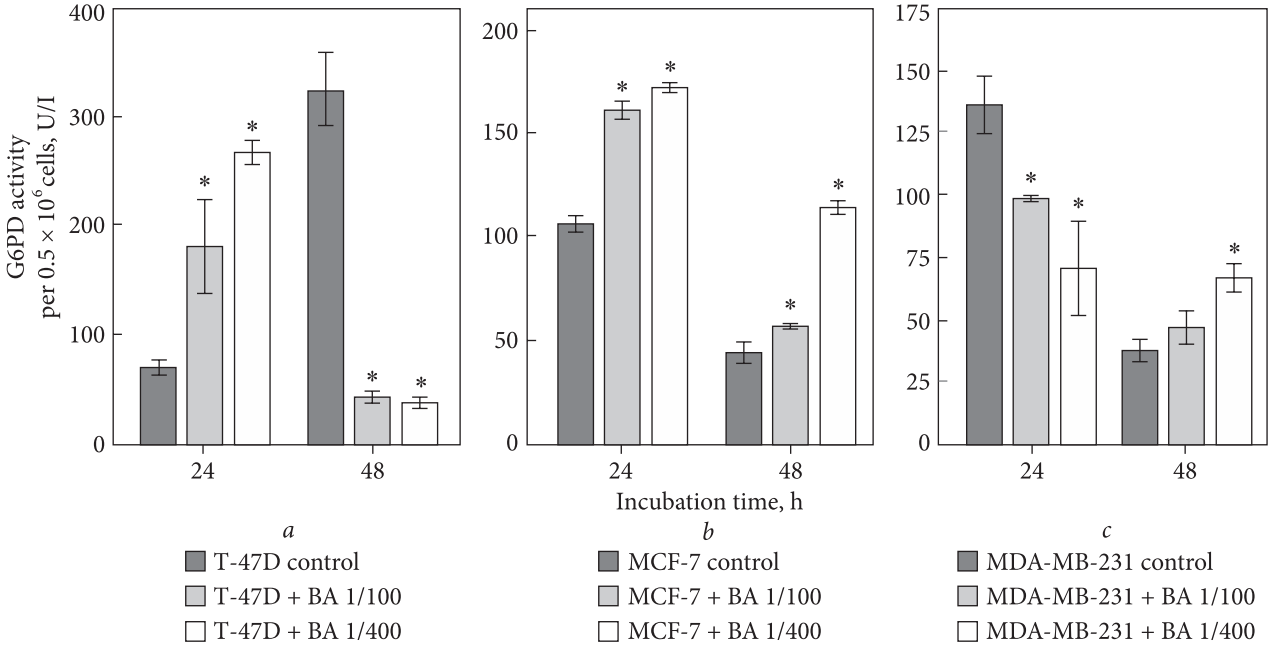

Fig. 5. G6PD activity in T-47D (*a*), MCF-7 (*b*), and MDA-MB-231 (*c*) cells after their co-cultivation with *B. animalis*. *$p < 0.05$ compared to the control cells

lis, a significant increase in enzyme activity by 155.4—277.6% was observed after 24 h of incubation and a decrease in its activity by 70.0—83.5% after 48 h, compared to the control cells (Fig. 5, *a*). In MCF-7 cells, after their exposure to *B. animalis* for 24—48 h, an increase in G6FD activity by 52.6—62.5% and 30.7—164.2% ($p < 0.05$ vs. the control cells) was recorded (Fig. 5, *b*). In MDA-MB-231 cells treated with *B. animalis* for 24 h, the enzyme activity decreased by 27.8—48.0%, and after 48 h of exposure, an increase in G6PD activity by 24.3—75.7% was observed compared to the control cells (Fig. 5, *c*).

Analysis of the main pathways of glucose metabolism in cancer cells indicates an important role of the glucose transporter GLUT1. It has been

