

<https://doi.org/10.15407/exp-oncology.2023.02.242>

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EFFECT OF LACTATE DEHYDROGENASE INHIBITION BY OXAMATE ON LEWIS LUNG CARCINOMA CELLS WITH DIFFERENT METASTATIC POTENTIAL

Background. Today, the ability for metabolic reprogramming is considered one of the distinguishing features of metastatically active tumor cells, a classic example of which is aerobic glycolysis. Despite a large number of studies in this direction, the question of the relationship between the intensity of aerobic glycolysis and the metastatic potential of tumor cells remains almost completely open. The work **aimed** to investigate the effect of the lactate dehydrogenase (LDH) inhibitor on the viability and several characteristics of Lewis lung carcinoma cells with different metastatic potential. **Materials and Methods.** High-metastatic (LLC) and low-metastatic (LLC/R9) variants of Lewis lung carcinoma cells were used. After 24 h of tumor cells incubation with or without 40 mM sodium oxamate, cell viability, the concentration of glucose and lactate in the incubation medium, distribution of cells by the cell cycle phases, and intracellular ROS production were estimated. **Results.** It was revealed that regardless of the metastatic potential, LLC cells are heterogeneous in terms of both the involvement of aerobic glycolysis in their growth and survival processes and the sensitivity to the cytotoxic/cytostatic action of an LDH inhibitor. 35% of cells of either LLC variant form an oxamate-resistant subpopulation while 65% are oxamate-sensitive. The rate of glucose consumption of LLC/R9 cells in the absence of oxamate is almost twice higher compared to LLC and, as a result, the sensitivity of these cells to the cytotoxic/cytostatic effect of oxamate also is significantly higher (the IC₅₀ for LLC/R9 cells is by 35.8% lower than that for LLC cells, $p < 0.05$). Approximately one-third of the cells of both LLC and LLC/R9 variants can survive and proliferate when aerobic glycolysis is completely inhibited by oxamate. This indicates metabolic reprogramming (either pre-existing or dynamically arising in response to inhibition of glycolysis) of this subpopulation of cells, within which not only the survival of cells but also their proliferative activity is most likely based on glutamine metabolism. **Conclusions.** Such metabolic heterogeneity

Citation: Kolesnik DL, Prokhorova IV, Pyaskovskaya ON, Solyanik GI. Effect of lactate dehydrogenase inhibition by oxamate on Lewis lung carcinoma cells with different metastatic potential. *Exp Oncol.* 2023; 45(2): 242-251. <https://doi.org/10.15407/exp-oncology.2023.02.242>

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of metastatically active cells indicates that inhibition of glycolysis as monotherapy is insufficient for effective anti-metastatic therapy. Presumably, more effective would be to involve various inhibitors of metabolic processes that ensure the metabolic plasticity of metastatic cells.

Keywords: lactate dehydrogenase inhibitor, oxamate, cancer cells, antimetastatic therapy.

Despite the intensive development and implementation of new and effective anticancer agents in oncological practice, the metastasis of malignant neoplasms remains one of the main factors of mortality, causing more than 90% of deaths among cancer patients. Our understanding of the mechanisms of metastasis and the development of effective antimetastatic drugs significantly stands behind our knowledge of the biology of the primary tumor and the pharmacological methods of inhibiting its growth. This is caused by the extremely complex and dynamic nature of the metastatic cascade, which comprises the entire body, from the primary tumor to the formation of metastatic tumors in distant organs and tissues [1]. Each of the stages of this cascade depends on many factors often little interconnected between themselves.

One of the features of the metastatic cascade is the highly variable metabolic microenvironment of metastatic cells, which is different at each stage of the cascade: from (i) the variable and deficient content of plastic and energy substrates in the primary tumor microenvironment [2], (ii) the relatively high and stable oxygen and glucose content in the bloodstream after intravasation of metastatic cells [3] to (iii) a high level of humoral factors produced by normal differentiated cells and a significantly lower level of energy substrates (compared to the bloodstream) after extravasation of metastatic cells into the target organ [4].

Thus, to implement the entire metastatic cascade, tumor cells should possess exceptional metabolic plasticity, capable of ensuring their adaptation and survival to constantly changing conditions [5]. The ability for metabolic reprogramming is now recognized as a distinctive feature of metastatically active tumor cells [6].

