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ROLE OF TUMOR/ENDOTHELIAL CELL INTERACTIONS IN TUMOR GROWTH AND METASTASIS

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***Background*: It is known that interactions between tumor and endothelial cells have a significant influence on the growth and metastasis of malignant tumors. *Aim:* To study the reciprocal effect of Lewis lung carcinoma (LLC) and endothelial cells on the growth rate of each other upon their co-cultivation *in vitro* and to assess the contribution of such tumor/endothelial cell crosstalk to *in vivo* LLC growth and metastasis. *Materials and Methods:* Two variants of Lewis lung carcinoma cells, high-metastatic (LLC) and low-metastatic (LLC/R9), and murine aorta endothelial cell line (MAEC) were used. Kinetics of tumor cell growth *in vitro* and *in vivo,* electrokinetic properties of tumor cells and their adhesion to endothelial monolayer, and the number of tumor and endothelial viable cells after 1-day contact or non-contact co-cultivation were estimated. *Results:* LLC/R9 had significantly higher growth rate *in vivo* (as opposed to *in vitro*) than LLC. However, the number and volume of lung metastatic lesions in LLC/R9-bearing mice were 4.5-fold ($p < 0.05$) and 3.6-fold lower ($p < 0.05$), respectively, compared to those in LLC-bearing mice. Non-contact co-cultivation of LLC/R9 + MAEC caused more than a 34% ($p < 0.05$) LLC/R9-induced increase in the number of MAEC and a 60% ($p < 0.05$) MAEC-induced increase in the number of LLC/R9 cells as compared to those of corresponding controls (cells cultured alone). In contrast, in the case of LLC + MAEC, both the number of LLC and MAEC cells after their non-contact co-cultivation and cultivation alone did not differ significantly. Contact co-cultivation LLC+MAEC (in contrast to LLC/R9+MAEC) caused more than a 50% ($p < 0.01$) LLC-induced decrease in the number of MAEC and a 50% decrease ($p < 0.05$) MAEC-induced in the number of LLC cells as compared to the corresponding controls. Both tumor cell variants showed a bimodal distribution of cells by ξ-potential, but in the case of LLC there was observed a shift towards high values due to 52% of cells with a surface charge density > 10 C/m^2, while in the case of LLC/R9 such a subpopulation was absent and 19% of cells had a surface charge < 5 C/m^2. The number of LLC cells that adhered to the monolayer of endothelial cells was by 65% ($p < 0.05$) higher than that of LLC/R9 cells. *Conclusion*. Obtained data demonstrated that the tumor/endothelial cell relationships might reflect the features of tumor growth and metastasis of a malignant tumor.**

***Key Words:* tumor-endothelial cell interactions, metastatic potential.**

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Metastasizing of a malignant tumor is a very complex and dynamic cascading process that remains one of the main factors in cancer-related mortality [1–4]. The success of each metastatic cascade, including the induction of tumor angiogenesis, intravasation and entry of tumor cells into the bloodstream, their dissemination, extravasation, and formation of metastases in distant organs as well as metastasis outcome largely depends on the interaction between tumor and endothelial cells, fibroblasts, cells of the immune system and other components of the tumor microenvironment [5–7].

The intercellular communication within tumor occurs directly between cells or via a large variety of bioactive molecules secreted, such as growth factors, cytokines, chemokines, and microRNAs, etc. This secretome, known as the set of proteins expressed by an organism and secreted into the extracellular microenvironment, derives not only from tumor cells but also from other tumor-associated cells and is an important source of key regulators of the tumorigenic process [5, 6]. A special place among intercellular communication within a tumor is occupied by tumor/endothelial interactions, which can not only enhance the vascularization of the malignant tumor but also significantly affect the proliferative characteristics and metastatic potential of malignant cells. And indeed, it is known that tumor cells due to their ability to secrete a large number of proangiogenic and antiangiogenic factors, such as vascular endothelial growth factor (VEGF), proteolytic enzymes, chemoattractants, etc. can activate the proliferation and migration of endothelial cells, enhancing tumor angiogenesis. In turn, endothelial cells can affect the adhesion of tumor cells, and increase their proliferation and invasion [5–11]. The work aimed to study the reciprocal effect of Lewis lung carcinoma (LLC) and endothelial cells on the growth rate of each other upon their co-cultivation in vitro and to assess the contribution of such tumor/endothelial cell crosstalk to in vivo LLC growth and metastasis. High- (LLC) and low-metastatic (LLC/R9) variants of Lewis lung carcinoma cells were used as a tumor model [12, 13].