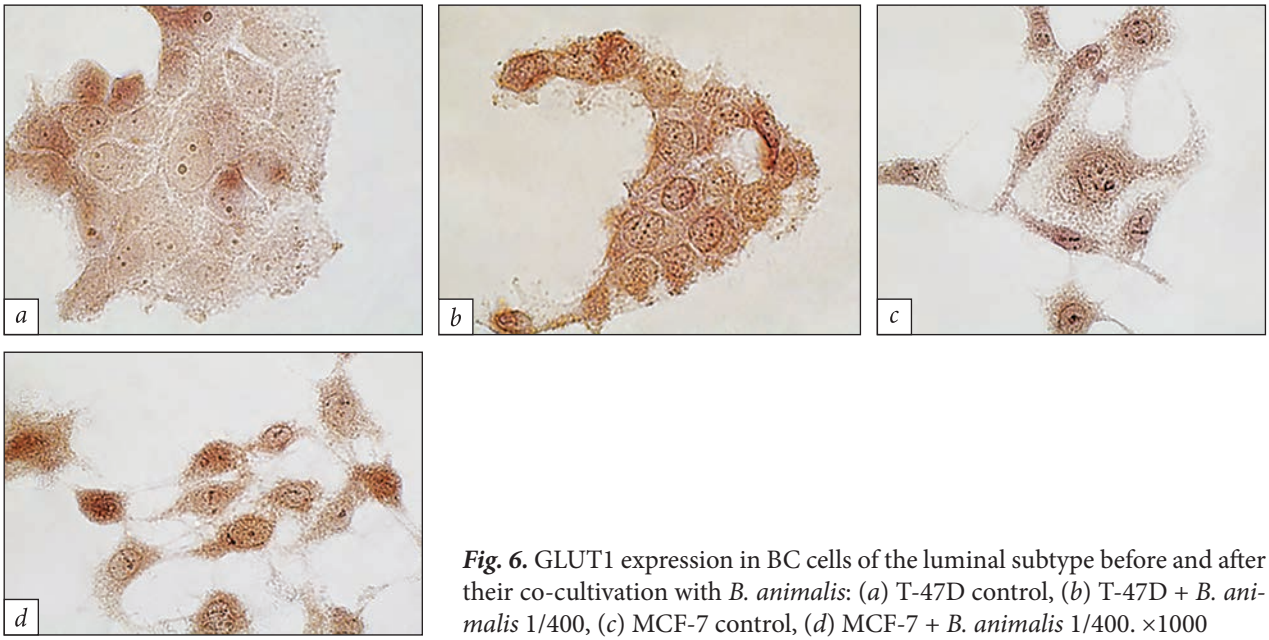

Fig. 6. GLUT1 expression in BC cells of the luminal subtype before and after their co-cultivation with *B. animalis*: (*a*) T-47D control, (*b*) T-47D + *B. animalis* 1/400, (*c*) MCF-7 control, (*d*) MCF-7 + *B. animalis* 1/400. ×1000

shown that human BC tissues are characterized by an increased level of GLUT1 expression [25].

Co-cultivation of T-47D or MCF-7 cells with *B. animalis* resulted in a significant increase in GLUT1 expression by 29.6—59.3% and 40.0—80.0%, respectively, compared to the control cells (Table 2). Such a rise occurred due to an increase in the percentage of cells with a high level of GLUT1 expression in the cell population (Fig. 6). In MDA-MB-231 cells with high basal level of GLUT1 expression, no change in its expression was observed after co-cultivation with *B. animalis* (Table 2).

The complex and multifactorial changes in the metabolic phenotype of *B. animalis*-co-cultivated

Table 2. **GLUT1 expression in BC cells after their co-cultivation with *B. animalis***

Cells	GLUT1 expression, points (M ± SD)
T-47D control	135 ± 15
T-47D + *B. animalis* 1/100	175 ± 10*
T-47D + *B. animalis* 1/400	215 ± 12*
MCF-7 control	100 ± 5
MCF-7 + *B. animalis* 1/100	140 ± 7*
MCF-7 + *B. animalis* 1/400	180 ± 10*
MDA-MB-231 control	260 ± 10
MDA-MB-231 + *B. animalis* 1/100	250 ± 15
MDA-MB-231 + *B. animalis* 1/400	275 ± 15

Note: *$p < 0.05$ compared to the control cells.

BC cells are regulated by a complex signaling cascade that includes transcription factors. Therefore, we studied the expression of STAT6 in BC cells after their exposure to *B. animalis*. STAT6 is a cytoplasmic protein that dimerizes upon phosphorylation by JAK kinases and is then transported to the nucleus to regulate gene transcription. There are several points in this signaling pathway where STAT6 influences the cell survival, death, proliferation, and metabolic activity [26].

It was shown that in T-47D and MCF-7 cells treated with *B. animalis*, STAT6 expression increased by 26.7—73.3% and 54.2—66.7%, respectively, compared to control cells. In MDA-MB-231 cells, a significant increase in STAT6 expression (by 82.4%) was recorded only after their co-cultivation with *B. animalis* in a ratio of 1/400 (Table 3, Fig. 7).

