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MODIFICATION OF BREAST CANCER CELLS' SENSITIVITY TO METFORMIN DUE TO CO-CULTIVATION WITH *Bifidobacterium animalis*

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Glucose metabolism (GM) disturbances are well-known risk factors for the development of breast cancer (BC). The GM regulator metformin is used as an adjunctive therapy for BC. Another potent modulator of GM in BC cells is the microbiota, particularly bifidobacteria. The combined action of these factors may lead to unpredictable effects on the sensitivity of malignant cells to antitumor agents.

Aim. To investigate the effect of *Bifidobacterium animalis* on the sensitivity of BC cells to the antiproliferative effects of metformin.

Materials and Methods. The impact of *B. animalis* on GM in BC cells was determined by biochemical methods (glucose consumption and lactate production rate, intracellular lactate dehydrogenase activity). Cell viability was evaluated using the trypan blue exclusion test.

Results. Co-cultivation of BC cells with *B. animalis* leads to enhanced glycolysis in malignant cells. These metabolic phenotype changes are accompanied by alterations in the sensitivity of BC cells to metformin. Only in *MCF-7* cells treated with *B. animalis* was a significant enhancement of the antitumor effects of metformin observed compared to cells incubated with either metformin or *B. animalis* alone.

Conclusions. Exposure of *MCF-7* cells to *B. animalis* increases their sensitivity to the antiproliferative effects of metformin, which is a result of GM reprogramming.

Keywords: breast cancer, *Bifidobacterium animalis*, metformin, glucose metabolism.

Breast cancer (BC) is the most common oncological disease and the leading cause of cancer-related mortality among women worldwide. Metabolic disorders are well-established risk factors for breast cancer [1]. Metformin is one of the known regulators of glucose metabolism, which can attenuate metabolic disorders and act as a therapeutic agent against BC. Another powerful modulator of glucose metabolism by BC cells is the microbiota. Among the primary constituents of the human breast microbiota are lactic acid bacteria, particularly bifidobacteria, which can interfere with the lactate cycle in BC cells and influence their sensitivity to agents with antitumor activity [2]an aberrant composition of the microbiome, characterizes breast cancer. In this review we discuss the changes to the metabolism of breast cancer cells, as well as the composition of the breast and gut microbiome in breast cancer. The role of the breast microbiome in breast cancer is unresolved, nevertheless it seems that the gut microbiome does have a role in the pathology of the disease. The gut microbiome secretes bioactive metabolites (reactivated estrogens, short chain fatty acids, amino acid metabolites, or secondary bile acids. Given the potential of bifidobacteria to modulate tumor cell metabolism, it is

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of particular relevance to investigate the sensitivity of BC cells to the antitumor effects of metformin under conditions of interaction with the microbiota and its metabolites.

Aim. To investigate the effect of metformin on the viability of intact and *Bifidobacterium* animalis-treated human breast cancer (BC) cells *in vitro*.

Methods. Human breast cancer (BC) cells (luminal T47D, MCF-7; basal MDA-MB-231) were seeded at 1×10^6 cells per 75 cm^2 in DMEM with 10% FBS without antibiotics and incubated for 10 hours in a 5% CO_2 atmosphere. Cells were then treated with *Bifidobacterium animalis subsp. lactis BB-12* (1:400 eukaryotic/bacterial ratio). Untreated cells served as controls. After 72 hours, cells were washed, counted, and analyzed for glucose consumption rate (GCR), lactate production rate (LPR), and lactate dehydrogenase (LDH) activity using biochemical methods and standard formulas [3]. Additionally, 1.5×10^5 untreated and *B. animalis*-treated BC cells were incubated for 24 hours, followed by metformin treatment (50 mM for T47D, 10 mM for MCF-7, and MDA-MB-231). After 48 hours, cell viability was assessed using the trypan blue exclusion assay. Statistical analysis was performed with GraphPad Prism 8.0.1 ($P < 0.05$, Welch's t-test).

Results and Discussion. After 72 hours of incubation of BC cells with *B. animalis*, a decrease in the number of viable T47D and MCF-7 cells by 40–50% ($P < 0.05$) and MDA-MB-231 cells by 25% ($P < 0.05$) was observed compared to untreated cells without bacteria. A shift in the metabolic phenotype of *B. animalis*-treated BC cells towards enhanced glycolysis was also noted, as evidenced by a statistically significant increase in GCR and LPR 2-4 times in T47D and MCF-7 cells and 1.8–2 times in MDA-MB-231 cells compared to untreated cells. The intracellular LDH activity further confirmed these changes in glucose metabolism in B. animalis-treated BC cells. An increase in enzyme activity by 80–90% ($P < 0.05$) in luminal subtype cells and 40% ($P < 0.05$) in basal subtype cells compared to untreated cells was observed. These results suggest that the most significant changes in the metabolic phenotype of BC cells occur in the luminal subtype, likely because the MDA-MB-231 cells preferentially utilize the glycolytic pathway for glucose consumption [4].