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Abbreviations used: LLC – wild-type high-metastatic variant of Lewis lung carcinoma; LLC/R9 – low-metastatic variant of Lewis lung carcinoma; MAEC – murine aorta endothelial cells; VEGF – vascular endothelial growth factor.

MATERIALS AND METHODS

Experimental animals and cell cultures. The studies were performed on C57Bl/6 mice

2–2.5 months old, weighing 18.5–21.5 g, bred in the vivarium at the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine. The research on animals was carried out in accordance with the provisions of the General Ethical Principles of Animal Experiments adopted by the First Congress on Bioethics (Kyiv, 2001) and international requirements in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1986).

Tumor and endothelial cell cultures were obtained from the National Bank of Cell Lines and Tumor Strains of IEPOR.

The LLC and LLC/R9 obtained via the multistage experimental progression of LLC *in vivo* towards the development of drug resistance to cisplatin [12] were used as tumor cell models. Murine aorta endothelial cell line (MAEC) [14, 15] was used as an endothelial cell model.

Tumor and endothelial cells were maintained in RPMI-1640 medium supplemented with 10% FBS (Sigma, USA), 2 mM L-glutamine (Sigma, USA), and 40 μg/ml gentamicin at 37 °C in a humidified atmosphere containing 5% CO_2.

Tumor/endothelial interactions were assessed by their mutual effects on proliferation under conditions of contact and non-contact co-cultivation according to [16]. These effects were calculated as the change in the number of carcinoma cells and endothelial cells after 4 h of their preincubation (point "0") and after 24 h of their non-contact and contact co-cultivation. In the case of non-contact co-cultivation, tumor cells ($1.5 \cdot 10^5$ cells/well) were placed on the bottom of a 6-well plate, and endothelial cells ($5 \cdot 10^4$ cells/well) were placed in diffusion chambers with nitrocellulose filters with a pore size of 0.22 μm. In the case of contact co-cultivation, MAEC cells were stained with a vital dye (5(6)-carboxyfluorescein diacetate N-succinimidyl ester (CFSE)) according to the standard procedure [17]. After co-cultivation, the portion of endothelial CFSE-stained cells was estimated by flow cytometry. In all other cases, tumor and endothelial cell numbers were evaluated by direct cell counting in a hemocytometer.

For *in vivo* experiments, LLC and LLC/R9 were grown *in vitro* under standard conditions and inoculated to the mice intramuscularly (10^6 cells in 0.1 ml of Hanks solution per animal).

Evaluation of tumor cell growth kinetics in vitro. LLC and LLC/R9 cells in the exponential growth phase were mechanically removed from the plastic, seeded into 6-cm Petri dishes at a density of $2.5 \cdot 10^5$ cells, and incubated under standard conditions overnight. At the end of the preincubation period, the attached cells were washed with PBS (pH 7.4), then a fresh medium was added and the cells were incubated for 2 days. The number of viable cells after 0, 24, and 48 h was counted in a hemocytometer using trypan blue staining.

Assessment of the growth kinetics of the primary tumor in vivo. LLC and LLC/R9 primary tumor volumes were calculated based on their diameter measured using caliper each 3rd day starting from the 10th day after tumor cell inoculation, by the formula:

$V = 0.52 \cdot d^3$,

where d — diameter of a tumor (mm).

The growth kinetics of LLC and LLC/R9 *in vivo* was analyzed using a modified Weibull's mathematical model:

$$V(t)=V_o \cdot \exp(\alpha \cdot (t-t_o)^{X(t)}) \quad (1)$$

$$X(t)=1-\beta \cdot V(t)^{2/3}, \quad (2)$$

where **V(t)** is the volume of the tumor at time **t**.

$\mathbf{V}_o$ is the initial tumor volume.