A classic example of the metabolic reprogramming inherent in almost all malignant cells is the aerobic glycolysis that leads to an increased rate of glycolysis and lactate production even in the presence of oxygen (the so-called “Warburg effect”) [7, 8]. Such a metabolic shift provides advantages in the survival of tumor cells as they can produce energy and maintain a high level of anabolic processes, even in the case of short-term or long-term hypoxia. Moreover, aerobic glycolysis can guarantee a high rate of synthesis of glycolysis intermediates, which are necessary as a building material for actively proliferating cells. The intensification of lactate production, in turn, significantly affects the microenvironment of cells in the tumor, causing the remodeling of the extracellular matrix and promoting the invasion and metastasis of malignant cells [9]. That is why inhibition of glycolysis in tumor cells is considered a promising strategy for antitumor therapy [10, 11].

Meanwhile, the dominance of aerobic glycolysis cannot fully ensure the metabolic plasticity of metastatic cells, the mechanisms of which may be different at different stages of the metastatic cascade and under different conditions of the tumor microenvironment. The latter can significantly affect the sensitivity of metastatically active tumor cells to the effect of modifiers of energy metabolism including inhibitors of glycolysis [12, 13]. Despite a large number of studies on the role of the Warburg effect in the growth of malignant cells and cancer progression, the question of the relationship between the intensity of aerobic glycolysis and the metastatic potential of tumor cells remains almost completely open [8, 14].

The work aimed to investigate the cytotoxic/cytostatic effect of the lactate dehydrogenase

(LDH) inhibitor (as one of the key enzymes of aerobic glycolysis) on Lewis lung carcinoma cells with different metastatic potential.

Materials and Methods

Experimental tumor models. In the work, two variants of Lewis lung carcinoma cells (LLC and LLC/R9), which differ in their metastatic potential *in vivo*, were used [15]. LLC/R9 cells are characterized by triply lower metastatic potential compared to LLC cells: upon transplantation *in vivo*, they form 3 times lower number and volume of lung metastases compared to LLC.

Both cell variants were maintained *in vitro* under standard conditions in the RPMI-1640 medium (Sigma-Aldrich, USA) supplemented with 10% fetal calf serum (Sigma-Aldrich, USA), 2 mM L-glutamine, and 40 µg/ml gentamicin (Zdorovia, Ukraine) at 37 °C in humidified conditions, 5% CO₂.

In vitro study of cytotoxic/cytostatic effect of oxamate on LLC and LLC/R9 cells. To evaluate the cytotoxic/cytostatic effect of oxamate *in vitro*, LLC and LLC/R9 cells were seeded into the wells of a 96-well plate (1.3×10^4 cells/well) and incubated overnight under standard conditions. After preincubation, the medium containing oxamate (Sigma-Aldrich, USA) in concentrations within 0–150 mM at two-fold dilutions was added to the cells, and incubation continued for 24 h. Each concentration of oxamate was studied in 3 replicates. The control cells were incubated under the same conditions by adding a fresh nutrient medium without oxamate. After 24 h of incubation with oxamate, the number of viable cells was assessed using crystal violet (Sigma—Aldrich, USA) on a Stat Fax 2100 photometer (Awareness Technology, USA) at a wavelength of 595 nm or in a hemocytometer using a 0.4% solution of the vital dye trypan blue (Sigma-Aldrich, USA).

Glucose consumption rate and lactate production. Determination of glucose and lactate

levels in the incubation medium of tumor cells was performed on a ChemWell 2910 biochemical analyzer (Awareness Technology, USA) using commercial kits for the determination of glucose based on glucose oxidase (Global Biomarketing Group, Inc, USA) and lactate based on lactate oxidase (Global Biomarketing Group, Inc, USA) according to the manufacturer's protocol.

The glucose consumption rate (GCR in 10^{-9} mmol/cell/day) was calculated according to the formula:

$$GCR = \frac{(C_{gl}(t_0) - C_{gl}(t_1)) \cdot V}{N(t_0) - N(t_1)}$$

where $C_{gl}(t_0)$ and $C_{gl}(t_1)$ are the concentrations of glucose in the incubation medium at the beginning of cell incubation and after 1-day incubation, respectively, $N(t_0)$ and $N(t_1)$ are the counts of living cells at the beginning of cell incubation and after 1-day incubation, respectively; V is the volume of the incubation medium, which in the studies was equal to 6×10^{-3} L.

