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RELATIONSHIP BETWEEN GLUCOSE METABOLISM IN CANCER CELLS WITH DIFFERENT METASTATIC POTENTIAL AND CELL SENSITIVITY TO ANOIKIS

Abstract. *Despite the significant intensification of research on the mechanisms of anoikis, the question of how metastatic cells avoid death upon separation from the extracellular matrix remains open. The aim of the study was to investigate in vitro glucose metabolism in the cells of two variants of Lewis lung carcinoma with different metastatic potential (highly metastatic LLC cells and low metastatic LLC/R9 cells) and to analyze the relationship between metastatic potential, glucose oxidation intensity and cell sensitivity to anoikis. A comparative analysis of the studied indices showed that in conditions of deadhesive growth (which simulated the process of dissemination of metastatically active cells) LLC/R9 cells (as opposed to LLC cells) were characterized by a decreased glycolysis intensity and decreased sensitivity to anoikis. The stability of the glycolysis*

intensity in LLC cells during the transition to deadhesive growth conditions determined both the resistance (at least in part) of these cells to anoikis and their high metastatic potential.
Keywords: *cancer cells, anoikis, glucose metabolism, metastatic potential.*

Despite intensive research aimed at developing new effective strategies for anticancer therapy, a significant improvement in the overall survival rate of cancer patients with metastases has not been achieved. Presently the metastasis of malignant neoplasms remains one of the main causes of mortality, causing more than 90% of deaths among cancer patients. On the one hand, the reason for this is that the treatment strategy for cancer patients is primarily aimed at inhibiting the growth of the primary tumor, rather than metastatic lesions. On the other hand, low progress in the treatment of metastases is due to the extremely complex and dynamic nature of the metastatic cascade, each of the stages of which depends on many and often unrelated factors. Without an understanding of the molecular and cellular mechanisms that determine each stage of the metastatic process and can serve as targets for therapeutic intervention, it is impossible to develop an effective treatment strategy for patients with metastatic disease [1].

In the early stages, metastatically active cells detach from the extracellular matrix (ECM) and/or neighboring cells and move both inside and outside the tumor [2, 3]. Unlike normal cells, the separation of which from ECM activates anoikis (apoptotic cell death due to detachment from the substrate) and causes their death, metastatic cells are characterized by resistance to anoikis, which ensures their survival in the early stages of metastasis [4]. Although there has been a significant intensification of research on the mechanisms of anoikis over the past few years, the question of how metastatic cells avoid death while detaching from ECM remains open.

The aim of the study was to investigate the indices of glucose metabolism in cancer cells with different metastatic potential and to analyze the relationship between the metastatic potential of cancer cells, the intensity of glucose oxidation, and their sensitivity to anoikis.

In the study, there were used two variants of Lewis lung carcinoma cells with different metastatic potential (LLC and LLC/R9) kindly provided by the National

Bank of Cell Lines and Transplanted Tumors of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine (Kyiv, Ukraine) [5, 6]. LLC/R9 variant obtained by the experimental progression of wild strain LLC toward the formation of drug resistance to cis-diamminedichloroplatinum is characterized by near 3-fold lower metastatic potential (expressed as the number and volume of lung metastases) compared to parental LLC.

The objectives of the study were: (i) to conduct a comparative assessment of the survival of LLC and LLC/R9 cells in the conditions of their growth *in vitro* on a deadhesive substrate (experimental model of anoikis) and adhesive substrate; (ii) to investigate glucose metabolism in LLC and LLC/R9 cells under different growth conditions; (iii) to analyze the relationship between the intensity of glucose oxidation in cancer cells with different metastatic potential and their sensitivity to anoikis.

