

## DECREASE IN MIGRATION CAPACITY OF LEWIS LUNG CARCINOMA CELLS DURING TUMOR GROWTH AND METASTASIS

*Yu.R. Yakshibaeva\**, *O.N. Pyaskovskaya*, *G.I. Solyanik*, *V.F. Chekhun*  
*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,*  
*National Academy of Sciences, Kyiv 03022, Ukraine*

## СНИЖЕНИЕ МИГРАЦИОННОЙ СПОСОБНОСТИ КЛЕТОК КАРЦИНОМЫ ЛЬЮИС В ПРОЦЕССЕ ЕЕ РОСТА И МЕТАСТАЗИРОВАНИЯ

*Ю.Р. Якшибаева\**, *О.Н. Пясковская*, *Г.И. Соляник*, *В.Ф. Чехун*  
*Институт экспериментальной патологии, онкологии и радиобиологии*  
*им. Р.Е. Кавецкого НАН Украины, Киев, Украина*

Cell migration capacity during Lewis lung carcinoma (LLC) growth and metastasis has been investigated. The inverted correlation between the tumor cell migration capacity and the number of lung metastases has been revealed. It has been shown that during tumor growth LLC cell migration capacity decreased while the number of lung metastases increased.

**Key Words:** Lewis lung carcinoma, cell migration capacity, metastasis.

Была изучена миграционная способность клеток карциномы Льюис на разных этапах ее роста и метастазирования. Выявлена достоверная обратная корреляция между миграционной способностью клеток карциномы Льюис и количеством метастазов в легких. В процессе роста карциномы миграционная способность опухолевых клеток прогрессивно уменьшалась наряду с увеличением количества метастазов в легких.

**Ключевые слова:** карцинома Льюис, миграционная способность, метастазирование.

Metastasis as a stage of tumor progression is based on the main fundamental property of neoplasm – its cellular heterogeneity [1]. The dominance of few metastatic cells within the tumor is one of the early events in tumor progression [2, 3]. Numerous publications concern the growth advantage of metastatic cells; however, this problem is far from being clear yet. From our point of view [4, 5] and in agreement with the data of other authors [6–8] the dominance of metastatic cells is realized mainly through their high migration capacity. Earlier we have shown that high migration capacity of malignant cells give them incontestable growth advantage in comparison with the cell subpopulations that have none (or low) migration capacity [9].

The present research was aimed on the study of the migration capacity of the Lewis lung carcinoma cells during LLC growth and metastasis.

C57Bl/6 mice bred at the vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine (Kyiv, Ukraine) were used. Tumor transplantation was performed by standard method. After trypsin treatment of tumor tissue LLC cells ( $1 \cdot 10^6/0.1$  ml of Hanks solution per animal) were inoculated intramuscularly in the thigh. Metastasis of LLC cells was estimated on 17, 21, 24 and 28 days after tumor transplantation (series I–IV, respectively, 5–7 animals per series). The number of lung metastases was counted visually.

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\*Correspondence: Fax: 263–94–16;  
 E-mail: gis@onconet.kiev.ua

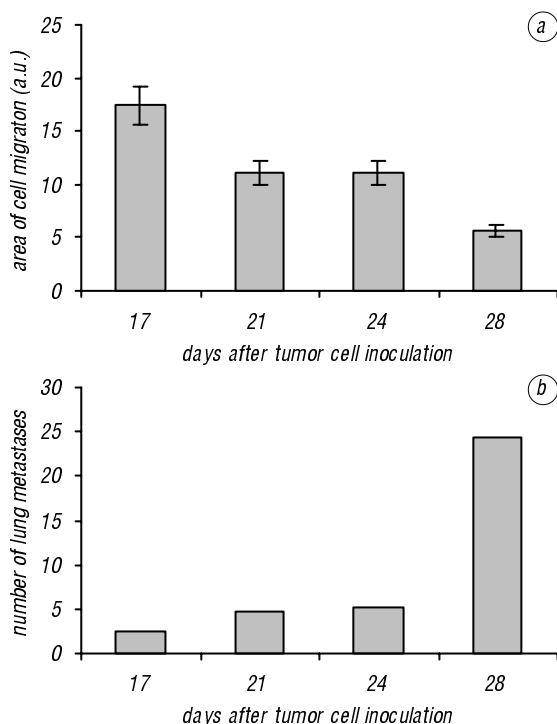
**Abbreviations used:** LLC — Lewis lung carcinoma; TTS — tumor tissue section.