The lactate production rate (LPR in 10^{-9} mmol/cell/day) was calculated according to the formula:

$$LPR = \frac{(C_l(t_1) - C_l(t_0)) \cdot V}{N(t_1) - N(t_0)}$$

where $C_l(t_0)$ and $C_l(t_1)$ are the concentrations of lactate in the incubation medium at the beginning of cell incubation and after a 1-day incubation, respectively; are the counts of living cells at the beginning of cell incubation and after 1-day incubation, respectively; V is the volume of the incubation medium, which in the studies was equal to 6×10^{-3} L.

Analysis of cell distribution by the cell cycle phases. The distribution of cells by cell cycle phases was determined using flow cytometry according to [16]. In brief, the cells were resuspended in a hypotonic lysis buffer with the addition of 5 µg/ml propidium iodide and RNase (Sigma-Aldrich, USA), and the DNA

content was analyzed on a Calibur flow cytometer (Becton Dickinson, USA) with an argon laser of 488 nm and a filter of 582/42 nm. Flow cytometry data were analyzed using the Mod Fit LT 3.0 program (BDIS, USA).

Determination of ROS production by tumor cells. The level of the intracellular ROS in tumor cells was determined with 2,7-dichlorofluorescein diacetate using flow cytometry [17]. Cells were washed twice with phosphate-buffered saline (pH 7.4) and incubated in RPMI-1640 medium without FTS with the addition of 2,7-dichlorofluorescein diacetate at 37 °C for 30 min. After that, cells were washed twice with phosphate-buffered saline and analyzed on a FACS Calibur flow cytometer (Becton Dickinson, USA) with a 488 nm argon laser and a 530/30 nm filter. For each sample, 20,000 events were analyzed, while the percentage of cell staining was about 100% (99.60%–99.95%). As a measure of the intensity of ROS production by a single cell, the GeoMean score was used for calculation by the flow cytometer software.

Statistical analysis. Statistical analysis was carried out using descriptive statistics, nonlinear regression analysis, Student's *t*-test, Mann–Whitney *U*-test using Microsoft Excel, Origin Pro v.9.5 and Statistica programs. The obtained data are presented as $M \pm SE$, where *M* is the mean value, and *SE* is the standard error of the mean value.

Results and Discussion

Fig. 1. presents the effect of the LDH inhibitor oxamate (in a wide range of concentrations) on the survival of LLC and LLC/R9 cells. An increase in the oxamate concentration to 50–60 mM causes a progressive exponential decrease in the number of living cells, both LLC and LLC/R9. However, at higher concentrations of oxamate, 30% to 40% of cells of both lines retain their viability, indicating that in addition to cells whose survival is significantly dependent on the

concentration of the LDH inhibitor, in cells of both lines, there is a subpopulation of cells at least insensitive or resistant to oxamate. This suggests the heterogeneity of LLC and LLC/R9 cells in terms of their sensitivity to the cytostatic/cytotoxic action of oxamate.

To analyze the experimental data, we used an exponential model that considers the presence of two subpopulations (sensitive and resistant) among the cells of each of the studied lines and describes the dependence of cell survival (*N* — the number of living cells in % of the control) on the concentration of oxamate (*C*) as follows:

$$N = N_R + N_S \times \exp\left(-\frac{C - C_0}{\alpha}\right)$$

In this model, N_R is the percentage of cells resistant to oxamate (the number of which does not depend on the concentration of oxamate); N_S is the percentage of cells sensitive to oxamate; C_0 is the highest non-cytotoxic concentration of oxamate, and α is a parameter characterizing the degree of sensitivity of cells of a sensitive subpopulation to the cytotoxic/cytostatic action of oxamate: the smaller this parameter, the greater the sensitivity of cells to the action of oxamate.

The parameters of the model were determined from the best fit of the model to the experimental data using the nonlinear regression analysis.

As seen in Fig. 1, and Table 1 the degree of heterogeneity (in terms of sensitivity to the action of the inhibitor) of LLC and LLC/R9 cells during their growth is almost the same: approximately 35% of cells form a resistant subpopulation (parameter N_R of the model), and 65% are sensitive (parameter N_S of the model).