α is the tumor growth rate in the exponential phase which depends both on the proliferative activity of tumor cells (doubling time in experiments *in vitro*) and the tumor cell supply with nutrients within a tumor. The latter is determined by the degree of tumor vascularization (its angiogenic potential); the higher the tumor angiogenic potential is, the better tumor is vascularized, and the higher the parameter **α** is. $\mathbf{t}_o$ is the latent growth phase. **X(t)** reflects the effect of neovascularization on tumor growth. **β** reflects the balance between the rate of tumor growth and the rate of vasculature growth within a tumor.

The number of visible lung metastases and their diameter were evaluated according to the conventional method using a binocular microscope and a millimeter scale. Total metastatic volume was calculated according to the formula:

$$V = \sum (n_i \cdot \frac{3.14 \cdot d_i^3}{6}); \; d_i = 0.5; \; 1.0; \; 1.5; \; ...; \quad (3)$$

where $\boldsymbol{n}_i$ — the number of metastases with a diameter $\boldsymbol{d}_i$.

Evaluation of electrophoretic mobility of tumor and endothelial cells was performed according to [18]. Endothelial and tumor cells were seeded in 6 cm Petri dishes at an initial density of $5 \cdot 10^5$ and $3 \cdot 10^5$ cells/dish, respectively, and incubated under standard conditions for 16 h. After that, cells were washed with PBS (pH = 7.6), the fresh nutrient medium was added and incubation was continued for 2 days.

The linear velocity of cells in a constant electric field after their 2-day incubation was measured using an instrument in which cells resuspended in 0.1 M phosphate buffer at pH 7.0 and t = 27 °C moved inside a flat-walled quartz capillary.

The values of the electrokinetic potential (ζ-potential) for each cell were calculated according to the formula:

$$\zeta=14 \cdot U, \text{ mV} \quad (4)$$

where U is the electrophoretic mobility of cells, which was defined as the ratio of the linear velocity (μm/sec) of cell motion in an electric field (V, μm/sec) to the field voltage (E, V/cm).

The surface charge density (q_s) was determined according to the Quincke-Helmholtz equation:

$$q_s = \zeta \cdot \varepsilon_a/\delta, \text{ C/m}^2 \quad (5)$$

where δ is the height of the double electric layer, which is equal to 10^{-10} m, and ε_a is the absolute dielectric permittivity, which is equal to the difference between the electric constant (ε_0) and the dielectric permittivity of water at 37 °C (ε_{H2O}).

Taking into account the heterogeneity of cancer cells, in each experimental study the motility of at least 50 cells was determined. The analysis of the distribution of cancer cells by the value of the surface charge was carried out using nonlinear regression analysis (Statistical package Origin Pro v.9.5).

Evaluation of the adhesive ability of tumor cells to the endothelium. To estimate the adhesive capacity of tumor cells, MAEC cells were seeded in 6-well plates ($1 \cdot 10^5$ cells/well) and incubated until they reached a monolayer. After medium aspiration, the endothelial cell monolayer was washed with PBS, and tumor cells (LLC or LLC/R9) were added to the wells ($1 \cdot 10^6$ cells/well) in the fresh nutrient medium. The cells were incubated for 40 min at room temperature on a shaker with gentle rotation (100 rpm). At the end of the incubation period, the cells were washed three times with PBS. The attached cells were removed from the substrate using a 0.05% trypsin solution and were counted using a hemocytometer.

Mathematical and statistical analysis. The parameters of the mathematical model were estimated from the best fit of the model to experimental data using nonlinear regression analysis (Origin v.9.5). Statistical analysis was performed using descriptive methods and the Mann — Whitney test. Data are presented as M ± SE, where M is the mean value; SE is the standard error of the mean value.

RESULTS

Kinetics of LLC and LLC/R9 growth in vitro and in vivo. A comparative analysis of the growth kinetics of tumor cells showed that the growth rate of LLC cells *in vitro*, at least in the phase of exponential growth, did not exceed that of LLC/R9 cells (Fig. 1). The doubling time of the LLC and LLC/R9 cell populations was 13.3 ± 0.8 h and 14.4 ± 1.2 h, respectively.

At the same time, the growth rate of the LLC/R9 variant *in vivo* was significantly higher than that of the LLC variant, which suggests a significant dependence of LLC/R9 growth on tumor angiogenesis (Fig. 2). The kinetic parameters of primary tumor growth of both variants are presented in Table 1.