Materials and Methods. Cancer cells were maintained *in vitro* under standard conditions in RPMI 1640 medium (Sigma, USA) with the addition of 10% fetal calf serum (FTS, Sigma, USA), 2 mm L-glutamine, and 40 μg/ml gentamicin at 37 °C in humidified conditions, 5% CO_2. Standard Petri dishes with a diameter of 60 mm for cell culture (Sarstedt) were used for cell culture in conditions of cell adhesion to the substrate. The same 60 mm diameter Petri dishes coated with a layer of poly (2-hydroxyethyl methacrylate) (Poly-HEMA) according to the manufacturer's protocol (Sigma) were used to simulate the deadhesive growth of cancer cells. Cells were incubated for 3 days. All studies were performed in triplicate. The number of cells in the suspension and their viability were assessed routinely by counting them directly in a hemocytometer using a 0.4% solution of vital trypan blue dye. The level of apoptosis was determined by flow cytofluorimetry according to [7]. In brief, the cells were resuspended in hypotonic lysis buffer with the addition of 5 μg/ml propidium iodide, and the DNA content was analyzed at an argon laser wavelength of 488 nm. The level of apoptosis was assessed by the number of cells with hypodiploid DNA content. Measurement of glucose and lactate levels in the incubation medium (using enzymatic methods for the determination of glucose oxidase and lactate oxidase, respectively) and determination of lactate dehydrogenase (LDH) activity in the supernatant was performed on a biochemical

analyzer Chem Well (Awareness Technology, USA) using standard kits according to the manufacturer's protocol (Global Scientific, USA). The results of measuring the LDH activity were recalculated depending on the protein concentration in the supernatants, and the total protein content was determined using the method of Bradford. Statistical analysis included descriptive statistics, nonparametric Mann-Whitney test using the statistical package Microcal Origin (v. 9.5). The obtained data are presented as M ± SE, where M is the mean value, SE is the standard error.

Results. The study has shown that the growth of both variants of cancer cells for 3 days as a deadhesive culture was accompanied by a significant decrease in the number of viable cells by more than 33% ($p < 0.01$) compared with the corresponding index for their adhesive growth (Fig. 1).

The deadhesive growth of highly metastatic LLC cells caused a decrease in the number of both dead cells (by 56%, $p < 0.01$) and apoptotic cells (by 29%, $p < 0.01$) compared with adhesive growth conditions, indicating the presence of the subpopulation of the cells which are resistant to anoikis (Fig. 1A).

In contrast to LLC, deadhesive growth of low-metastatic LLC/R9 cells caused an increase in the number of apoptotic cells by 80% ($p < 0.005$), without affecting the number of necrotic cells (Fig. 1B).

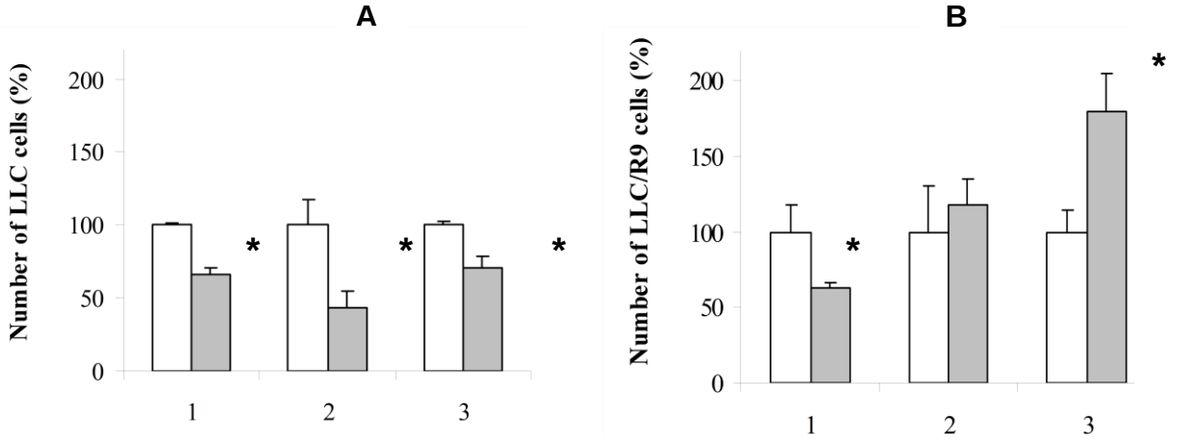

Fig. 1. **The number of viable cells (1), dead cells (2), and apoptotic cells (3) after 3 days incubation of LLC (A) and LLC/R9 (B) cells growing as adhesive (white column) and deadhesive (grey column) culture (in percent of adhesive variant)**