The most significant differences between LLC and LLC/R9 cells are represented by IC_{50} value for oxamate, which is by 35.8% lower for LLC/R9 indicating that the sensitivity of LLC/R9 cells to the cytotoxic/cytostatic effect of oxamate is significantly higher than that of LLC cells, although the fraction of the oxamate-resistant subpopulation is practically the same in these

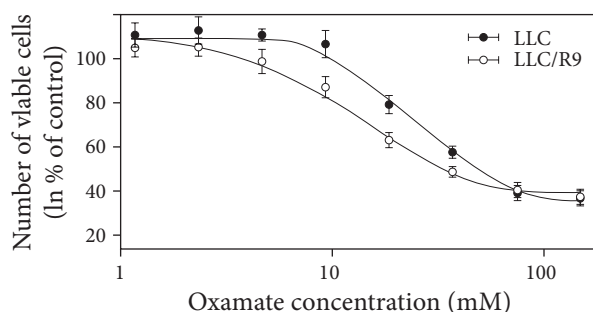


Fig. 1. Cytotoxic/cytostatic effect of oxamate against highly metastatic (LLC) and low metastatic (LLC/R9) Lewis lung carcinoma cells

Table 1. Model parameters of oxamate cytotoxicity against LLC and LLC/R9 cells

Model parameters	LLC	LLC/R9
NR (%)	32.4 ± 3.9	34.9 ± 1.5
NS (%)	71.3 ± 4.8	70.1 ± 3.6
α (mM)	26.6 ± 5.0	16.7 ± 1.8
C_0	4.7 ± 0.4	1.3 ± 0.2
IC ₅₀ (mM)	41.9 ± 7.1	26.9 ± 3.2

two variants of cells. Therefore, it can be assumed that oxamate exerts neither a cytotoxic nor a cytostatic effect on the cells of the resistant subpopulations of both variants, and the recorded difference in sensitivity to oxamate is completely determined by the differences in the properties of the sensitive subpopulations of LLC and LLC/R9 cells.

For a more detailed analysis of the effect of oxamate on some cellular characteristics of LLC and LLC/R9, the cells of each line were planted in 60 mm Petri dishes and incubated for 1 day in a medium with 40 mM oxamate. This concentration of oxamate ensures the partial survival of both LLC and LLC/R9 cells among which oxamate-resistant cells predominate. After a one-day incubation of LLC/R9 cells with oxamate at a concentration of 40 mM (which significantly exceeds the IC₅₀), almost 100% of the surviving cells become resistant (Fig. 1). In the case of LLC, among the survivors of 40 mM

Table 2. Number of viable and dead cells after 24 h incubation of LLC and LLC/R9 with 40 mM oxamate and without oxamate

Incubation time (h)	Oxamate concentration (mM)	LLC		LLC/R9	
		Viable cells ($\times 10^6$)	Dead cells ($\times 10^6$)	Viable cells ($\times 10^6$)	Dead cells ($\times 10^6$)
0	0	0.81 ± 0.08	0.03 ± 0.01	0.72 ± 0.11	0.02 ± 0.003
24	0	2.01 ± 0.05	0.07 ± 0.01	1.52 ± 0.15	0.06 ± 0.001
24	40.0	1.07 ± 0.19	0.19 ± 0.03	0.69 ± 0.03	0.15 ± 0.01

Table 3. Glucose and lactate concentrations in supernatant after 24 h incubation of LLC and LLC/R9 with 40 mM of oxamate and without oxamate

Incubation time (h)	Oxamate concentration (mM)	Concentrations in supernatant (mmol/L)			
		LLC		LLC/R9	
		Glucose	Lactate	Glucose	Lactate
0	0	10.72 ± 0.1	1.69 ± 0.04	10.72 ± 0.1	1.69 ± 0.04
24	0	8.8 ± 0.05 *	5.3 ± 0.06 *	8.2 ± 0.07 *,#	6.2 ± 0.08 *,#
24	40.0	10.58 ± 0.09&	1.9 ± 0.02&	10.63 ± 0.08&	1.98 ± 0.01&

Note: * $p < 0.05$ — significant difference compared to the corresponding indices at the beginning of incubation (incubation time=0); & $p < 0.05$ — significant difference compared to the corresponding indices of cells cultured without oxamate (incubation time = 24 h); # $p < 0.05$ — significant difference compared to the corresponding indices of LLC cells

oxamate exposure (corresponding to the IC_{50}), more than 70% of the cells are oxamate-resistant.