Using a mathematical model, we found that although the latent period of growth of the primary tumor in the case of LLC/R9 was 1.5 times ($p < 0.01$) longer than in the case of LLC, the growth rate of the LLC/R9 variant at the exponential phase, which is reflected by the parameter α, exceeded by 90% ($p < 0.001$) the growth rate of the LLC variant (Table 1). Taking into account the same doubling time of LLC and LLC/R9 cells *in vitro*, an almost twofold increase in the LLC/R9 tumor growth rate (compared to LLC) directly reflects its high angiogenic potential, which can provide a high level of nutrients to relatively small volumes of the tumor. Meanwhile, the value of the parameter β in the case of LLC/R9 was almost 65% ($p < 0.01$) higher than in the case of LLC (Fig. 2, Table 1), which is manifested in significant inhibition of the growth of this tumor starting from the 17th day. The high level of such inhibition was caused by much higher LLC/R9 primary tumor volume compared to LLC.

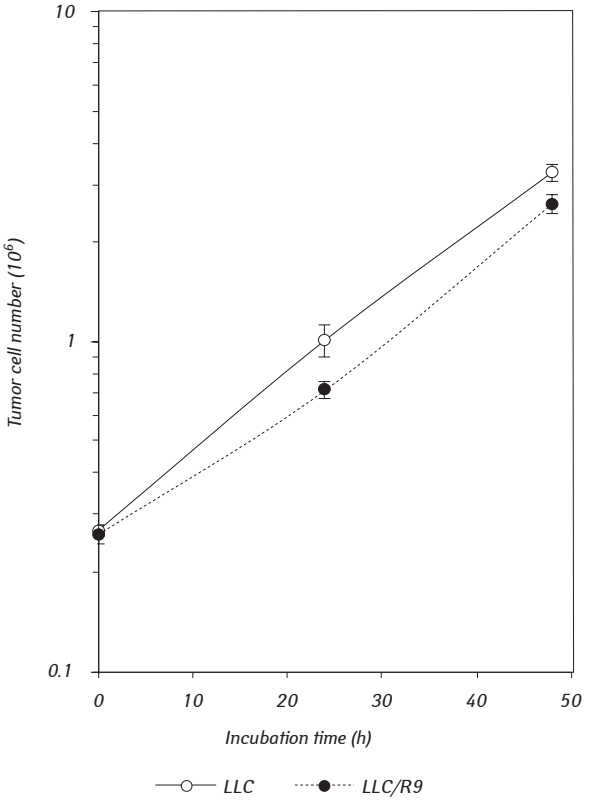

Fig. 1. LLC and LLC/R9 cell growth kinetics *in vitro*

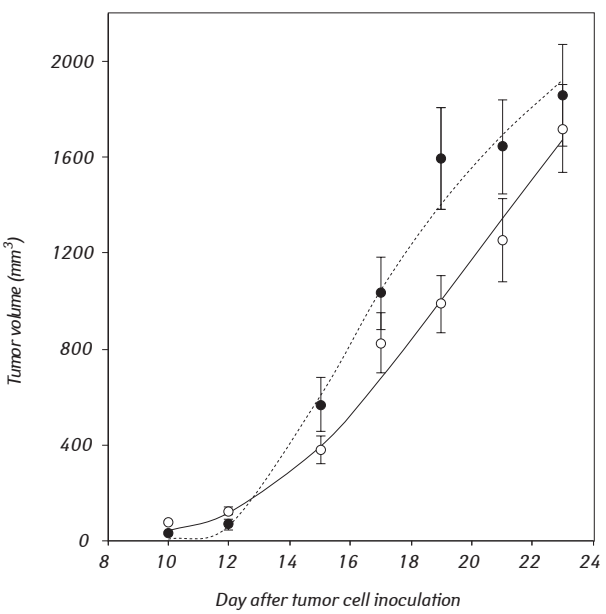

Fig. 2. LLC and LLC/R9 primary tumor growth kinetics *in vivo.* Symbols — experimental data (indicated by black (LLC/R9) and open (LLC) circles); solid (LLC/R9) and dashed (LLC) lines — model approximation obtained from the best fit of the mathematical model (1) to experimental data

Table 1. Kinetic parameters of the primary tumor growth of LLC/R9 and LLC

Parameters of the mathematical model		Units	Tumor variant	
			LLC	LLC/R9
Latent period	t_o	day	6.3 ± 0.9	9.5 ± 0*
Growth rate	α	$(day)^{-1}$	0.76 ± 0.11	1.44 ± 0.04**
Dependence on angiogenesis	β	10^{-3} (mm^{-2})	1.57 ± 0.24	2.57 ± 0.1*

Note: the difference is significant compared to LLC: *$p < 0.01$; **$p < 0.001$.