The results of the viability analysis of untreated and *B. animalis*-treated BC cells after exposure to metformin show that combined treatment of T47D cells inhibits the antiproliferative effects of *B. animalis* and metformin, which are observed in the monotherapy (Fig. *A*). In MDA-MB-231 cells treated with both agents, no statistically significant difference in cell viability was found compared to cells treated with either metformin or *B. animalis* alone (Fig. *C*). Only in MCF-7 cells treated with *B. animalis*, a statistically significant enhancement of the antiproliferative effect of metformin was observed compared to cells incubated with metformin or *B. animalis* separately (Fig. *B*).

Such an effect of metformin on the viability of *B. animalis*-treated BC cells can be explained by changes in the expression of the insulin receptor in the studied cells. According to the literature, one of the primary mechanisms behind the antitumor effect of metformin is its ability to reduce insulin resistance in malignant cells by increasing the expression of the insulin receptor [5]. Our previous studies [6] have shown that co-cultivation of BC cells with *B. animalis* leads to a decrease in the number of RI+ cells and the receptor expression level in T47D cells. Conversely, it increases both the number of RI+ cells and the level of receptor expression in MCF-7 cells. Furthermore, the increased sensitivity of *B. animalis*-treated MCF-7 cells to the effects of metformin may also be attributed to

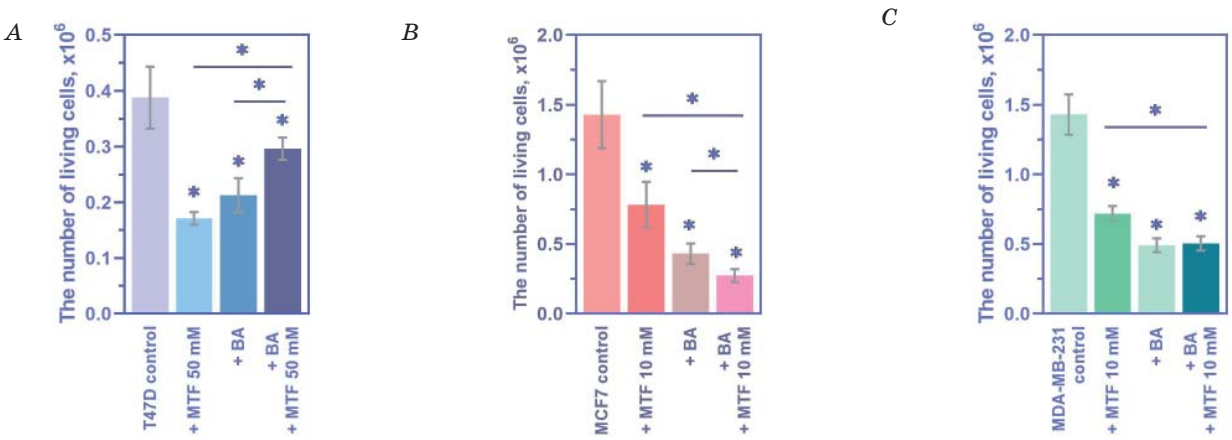

Figure. **Viability of untreated and *B. animalis*-treated human BC cells after incubation with metformin**
$P < 0.05$ to the intact control cells; $P < 0.05$ between the two experimental groups.

the fact that the metabolic phenotype of these cells undergoes the most significant changes as a result of co-cultivation with *B. animalis*.

Conclusions. Pre-treatment of MCF-7 cells with *B. animalis* enhances their sensitivity to the antiproliferative effects of metformin. These changes result from the reprogramming of glucose metabolism in BC cells after co-cultivation with *B. animalis*, which is reflected at the level of receptor expression and metabolite production. The obtained results contribute to our understanding of the role of the microbiota as a component of therapeutic strategies to enhance the efficacy of antitumor therapy.

Authors' contribution

TPK maintained cell culture, conducted experiments with BC cells, *B. animalis*, and metformin, statistical analysis; OOL cell viability assessment and analyzed the results; PAV biochemical analysis; VFC development of research ideas and experimental design.

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