The number of viable and dead LLC and LLC/R9 cells, as well as concentration of glucose and lactate in the incubation medium one day after incubation in a medium without oxamate and with oxamate are presented in Tables 2 and 3.

The calculation of GCR by tumor cells taking into account the change in their number per day of incubation without oxamate showed that this index for LLC/R9 is significantly higher by almost 90% than that for LLC ($18.2 \times 10^{-9} \pm 3.5 \times 10^{-9}$ mmol/cell/day for LLC/R9 and $9.6 \times 10^{-9} \pm 1.4 \times 10^{-9}$ mmol/cell/day for LLC). In proportion to the statistically significant increase in GCR by LLC/R9 cells compared to LLC, the rate of lactate production (LPR) by these cells also increases by more than 80% ($32.6 \times 10^{-9} \pm 6.8 \times 10^{-9}$ mmol/cell/day for LLC/R9 and $18.05 \times 10^{-9} \pm 2.1 \times 10^{-9}$ mmol/cell/day for LLC).

It should be noted that the high intensity of glycolysis in LLC/R9 cells in the absence of oxamate (compared to LLC) does not cause activation of their proliferative activity. The doubling time of LLC cells (in the absence of oxamate) is 18.5 ± 1.8 h and does not statistically differ from the doubling time of LLC/R9 cells, which is 22.3 ± 2.8 h. This is also confirmed by the almost identical distribution of cells of both variants by the phases of the cell cycle (Table 4).

The discrepancy between the rate of glucose uptake by LLC/R9 cells and their proliferative potential means that only a part of the consumed glucose ensures the synthesis of plastic substrates and a part is most likely redirected to the pentose phosphate shunt. This gives a reason to assume the active involvement of glucose in the antioxidant defense system of LLC/R9 cells, which is confirmed by the results of the study of the intracellular level of ROS in tumor cells, the level of which in LLC/R9 cells in the absence of oxamate exposure is significantly lower, by 15.8%, than the corresponding index of LLC cells (Fig. 2).

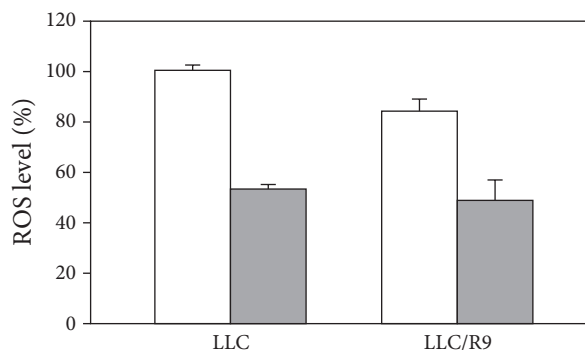


Fig. 2. ROS level in LLC and LLC/R9 cells after 24 h of incubation without oxamate (white columns) and with 40 mM oxamate (grey columns). The data are presented in % of ROS level in LLC cells after 24 h of incubation without oxamate

One-day incubation of cells of both lines with oxamate at a concentration of 40 mM causes a slight, although statistically significant, increase in the number of dead cells, most likely due to the effect on a small part of the cells of the oxamate-sensitive subpopulation.

Analysis of the effect of oxamate on the distribution of LLC cells by cell cycle phases showed a tendency for increasing the percentage of cells in the G_1/G_0 phase and decreasing the percentage of cells in the S phase, however the differences were not statistically significant (Table 4). Unlike LLC, one-day incubation with oxamate of LLC/R9 cells causes a statistically significant

Table 4. Number of cells after 24 h incubation of LLC and LLC/R9 with 40 mM oxamate and without oxamate at different cell cycle phases