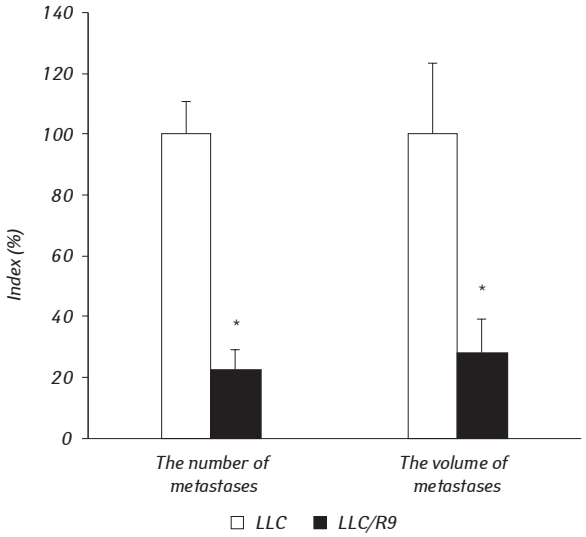

Fig. 3. The number and volume of lung metastatic lesions in LLC- and LLC/R9-bearing mice. Indices are expressed as a percent from the corresponding value in LLC, *$p < 0.05$

The number and volume of metastases in the lungs of mice with transplanted LLC and LLC/R9 on the 23rd day after tumor transplantation are shown in Fig. 3. The number and volume of lung metastatic lesions in LLC/R9 bearing mice were 4.5-fold ($p < 0.05$) and 3.6-fold lower ($p < 0.05$), respectively, compared to those in LLC bearing mice. It should be noted that such significant differences between the metastatic potential of LLC and LLC/R9 are reproduced *in vivo* from experiment to experiment, which confirms the data of previous works [13, 19].

Tumor/endothelial interactions. In the study, the results obtained confirmed the previously described data on the dependence of the mutual effects of endothelial and tumor cells on each other's proliferation and the metastatic potential of tumor cells [16] (Table 2).

As it can be seen in Table 2, the number of MAECs in the case of their 1-day non-contact co-cultivation with LLC and LLC/R9 cells increased by 21% ($p > 0.05$) and more than 34% ($p < 0.05$), respectively, as compared to the corresponding control (without tumor cells). In turn, the number of LLC and LLC/R9 cells in the case of their 1-day non-contact co-cultivation with MAECs did not change and increased by 60% ($p < 0.05$), respectively, as compared to the corresponding control (without endothelial cells).

During 1-day contact co-cultivation, LLC cells in contrast to LLC/R9 cells caused a more than 50% ($p < 0.01$) decrease in the number of MAEC cells. In turn, MAEC cells caused a 50% decrease ($p < 0.05$) in the number of LLC cells compared to the corresponding control and did not significantly affect the number of LLC/R9 cells.

Adhesive and electrokinetic properties of tumor cells. Comparative evaluation of the adhesive properties of tumor cells with different metastatic potential showed that the ability of LLC cells to adhere to a monolayer of endothelial cells was significantly higher than that of LLC/R9 cells. The number of LLC cells that adhered to the monolayer of endothelial cells, even in the case of their short-term direct interaction, was by 65% ($p < 0.05$) higher than that of LLC/R9 cells (Fig. 4).

As it was shown previously, only a part of endothelial cells (in contrast to tumor cells) showed electrophoretic mobility in a constant electric field, moving in the direction of the cathode (i.e., had a positive surface charge), and demonstrating unimodal ζ-potential distribution [20]. All tumor cells were characterized by a negative surface charge and electrophoretic mobility.

The analysis of electrophoretic mobility of both LLC and LLC/R9 showed a bimodal ζ-potential distribution, which indicated the existence of at least two subpopulations of cancer cells that differed by the value of surface charge density.

