Sensitivity of Lewis Lung Carcinoma to Cisplatin Undergoes Considerable Variations during Growth and Metastasizing

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Growth and metastasizing of Lewis lung carcinoma were accompanied by complex changes in tumor sensitivity to cisplatin. On day 17 of growth, tumor sensitivity was much lower than immediately after transplantation. Further growth of Lewis lung carcinoma was accompanied by a progressive increase in its sensitivity to cytostatic treatment. The study of migration activity of the tumor and dynamics of metastasizing showed that metastatically active carcinoma cells dominated on day 17 of tumor growth, but then the number of these cells progressively decreased to the 29th day of tumor growth. Growth-related changes in the sensitivity of Lewis lung carcinoma were probably due to complex variations in the number of metastatically active tumor cells with high resistance to cisplatin.

Key Words: *Lewis carcinoma; cisplatin; resistance; metastatic potential; migration activity of tumor cells*

Most cancer patients die from metastases that are formed during migration of individual tumor cells from the primary tumors and colonization of normal organs and tissues. Metastasizing is a form of tumor progression, which is associated with cellular heterogeneity of the neoplasm. It results from genetic and phenotypic instability of tumor cells [1,8,11]. It should be emphasized that metastases appear after several successive selective stages of tumor progression. Predominance of a small number of metastatically active cells is one of the first and necessary stages [3,9,12]. High migration activity of metastatic cells coupled with increased secretion of proteolytic enzymes and reduced adhesiveness provides growth advantages even in the absence of selective pressure and determines predominance of these cells in the heterogeneous tumor [4,10]. Metastatic cells leave the primary tumor and invade normal organs and tissues. A progressive increase in the number of metastatically active tumor cells during growth (phenomenon of predominance [3,9,12]) is followed by a progressive decrease in the count of metastatic cells (tumor depletion of metastatic cells [13]). The data indicate that growth of the metastatic tumor is accompanied by changes in its cellular composition. This is associated with considerable and different variations in the pool of metastatic cells. Changes in the cellular composition of metastatic tumors probably determine differences in tumor sensitivity to antitumor agents at various stages of growth and metastasizing. The present work was designed to test this hypothesis.

MATERIALS AND METHODS

Experiments were performed on cisplatin-resistant Lewis lung carcinoma variant LLC/R_{19} (Cell Line Bank, R. E. Kavetskii Institute of Experimental Pathology, Oncology, and Radiobiology).

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The sensitivity of LLC/R_{19} to cisplatin and metastatic potential of tumor cells were studied on days 0, 13, 17, 23, and 29 of growth. Tumor cells were intramuscularly inoculated (1×10^6) into the femur of C57Bl/6 mice. The animals were obtained from the vivarium of R. E. Kavetskii Institute of Experimental Pathology, Oncology, and Radiobiology. Each series involved at least 3 mice.

The animals were killed under ether anesthesia. The volume of primary tumors and number of lung metastases were determined by standard methods.

The sensitivity of LLC/R_{19} to cisplatin was studied on primary cell culture. The culture was obtained routinely by trypsinization of primary tumor tissue. The cytostatic test was performed in a 96-well plate (Nunclon, 10^5 tumor cells/well). The cells were preincubated in 100 μl RPMI-1640 complete nutrient medium containing 10% fetal bovine serum (Biomark), 2 mM L-glutamine, and 80 μg/ml gentamicin sulfate at 37°C and 5% CO_2 (humid atmosphere) for 3 h. The medium with cisplatin (100 μl, EBEWE) was added. Cell viability was assayed after 24-h incubation with decreasing concentration (0.5-0.0005 mg/ml) of the cytostatic using the MTT colorimetric test [7]. All concentrations were tested in triplicates. IC_{50} served as a marker of the sensitivity of tumor cells to cisplatin. The cytostatic in this concentration caused death of 50% tumor cells. IC_{50} was determined by nonlinear regression analysis after the cytotoxic test.

The metastatic potential of LLC/R_{19} was *in vitro* estimated by migration activity in the biopsy test [13]. Samples of tumor tissue (2.0±0.1 mg, 6-8 samples from each animal) were transferred onto sterile nitrocellulose filters with a pore diameter of 0.22 μ (Millipore). Incubation was performed in 24-well plates (Nunclon) in complete nutrient medium for 2 days under standard conditions. The filters were fixed with an alcohol-formalin mixture and stained with Carazzi's hematoxylin. Migration of cells in the substrate was studied under a light microscope. The area of tumor cell migration over the surface of a filter was expressed in mm^2.