Tumor tissue sections (TTS) were obtained by routine method (> 3 TTS from one tumor). Tumor fragments were triply washed with PBS to remove blood and debris, and cut into peaces with the weight  $2 \pm 0.1$  mg. These peaces were placed on 0.22  $\mu$ m nitrocellulose filters (Millipore, USA). The cultivation of TTS *in vitro* was carried out in 24-well polystyrene plates (Nunc, Denmark) at 37 °C in humidified atmosphere with 5% CO<sub>2</sub> for 48 h in RPMI–1640 medium (Sigma, USA) containing 10% of fetal calf serum (Biomark, Ukraine), 2% L–glutamine (Sigma, USA) and 20  $\mu$ g/ml of gentamicine (Pharmachim, Bulgaria). The 2–day long cultivation was found to be optimal for maximal area of cell migration in the absence of cell proliferation.

Filters were fixed in ethanol–formalin mixture, stained with Karacchi hematoxylin and placed in cedar seed oil. The migration capacity of tumor cells was estimated by light microscopy as area of cell distribution (in arbitrary units — a.u.) around TTS including the area of the last. The round–shaped areas were analyzed only.

Statistical analysis was done using Student's *t*–test.

Our data have shown that during the growth of LLC the cell migration area around TTS was significantly reduced (Fig., a). The area of cell migration on the 21–th day after tumor transplantation was on 36% lower than that on the 17–th day and remained unchanged till the day 24. On the days 21–24 in the majority of cases the migrating cells were round–shaped. At the same time on 24–th day the migration of cells wasn't found in 3 TTS from 28 samples studied in the research. On the 28–th day the cell migration was absent in all TTS that were studied.



**Figure.** Migration capacity (a) and metastasis (b) of LLC cells during tumor growth

Thus, we have revealed the sharp decrease in the migration capacity of tumor cells during LLC growth. Such fact has been confirmed by the decrease of the cell migration area as well as by gradual increase of cell adhesiveness; the last event manifested itself as an increase of cell sprawling on the substrate checked by light microscopy (data not presented). Those results are in agreement with the data that evidenced the reverse relation between the migration rate of malignant cells in autologous matrix and their adhesiveness [10, 11].

During the analysis of migration capacity and metastatic level of LLC cells the high statistically significant inverse correlation ( $r = -0,84$ ) between the area of cell migration and lung metastases number was revealed (Fig., b). Controversy to the progressive reduction of cell migration capacity upon tumor development, the number of lung metastases has been increasing. On the 17-th day the relatively low level of metastasis injuries was observed; metastasis tended to increase lightly on the days 21–24 after tumor transplantation; on the 28-th day the number of metastases sharply raised in the absence of actively migrating cells in the primary tumor.

One should note that the area of tumor cell migration estimated in TTS test reflect in the given moment the migration properties of cells originated from LLC cell subpopulation with the most active migration capacity. The decrease of cell migration area during LLC growth more likely seems to be caused by alteration of composition of tumor cell population during LLC development than by decrease of migration capacity of the cells. On the 21-th day after LLC transplantation the cellular subpopulation which was characterized by

maximal migration capacity and which was detected on the 17-th day in the primary tumor, is nearly absent. On 28-th day the primary tumor completely lacked actively migrating cells.

As far as high migration capacity of tumor cells is mainly attributed to metastatic-active cells [6, 7, 9], the presented data point to progressive reduction of metastasis potential of LLC during tumor growth. Possibly, metastatic-active cell subpopulations after so-called intratumor stage of metastasis characterized by dominance events leave the tumor, penetrate blood vessels and normal tissues and initiate metastasis process in non-tumor environment.

It's necessary to note that in LLC the dominance of metastatic-active cells precedes to metastasis process and occurs on early stages of tumor growth; possibly, on these stages one may expect the increase in migration capacity of LLC cells. However, this hypothesis should be confirmed by experimental data.

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