A comparative analysis of the electrokinetic characteristics of tumor cells revealed that LLC cells are characterized by a shift in ζ-potential distribution toward high values due to the existence of a large subpopulation with an elevated level of surface charge density (Fig. 5). Meanwhile, none of the studied LLC/R9 cells had such a high level of a surface charge. Moreover, 19% of LLC/R9 cells have a low negative surface charge and, as a result, weak electrophoretic mobility.

Table 2. The number of endothelial and tumor cells after their one-day contact and non-contact co-cultivation (presented as a percentage of the number of corresponding cells after their monoculture)

Tumor cell variant	MAEC (%)		Tumor cells (%)	
	alone (control)	MAEC+ tumor cells	alone (control)	Tumor cells + MAEC
	1	2	3	4
Non-contact co-cultivation				
LLC	100 ± 11	121 ± 14	100 ± 9	87 ± 21
LLC/R9	100 ± 3	134 ± 9*	100 ± 11	160 ± 13*
Contact co-cultivation				
LLC	100 ± 10	46 ± 4*	100 ± 5	47 ± 4*
LLC/R9	100 ± 10	100 ± 4	100 ± 3	109 ± 5

Note: the differences are significant compared to the corresponding control: *$p < 0.05$.

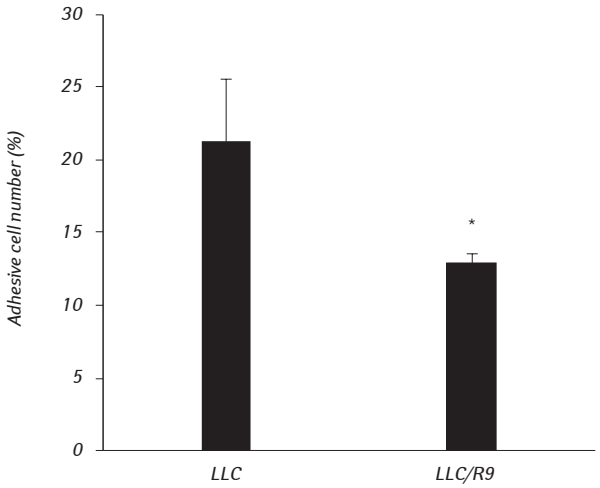

Fig. 4. The number of tumor cells that adhered to the monolayer of endothelial cells after 40 min of their co-cultivation. *p < 0.05 *vs* corresponding value of control

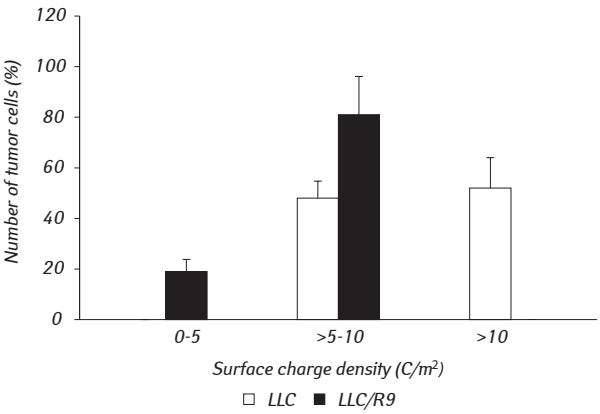

Fig. 5. Distribution of LLC and LLC/R9 cells by surface charge density in the range of low (0–5 C/m^2), medium (> 5–10 C/m^2) and high (> 10 C/m^2) values

DISCUSSION

To elucidate the features of tumor/endothelial cell interactions, two variants of Lewis lung carcinoma, LLC and LLC/R9, the cells of the same genesis, which differ in some biological properties, were used in this study. LLC is the parental strain; LLC/R9 is a variant obtained via experimental *in vivo* progression towards the development of resistance to cisplatin and subsequent adaptation of cells to *in vitro* growth [12]. These two cell variants differ significantly in the level of in *vitro* secretion of the key inducer of tumor angiogenesis, VEGF, as well as the level of circulating VEGF, the volume of the primary tumor, and the degree of metastatic lung disease in animals transplanted with LLC and LLC/R9 cells. LLC and LLC/R9 also demonstrate some differences in the functioning of the prooxidant/antioxidant system, energy metabolism, as well as the sensitivity of these cells to hypoxia, nutrient deficiency, antiangiogenic inhibitors, and modifiers of energy metabolism [13, 21, 22].