Discussion

The ability to reorganize metabolic processes is one of the key properties of cancer cells necessary to ensure high energy needs, survival, proliferation, metastasis, and resistance to anticancer drugs [27]. Therefore, changes in key biochemical and signaling pathways involved in glucose metabolism in BC cells under the influence of various factors are an informative marker of their metabolic state and, therefore, biological properties. BC cells uptake large amounts of glucose and metabolize it through glycolysis, producing lactate as the end-product. In-

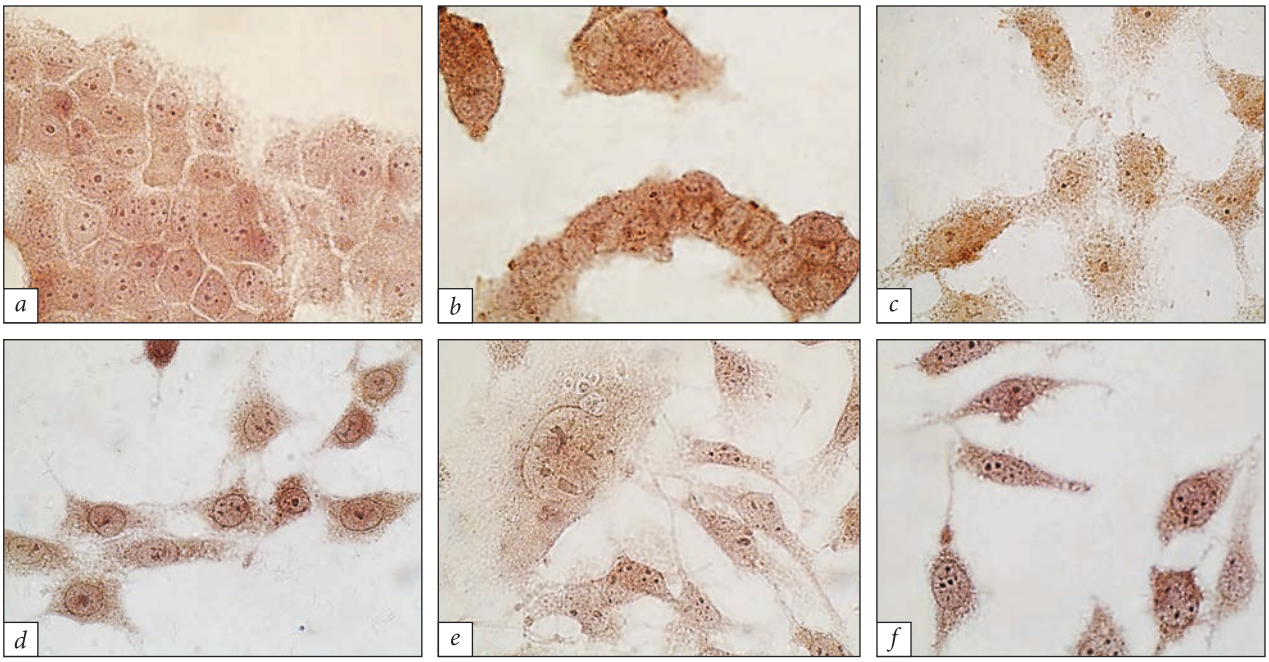

Fig. 7. STAT6 expression in BC cells after their co-cultivation with *B. animalis*. (*a*) T-47D control, (*b*) T-47D + *B. animalis* 1/400, (*c*) MCF-7 control, (*d*) MCF-7 + *B. animalis* 1/400, (*e*) MDA-MB-231 control, (*f*) MDA-MB-231 + *B. animalis*. × × 1000 magnification

creased lactate production is often observed in tumors, where it enhances glycolysis and promotes angiogenesis and cancer progression [17, 24]. Given such pro-tumorigenic activity of lactate, the need to study the effects of *B. animalis* on the main pathways of glucose metabolism in BC cells becomes especially relevant. It is known that lactic acid bacteria, in particular bifidobacteria, important components of the human intestinal and breast microbiota [10], use a pathway known as the "bifido shunt" for carbohydrate metabolism and produce acetate and lactate. According to some opinions, these compounds may enhance the Warburg effect in cancer cells, increasing their proliferation [13] and reducing the effectiveness of chemotherapy [28].

However, there is currently a lot of evidence of the in vitro and in vivo antitumor activity of bifidobacteria [12, 29—31], which is confirmed by the results of our previous studies [32]. Therefore, analysis of the effects of *B. animalis* on some indicators of energy metabolism in BC cells will help improve our understanding of the mechanisms of the microbiota influence on the biological properties of malignant cells and explain the antiproliferative and proapoptotic effects of bifidobacteria on these cells.