Cells	Cell cycle phases	Oxamate concentrations (mM)	
		0	40.0
LLC	G1	45.7 ± 1.3	50.2 ± 2.0
	G2/M	4.6 ± 0.5	$6.8 \pm 0.9^*$
	S	49.8 ± 1.7	44.0 ± 2.6
LLC/R9	G1	42.0 ± 0.3	46.2 ± 1.0
	G2/M	4.7 ± 0.09	$8.6 \pm 0.4^*$
	S	53.2 ± 0.2	$45.1 \pm 1.2^*$

Note: * $p < 0.05$ — significant difference compared to the corresponding indices of cells cultured without oxamate

decrease in the percentage of cells in the S phase by 15% ($p < 0.05$). It should be noted that the redistribution of cells between the G_1/G_0 phase and the S phase of the cell cycle causes in both cell lines a statistically significant increase in the percentage of cells in G_2/M phase (by 47.8% and 83.0% for LLC and LLC/R9, respectively). Usually, an increase in the percentage of cells in the G_2/M phase could be associated either with the activation of DNA repair after the action of DNA-damaging agents (which does not include oxamate), or with an insufficient amount of glucose for the synthesis of cell membrane components, which is necessary to complete the process of cell division and the formation of two daughter cells [18]. Similar results were obtained in the work [19]. It was shown that the inhibition of LDHA in cervical cancer HeLa and SiHa cells by oxamate resulted in a significant reduction in glucose intake and lactate production, as well as the induced G_2/M cell cycle arrest.

The results of our study on the dynamics of changes in the glucose concentration in the incubation medium after a one-day exposure to 40 mM oxamate also evidence in favor of the latter assumption. It is noteworthy that cells of both variants with a dominant content of oxamate-resistant subpopulation practically do not consume glucose. As seen from Table 3 the level of glucose in the incubation medium of LLC and LLC/R9 cells after one-day incubation with oxamate does not decrease. Meanwhile, these cells retain a fairly high proliferative activity, which, unlike oxamate-sensitive cell subpopulations, is not provided by glycolysis, but most likely, by the non-canonical pathway of glutamine metabolism. As a part of this pathway, aspartate derived from glutamine in the Krebs cycle is transferred to the cytoplasm, where it is converted into oxaloacetate with the involvement of aspartate aminotransferase. The latter is metabolized into pyruvate, which provides the cell with anabolic intermediates and increases

the NADPH/NADP ratio, which supports the redox potential of cells [20, 21]. This path significantly slows down the synthesis of plastic substrates, especially phosphorus-containing lipids as the main structural units of membranes, which may explain the increase in the proportion of cells in the G_2/M phase of cell cycle.

The question of the nature of the heterogeneity of lung carcinoma cells in relation to their sensitivity to oxamate remains open. Of particular interest is undoubtedly the oxamate-resistant subpopulation of cells. The dynamic nature of the emergence of such resistance as a form of metabolic adaptability without genetic mutations is quite real. The key issue from our point of view is the reversibility or irreversibility of such resistance. The answer to this question requires further research. It is clear however that the oxamate-resistant cells of both LLC variants have a complete mitochondrial system that allows at least non-canonical glutamine metabolism to be implemented.

Is it possible that these cells possess in fact a higher ability to disseminate. Earlier it was shown that the cells capable of surviving under conditions of anchorage-independent growth exhibit increased mitochondrial biogenesis and activation of genes involved in TCA and the pentose phosphate pathway [22, 23]. Meanwhile, our studies did not reveal significant differences in the volume of the oxamate-resistant subpopulation between high- and low-metastatic variants of Lewis lung carcinoma. The main differences between the two Lewis lung carcinoma variants were related to the oxamate-sensitive (i.e. glycolytic) cell subpopulation. Further research will help to identify cell characteristics of resistant subpopulations that correlate with the metastatic potential of tumor cells.

In conclusion, our study has shown that, regardless of the metastatic potential of LLC cells, they are heterogeneous in relation to the involvement of aerobic glycolysis in the processes of their growth and survival. Only in 2/3 of the

cells of both variants (LLC and LLC/R9) plastic and energy metabolism is based on aerobic glycolysis, the inhibition of which by oxamate leads primarily to blocking proliferative activity (cytostatic effect) against the background of the relatively lower cytotoxic action. At the same time, the intensity of glycolysis in the cells of the low-metastatic variant is significantly higher than in the highly metastatic cells, which explains the higher efficiency of the cytotoxic/cytostatic action of oxamate in the LLC/R9 cells.