From the point of tumor/endothelial cell interactions, the most interesting differences between LLC and LLC/R9 are their angiogenic and metastatic potentials. LLC/R9 variant is characterized by a high angiogenic potential compared to LLC, as evidenced by the high VEGF-secretory activity of these cells *in vitro* and *in vivo*, as well as the high sensitivity of this tumor to inhibitors of tumor angiogenesis [12, 13, 23]. Interestingly, despite its high angiogenic potential, LLC/R9 variant is low-metastatic: the level of metastatic lung disease in LLC/R9-bearing mice is usually almost three times lower ($p < 0.05$) than in LLC-bearing mice [13].

Such differences between LLC and LLC/R9 may be due to both tumor-associated production of various cytokines and the direct interaction of tumor cells with other cells involved in the tumor process, first of all, endothelial cells. In the present study, the model of contactless and contact co-cultivation makes it possible to separate partially the effects of tumor/endothelial cell secretory cross-talking from their direct interaction.

According to the obtained data in the case of non-contact co-cultivation, the interactions of LLC and LLC/R9 cells with endothelial cells, which are mediated exactly through the secretion of soluble factors, led to the stimulation of endothelial cell proliferation. Moreover, the stimulating effect of LLC/R9 cells was almost twice stronger ($p < 0.05$) than the effect of LLC cells. In turn, endothelial cells significantly stimulated LLC/R9 cell proliferation and did not affect LLC cell proliferation.

Such enhancement of LLC/R9 cell proliferation, as well as the pronounced LLC/R9-induced stimulation of endothelial cell proliferation, were primarily associated with their angiogenic potential, in part, their much higher ability to secrete VEGF, able to promote the survival and proliferation of both target cells [24]. The VEGF-mediated stimulating effect of LLC/R9 cells and endothelial cells on each other's proliferation appears to have made a significant contribution to the high (compared to LLC) growth rate of this tumor *in vivo* and the high dependence of its growth on tumor angiogenesis. The latter was confirmed by a high value of angiogenic factor β, which for LLC/R9 was almost by 65% ($p < 0.05$) higher than for LLC.

In contrast, under contact co-culturing, the VEGF-mediated stimulatory effect of LLC/R9 cells on endothelial cell proliferation was fully compensated by inhibition of their growth due to their contact interaction. Moreover, in the case of LLC cells, the inhibitory effect of tumor and endothelial cells on each other's proliferation was much more pronounced than in the case of LLC/R9 cells.

It is known that a significant role in direct tumor/endothelial cell interactions plays tumor cell adhesion capacity toward endothelial cells as well as the electrokinetic characteristics of tumor cells as an integral index of charged molecules on their plasma membrane [25, 26]. Indeed, the pronounced inhibitory effect of LLC cells on the proliferation of endothelial cells during their contact co-cultivation was directly correlated with the high adhesive activity of these cells to a monolayer of endothelial cells, which was by 65% ($p < 0.05$) higher than that of LLC/R9 cells. A significant contribution to these processes was made by the

surface charge density of LLC cells, which in absolute value was significantly ($p < 0.05$) higher than that of LLC/R9 cells.

It is known that anoikis, apoptotic cell death due to loss of contact with the extracellular matrix (ECM) or neighboring cells in the primary tumor, is critical for the survival of tumor cells in the early stages of the metastatic cascade [27–29]. Therefore, the different metastatic potential of LLC and LLC/R9 cells may be due, *inter alia*, to their different sensitivity to anoikis. Indeed, LLC and LLC/R9 cells are characterized by different survival during their cultivation in the conditions of anchorage-independent growth, which simulated their detachment from ECM. In particular, anchorage-independent growth of low-metastatic LLC/R9 cells caused an increase in the number of apoptotic cells by 80% ($p < 0.005$) compared with adhesive growth conditions. On the contrary, anchorage-independent growth of highly metastatic LLC cells caused a decrease in the number of apoptotic cells by 29% ($p < 0.01$) compared with adhesive growth conditions, suggesting the presence of the subpopulation of the cells which are resistant to anoikis. From the point of tumor/endothelial interactions, it is interesting that tumor-associated endothelial cells in contact with tumor cells can protect the latter from anoikis in circulation and facilitate their movement to distant organs [8]. Given the higher adhesion of LLC cells to the endothelial monolayer, such tumor/endothelial interactions may promote the survival of circulating tumor cells and enhance their metastatic potential *in vivo*.