The increase in GCR and LPR in BC cells co-cultured with *B. animalis* compared to control cells, indicates an enhancement of the glycolytic pathway of glucose metabolism in this model system. The most pronounced changes in metabolic activity toward increased glycolysis were observed in the luminal subtype BC cells, probably because MDA-MB-231 cells of the basal subtype have already prefered this pathway of glucose metabolism [33]. A statistically significant increase in GCR and LPR in MDA-MB-231 cells was observed only after 48 h of exposure of these cells to the probiotic. It is believed that increased glucose metabolism, especially through glycolysis, occurs in tumor cells with high proliferative

Table 3. **STAT6 expression in BC cells after their co-cultivation with *B. animalis***

Cells	STAT6 expression, points (M ± SD)
T-47D control	150 ± 15
T-47D + *B. animalis* 1/100	190 ±10*
T-47D + *B. animalis* 1/400	260 ± 10*
MCF-7 control	120 ± 10
MCF-7 + *B. animalis* 1/100	185 ± 7*
MCF-7 + *B. animalis* 1/400	200 ± 10*
MDA-MB-231 control	85 ± 5
MDA-MB-231 + *B. animalis* 1/100	100 ± 10
MDA-MB-231 + *B. animalis* 1/400	155 ± 10*

Note: *$p < 0.05$ compared to the control cells.

activity [34]. However, the co-cultivation of BC cells with *B. animalis* resulted in a decrease in the number of viable cancer cells after 48 h of exposure. Probably, in our case, glycolysis fulfills another important function: it provides high levels of glycolytic intermediates to maintain anabolic reactions in BC cells aimed at preserving their viability [35].

To search for possible signaling mechanisms that ensure the increase in GCR in BC cells due to their co-cultivation with *B. animalis*, we studied its effect on the expression of IR in malignant cells. Indeed, the signaling pathways activated by IR regulate the glucose consumption and utilization and the expression of glycolytic genes in cells [36]. The detected changes in IR expression suggest that in the studied cells treated with *B. animalis*, the increase in glucose consumption is regulated through different proximal fragments of the PI3K/Akt insulin signaling pathway: in MCF-7 and MDA-MB-231 cells through IR, and in T-47D cells through activation of the Ras signaling [37].

It is through the PI3K/Akt signaling pathway that the expression of the glucose transporter and important regulator of glycolysis, GLUT1, can be regulated [38]. The phosphorylated form of Akt induces GLUT1 expression in BC cells, which leads to stimulation of glucose transport and an increase in their metabolic activity [38]. It was shown that co-cultivation of T-47D and MCF-7 cells with *B. animalis* was accompanied by a significant increase in GLUT1 expression. Such changes in GLUT1 expression correlated with changes in GCR and LPR in the studied cells. Increased expression, translocation to the plasma membrane, and activation of GLUT1 in BC cells following their exposure to *B. animalis* may be induced by lactate [39]. Glycolysis in tumor cells is regulated through changes in the activity of glycolytic enzymes [40]. We investigated the effect of *B. animalis* on intracellular LDH activity in BC cells. A significant increase in this parameter was found in BC cells, which correlated with changes in LPR and is additional evidence of increased glycolysis in the studied cells [41]. The most pronounced changes in the activity of this enzyme were observed in cells of the luminal subtype.

In this study, we also analyzed the activity of G6PD in BC cells after their exposure to *B. animalis*. G6PD is an enzyme of the oxidative branch of the PPP that generates ribulose-5-phosphate, CO_2, and NADPH. It is NADPH that malignant cells use as a powerful antioxidant maintaining redox homeostasis [42]. Changes in G6PD activity observed in BC cells after their co-cultivation with *B. animalis* are associated, in our opinion, with an increase in ROS levels in these cells, which was shown in our previous study [32]. The ability of *B. animalis* to affect G6PD activity in BC cells is an important finding, as it is the first rate-limiting enzyme of PPP, and its activity is associated with invasiveness, metastasis, and chemotherapy resistance of malignant cells [43].