The results were analyzed by descriptive methods, Student's *t* test, and nonlinear regression analysis.

RESULTS

The sensitivity of LLC/R_{19} to cisplatin significantly differed at various stages of growth and metastasizing (Table 1). On day 17 of tumor growth, IC_{50} was 5 times higher compared to that observed during transplantation of LLC/R_{19} ($p<0.01$). Further growth of LLC/R_{19} was accompanied by a progressive decrease in IC_{50}. On days 23 and 29 of tumor growth, IC_{50} was lower than on day 17 (by 5 and 9 times, respectively, $p<0.01$).

These data indicate that the resistance of LLC/R_{19} to cisplatin increased most significantly by the 17th day of tumor growth, but progressively decreased in the follow-up period. Hence, the sensitivity of LLC/R_{19} to cisplatin increased at later stages of observations.

The development of the resistance of malignant tumors to damaging agents, similarly to tumor invasion or metastasizing, is a form of tumor progression reflecting the direction of neoplasm development [6]. The theory of heterogeneity postulates that progression of multicellular tumors results from changes in the cellular composition toward the prevalence of cells with growth advantages. These advantages are induced in individual tumor cells by selective factors (induction mechanism) or result from selection of preexisting cell populations with growth advantages (selection mechanism) [2,5]. However, complex changes in the resistance of LLC/R_{19} cannot be explained by selection or induction mechanism.

The resistance of LLC/R_{19} to cisplatin increased by the 17th day. These results indicate that the cisplatin-resistant population of cells prevailing at this stage of tumor growth and determining low sensitivity to the cytostatic had growth advantages even in the absence of selective pressure with the antitumor agent. The resistance of LLC/R_{19} decreased after 17-day growth, which reflects a decrease in the volume of these cells in the follow-up period. Variations in the sensitivity to cisplatin suggest that cisplatin-resistant

TABLE 1. Biological Characteristics of LLC/R_{19} during Growth and Metastasizing (*M*±*m*)

Period after tumor transplantation, days	Volume of primary tumor, mm^3	Number of metastases	IC_{50}, mg/ml	Area of tumor cell migration, mm^2
Day of transplantation	0	0	0.026±0.002	1.00±0.05
13	4.5±0.5	0	—	1.00±0.02
17	50.1±2.3	4.1±0.5	0.135±0.070	6.73±0.83
23	267.0±12.6	15.6±0.8	0.024±0.001	1.1±0.1
29	410.0±18.5	27.5±2.7	0.015±0.001	2.23±1.30

cells have high migration, but not proliferative capacity. Therefore, these cells belong to the pool of metastatically active cells [4,10]. High migration activity contributes to the prevalence of these cells in the heterogeneous population even in the absence of selective pressure and determines the decrease in cell number in the primary tumor due to invasion of normal organs and tissues [13].

To test this hypothesis, we studied growth-related changes in migration activity of LLC/R_{19} and evaluated the relationship between this characteristic and sensitivity of tumor cells to cisplatin. A significant correlation was revealed between changes in the sensitivity of tumor cells to cisplatin and migration activity (r=0.91, p<0.05). Growth of LLC/R_{19} was accompanied by different changes in migration activity (Table 1). On day 13 of growth, the tumor practically did not exhibit migration activity. The area of cell migration was equal to the area of the bioptate. Migration activity peaked on day 17 of tumor growth. In this period the area of cell migration increased by more than 6 times (p<0.001). Further growth of LLC/R_{19} was accompanied by a significant decrease in migration activity (p<0.001). Metastatically active cells prevailed on day 17 of tumor growth. The volume of this subpopulation progressively decreased to the 29th day of growth. The decrease in the metastatic potential of primary tumors after 17-day growth was accompanied by a progressive increase in the number of lung metastases (Table 1). On days 24 and 29 the number of lung metastases surpassed that observed on day 17 by 5 and 7 times, respectively (p<0.005).

Our results suggest that different sensitivity of Lewis lung carcinoma to cisplatin during growth and metastasizing is determined by complex changes in the number of metastatically active cells. It should be emphasized that metastatically active cells are most resistant to cisplatin.

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