Therefore, the tumor/endothelial cell interactions might reflect the features of tumor growth and metastasis of a malignant tumor. The surface charge density of tumor cells, as well as their adhesive capacity toward endothelial cells directly correlated with their metastatic potential. The high adhesion of LLC cells to endothelial cells and their high negative charge can favor their survival at the different stages of metastatic cascade and provide these cells with high metastatic potential. Meanwhile, cross-talking between LLC/R9 cells and endothelial cells, which provides them with high angiogenic potential, makes a significant contribution to the primary tumor growth, but their low adhesion to endothelial cells stipulates low metastatic potential of these cells.

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РОЛЬ ВЗАЄМОДІЇ МІЖ ПУХЛИННИМИ ТА ЕНДОТЕЛІАЛЬНИМИ КЛІТИНАМИ В РОСТІ ТА МЕТАСТАЗУВАННІ ЗЛОЯКІСНОЇ ПУХЛИНИ

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Відомо, що взаємодія між пухлинними та ендотеліальними клітинами відіграє суттєву роль у рості та метастазуванні злоякісної пухлини. ***Мета:*** Вивчити взаємний вплив клітин карциноми легені Льюїс (LLC) та ендотеліальних клітин при їх спільному культивуванні *in vitro* та оцінити внесок взаємодії між пухлинними та ендотеліальними клітинами в ріст і метастазування LLC *in vivo*. ***Матеріали та методи:*** У роботі було використано два варіанти клітин карциноми легені Льюїс, високо- (LLC) та низькометастатичні (LLC/R9), а також лінію клітин мишачої аорти (MAEC). Оцінювали кінетику росту пухлинних клітин *in vitro* та *in vivo,* електрокінетичні властивості пухлинних клітин та їх адгезію до моношару ендотеліальних клітин, а також кількість пухлинних та ендотеліальних клітин після їх 1-денного контактного та безконтактного співкультивування. ***Результати:*** LLC/R9 відрізнялася від LLC значно вищими темпами росту *in vivo* (на відміну від *in vitro*). Натомість кількість та об'єм легеневих метастазів у мишей з LLC/R9 були в 4,5 раза ($p < 0,05$) та в 3,6 раза нижчими ($p < 0,05$) відповідно ніж такі у мишей з LLC. Безконтактне співкультивування (LLC/R9 + MAEC) призводило до 34% ($p < 0,05$) LLC/R9-індукованого збільшення кількості клітин MAEC та до 60% ($p < 0,05$) MAEC-індукованого збільшення кількості клітин LLC/R9 порівняно з такими у відповідних контрольних зразках (культивування поодинці). Натомість у разі LLC + MAEC як кількість клітин LLC, так і MAEC при їх безконтактному культивуванні суттєво не відрізнялися від таких при їх культивуванні поодинці. Контактне співкультивування LLC + MAEC (на відміну від LLC/R9 + MAEC) обумовлювало більше ніж 50% ($p < 0,01$) LLC-індуковане зменшення кількості клітин MAEC та 50% MAEC-індуковане зменшення ($p < 0,05$) кількості клітин LLC порівняно з відповідними контрольними зразками. Обидва варіанти пухлинних клітин демонстрували бімодальний розподіл клітин за ζ-потенціалом, однак у разі LLC спостерігали зсув у бік високих значень завдяки 52% клітин з щільністю поверхневого заряду >10 Кл/м^2, тоді як у випадку LLC/R9 така субпопуляція була відсутня і 19% клітин мали поверхневий заряд < 5 Кл/м^2. Кількість клітин LLC, які адгезували до моношару ендотеліальних клітин, була на 65% ($p < 0,05$) більшою, ніж у разі клітин LLC/R9. ***Висновки:*** Одержані дані продемонстрували, що особливості взаємодії між пухлинними та ендотеліальними клітинами можуть відображати особливості росту та метастазування злоякісної пухлини.

Ключові слова: пухлинні/ендотеліальні клітинні взаємодії, метастатичний потенціал.