Transcription factors are essential participants in signaling pathways that regulate the metabolic plasticity of BC cells. In our work, we decided to focus on the regulatory role of the STAT6 transcription factor, changes in the expression of which not only affect the energy metabolism of glucose in BC cells but also play a decisive role in controlling the proliferation of malignant cells. Increased nuclear and cytoplasmic expression of STAT6 was observed in the cells of all three BC cell lines after co-cultivation with *B. animalis*. Thus, STAT6 can regulate glucose energy metabolism in malignant cells by controlling the activity of glycolysis rate-limiting enzymes [44], or GLUT1 expression [45]. At the same time, it is known that increased STAT6 expression in BC cells is positively associated with a lower rate of their proliferation or induction of apoptosis. In this case, the antiproliferative and proapoptotic effects of STAT6 are mediated by stimulation of the expression of G1 cyclin-dependent kinase inhibitor $p21^{WAF1}$ [46]. It is the increase in the expression of this regulatory protein that we observed in BC cells after their co-cultivation with *B. animalis* in our previous studies [32]. It should be noted that STAT6 is activated in cells in response to IL-4/IL-13 [47], and lactate or *B. animalis* itself can promote the production of these cytokines [48].

Therefore, the co-cultivation of BC cells with *B. animalis* is accompanied by changes in glucose metabolism in cancer cells, in particular, increased glycolysis. Notably, *B. animalis* affects not only the components of glucose metabolism but also signaling pathways, modulating the level of IR expression and increasing the expression of GLUT1 and STAT6. In addition, the antiproliferative mechanisms of action of *B. animalis* may be translated through PI3K-mediated signaling pathways, and glycolysis is enhanced to support anabolic reactions [35, 49] or nuclear transport of anti- and pro-

apoptotic factors, which requires active metabolism and ATP energy [50] in BC cells.

In the present study, we have identified only some fragments of metabolic pathways that are activated in BC cells upon their co-cultivation with *B. animalis*. Further studies will allow us to understand the features of energy metabolism in BC cells due to the effects of bifidobacteria and create the basis for developing new approaches to effective treatment of this dangerous disease especially in conditions of metabolic syndrome development.

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ПЕРЕПРОГРАМУВАННЯ МЕТАБОЛІЗМУ ГЛЮКОЗИ
В КЛІТИНАХ РАКУ МОЛОЧНОЇ ЗАЛОЗИ ЛЮДИНИ
ПІСЛЯ СПІВКУЛЬТИВУВАННЯ З *BIFIDOBACTERIUM ANIMALIS*

Вступ. Здатність до реорганізації метаболічних процесів є однією з ключових властивостей злоякісних клітин, необхідною для забезпечення їхніх високих енергетичних потреб, виживаності, проліферації, метастазування і резистентності до дії протипухлинних препаратів. Молочнокислі бактерії, зокрема Bifidobacteria, є важливим елементом пухлинного мікрооточення при РМЗ і, як активні продуценти лактату, можуть впливати на метаболічний фенотип злоякісних клітин. **Мета.** Дослідити вплив *B. animalis* на деякі ланки метаболізму глюкози та експресію білків, асоційованих з цим процесом у клітинах ліній РМЗ людини різних молекулярних підтипів. **Матеріали та методи.** Дослідження проводили на клітинах РМЗ людини ліній T-47D, MCF-7 (люмінальний підтип) і MDA-MB-231 (базальний підтип) та живій культурі *Bifidobacterium animalis subsp. lactis (B. animalis*). У дослідженні використовували колориметричні ферментативні методи, проточну цитометрію, імуноцитохімічний аналіз, а життєздатність клітин визначали в тесті з трипановим синім. **Результати.** Співкультивування клітин РМЗ з *B. animalis* привело до статистично достовірного ($p < 0,05$) підвищення швидкості споживання глюкози в 1,2—4,7 рази і продукції лактату на 15—115%, а також активності ЛДГ на 15—160% у клітинах РМЗ відносно інтактних клітин. Найбільш виражені зміни спостерігали в клітинах люмінального підтипу. Зміни біохімічних показників метаболічного фенотипу клітин РМЗ люмінального підтипу супроводжувались підвищенням експресії транспортера глюкози GLUT1 на 30—80% у порівнянні з контролем. В оброблених *B. animalis* клітинах РМЗ всіх трьох ліній також виявили підвищення експресії фактора транскрипції STAT6. **Висновки.** Співкультивування клітин РМЗ з *B. animalis* супроводжується посиленням гліколізу в злоякісних клітинах. При цьому *B. animalis* впливає не лише на біохімічні ланки метаболізму глюкози, але й сигнальні шляхи, модулюючи рівень експресії рецепторів інсуліну і підвищуючи експресію транспортера глюкози GLUT1 і транскрипційного фактора STAT6.

Ключові слова: рак молочної залози, *Bifidobacterium*, глюкоза, лактат, STAT6, GLUT1.