Approximately one-third of the cells of both LLC and LLC/R9 variants can survive and proliferate when aerobic glycolysis is inhibited by oxamate. This indicates metabolic reprogramming (either pre-existing or dynamically arising in response to inhibition of glycolysis) of this subpopulation of cells, within which not only the survival of cells but also their proliferative activity is most likely based on glutamine

metabolism. There are reasons to suggest that metabolic reprogramming of a fraction of LLC cells (regardless of their metastatic potential) ensures their growth and survival at later stages of the metastatic cascade (for example, at the stage of dissemination). Such metabolic heterogeneity of metastatically active cells indicates that inhibition of glycolysis (as monotherapy) is insufficient for effective antimetastatic therapy. It also raises doubts that inhibition of glutamine metabolism alone can provide the desired effect [24, 25]. Most likely, only polychemotherapy with the involvement of various inhibitors of metabolic processes that ensure the metabolic plasticity of metastatic cells can be effective [26].

Funding

This work was supported by National Academy of Sciences of Ukraine (0121U113838) and National Research Fund of Ukraine (62/0229).

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Submitted: November 11, 2022

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ВПЛИВ ІНГІБІТОРА ЛАКТАТДЕГІДРОГЕНАЗИ ОКСАМАТУ НА КЛІТИНИ КАРЦИНОМИ ЛЕГЕНІ ЛЬЮїС З РІЗНИМ МЕТАСТАТИЧНИМ ПОТЕНЦІАЛОМ

На сьогоднішній день здатність до метаболічного перепрограмування вважається однією з відмінних рис метастатично активних пухлинних клітин, класичним прикладом якого є аеробний гліколіз. Попри велику кількість досліджень у цьому напрямку, питання про зв'язок між інтенсивністю аеробного гліколізу та метастатичним потенціалом пухлинних клітин залишається майже повністю відкритим. **Метою** даної роботи було дослідити вплив інгібітору лактатдегідрогенази на життєздатність і деякі клітинні характеристики клітин карциноми легені Льюїс з різним метастатичним потенціалом. **Матеріали та методи.** Були використані високометастатичний (LLC) та низькометастатичний (LLC/R9) варіанти клітин карциноми легені Льюїс. Життєздатність пухлинних клітин, концентрацію глюкози та лактату в інкубаційному середовищі, розподіл клітин за фазами клітинного циклу та внутрішньоклітинну продукцію АФК оцінювали через 24 години інкубації пухлинних клітин з 40 мМ оксамату натрію або без нього. **Результати.** Показано, що, незалежно від метастатичного потенціалу, клітини LLC гетерогенні з точки зору як участі аеробного гліколізу в процесах їх росту та виживання, так і чутливості до цитотоксичної/цитостатич-

ної дії інгібітору ЛДГ. 35% клітин кожної клітинної лінії утворюють оксаматрезистентну субпопуляцію, а 65% — оксаматчутливу. Швидкість споживання глюкози клітинами LLC/R9 за відсутності оксамату майже вдвічі вища порівняно з LLC і як наслідок, чутливість цих клітин до цитотоксичної/цитостатичної дії оксамату також значно вища (IC_{50} для клітин LLC/R9 на 35,8% нижча, ніж для клітин LLC, $p < 0,05$). При повному інгібуванні гліколізу оксаматом приблизно одна третина клітин обох варіантів LLC і LLC/R9 здатна виживати і розмножуватися. Це вказує на метаболічне перепрограмування (або існуюче раніше, або динамічне, що виникає у відповідь на інгібування гліколізу) цієї субпопуляції клітин, у межах якої не лише виживання клітин, але й їхня проліферативна активність, найвірогідніше, базується на метаболізмі глутаміну. **Висновки.** Метаболічна гетерогенність метастатично активних клітин свідчить про те, що інгібування аеробного гліколізу у вигляді монотерапії недостатньо для ефективної антиметастатичної терапії. Імовірно, ефективніше залучати декілька інгібіторів метаболічних процесів, що забезпечують метаболічну пластичність метастатичних клітин.

Ключові слова: інгібітор лактатдегідрогенази, оксамат, ракові клітини, антиметастатична терапія.