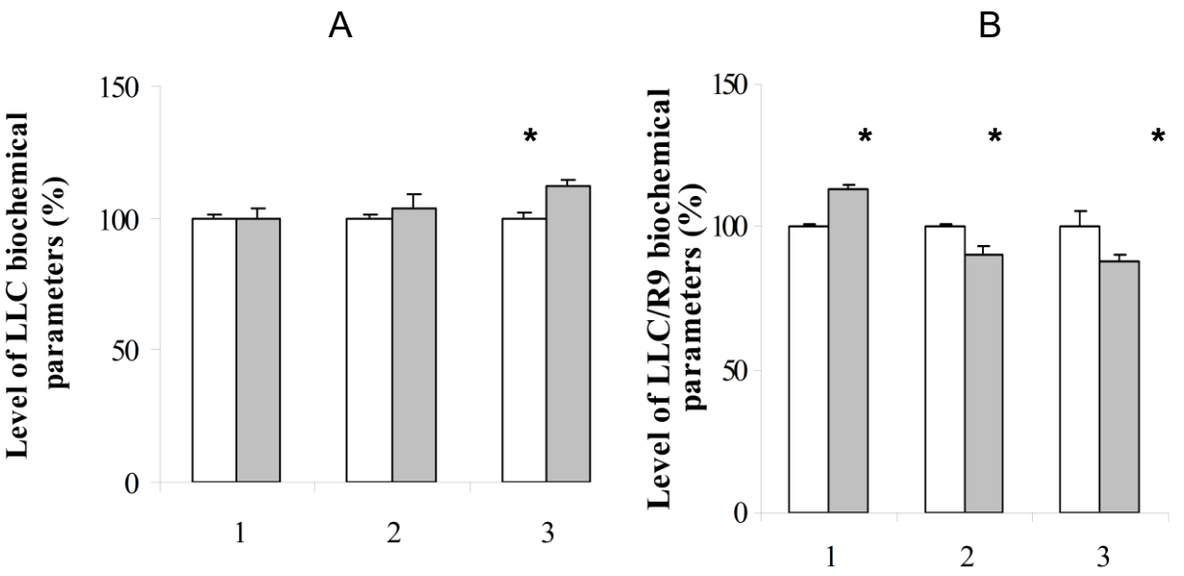

Fig. 2. **Level of glucose (1), lactate (2), and LDH activity (3) in the supernatant after 3 days incubation of LLC (A) and LLC/R9 (B) cells growing as adhesive (white column) and deadhesive (grey column) culture (in percent of adhesive variant)**

For both variants of Lewis lung carcinoma cells, the decrease in the number of viable cells after 3 days of deadhesive growth could be related to either blocking proliferative activity (by increasing the number of cells in $G1_0$ and/or $G2_0$ phases) or the survival of a subpopulation of cells with low proliferative potential (longer mitotic cycle)

Cultivation of LLC/R9 cells on a deadhesive surface *in vitro* was accompanied by a decrease in the glycolysis intensity, as evidenced by a decrease in both the level of glucose consumption and the level of lactate production by the studied cells. Thus, the level of glucose in the incubation medium was 12.6% ($p < 0.05$) higher, and the level of lactate 10% ($p < 0.05$) lower than the corresponding indices of these cells grown in adhesive culture (Fig. 2B). At the same time, the level of glucose and lactate in the incubation medium of LLC cells was almost the same in the conditions of their adhesive and non-adhesive growth (Fig. 2A).

One should note the divergent changes in LDH activity in the incubation medium of LLC/R9 and LLC cells during deadhesive growth. The activity of LDH

during the LLC/R9 deadhesive culturing decreased by 11.8% ($p < 0.05$) compared to the conditions of adhesive growth, while in LLC cells this index increased by 12% ($p < 0.05$).

Conclusions. A comparative analysis of the studied parameters for Lewis lung carcinoma cells with different metastatic potential showed that under conditions of deadhesive growth (simulating the process of dissemination of metastatically active cells) low metastatic LLC/R9 cells (as opposed to highly metastatic LLC cells) are characterized by reduced glycolysis and decreased sensitivity to anoikis. The stability of the glycolysis intensity in LLC cells during the transition to deadhesive growth conditions allows them to maintain a high level of synthesis of antioxidant enzymes and, consequently, to neutralize reactive oxygen species, causing both resistance of these cells (at least in part) to anoikis as well as their high metastatic potential.

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