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CHANGES IN VEGF LEVEL AND TUMOR GROWTH CHARACTERISTICS DURING LEWIS LUNG CARCINOMA PROGRESSION TOWARDS CIS-DDP RESISTANCE

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Aim: **To study the relationship between tumor angiogenic potential and its growth and metastasis using Lewis lung carcinoma (LLC) models with different degree of resistance to cis-diamminedichloroplatinum (cis-DDP).** ***Methods:*** **LLC and its two cis-DDP-resistant variants (LLC-9 и LLC-19), were used. For determination of angiogenic potential of LLC, LLC-9 and LLC-19, the level of VEGF production by these tumor cells *in vitro* and the level of circulating VEGF during tumor growth *in vivo* was measured by enzyme-linked immunosorbent assay.** ***Results:*** **Progressive decrease of LLC-9 and LLC-19 sensitivity to action of cis-DDP evidenced *in vitro* (IC_{50} = 0.0077 ± 0.0005 mg/ml and 0.0156 ± 0.0008 mg/ml respectively vs. 0.004 ± 0.0003 mg/ml for LLC, $p < 0.05$) and *in vivo* (index of primary tumor growth inhibition by cis-DDP was 26% and 3% respectively vs. 46%; index of metastasis inhibition — 46% and 11% vs. 65%, $p < 0.05$) was accompanied by the significant changes of tumor angiogenic potential. The level of VEGF production by primary culture of LLC-9 *in vitro* was 1.5 fold higher ($p < 0.05$) than that by primary culture of LLC, whereas there were no differences in the level of VEGF production between LLC-19 and LLC. The level of circulating VEGF drastically increased in the initial phase of LLC-9 and LLC-19 growth *in vivo*, whereas in LLC bearing mice the dynamic changes of VEGF level are characterized by the presence of long-term latent period (t_{lag} = 17.0 ± 0.3 days). In LLC bearing mice the character of changes of circulating VEGF level significantly correlated with the number of metastases ($p < 0.001$) but not with tumor volume; while in LLC-9 bearing mice — with tumor volume ($p < 0.01$) and the number of metastases ($p < 0.05$). Although maximum level of circulating VEGF was significantly ($p < 0.05$) higher in LLC-9 bearing mice than that in LLC bearing mice, maximum number of lung metastases was significantly ($p < 0.05$) lower in LLC-9 bearing mice vs LLC. In contrast to LLC-9, in LLC-19 bearing mice the level of metastatic injury was significantly elevated ($p < 0.05$) and the level of circulating VEGF considerably correlated with both tumor volume ($p < 0.01$) and metastatic index ($p < 0.01$).** ***Conclusion:*** **There is revealed a direct correlation between the level of circulating VEGF and all parameters of tumor progression observed only in the cases of highly resistant tumors, whilst elevation of circulating VEGF level during tumor growth *in vivo* could be considered as a marker of metastasis not dependent on a drug resistance of tumor.**

Key Words: **VEGF, Lewis lung carcinoma, cis-DDP resistance.**

It is known that tumors progression is manifested in three main directions — enhancement of invasion, metastasis, and formation of drug resistance. Metastasis is the main cause of mortality of oncological patients. At the same time drug resistance of tumor results in low efficacy of anticancer agents and continues to be a major and largely unsolved problem of cancer therapy. Despite the availability of approximately 60 different agents for the systemic therapy of cancer, resistance to treatment is developed in the majority of cases.

Presently it is well documented that progression of malignant tumors can't occur without tumor angiogenesis. According to common point of view, higher stages of disease progression are linked to more intense processes of angiogenesis [1, 2]. For example, in a number of solid tumors there has been shown a relationship between clinical stages of cancer and angiogenic potential of tumor, which was evaluated or by the level of circulating VEGF, or/and by the level of its expression in tumor tissue, and/or by the density of vessels in tumor [3–8]. At the same time some researchers have documented the absence of such correlation [9, 10]. The ambiguous results on direct correlation between tumor progression and angiogenesis were obtained also in preclinical and clinical trials on anticancer action of antiangiogenic agents [11]. Such ambiguousness is caused by wide variability of pathophysiologic characteristics of cancer process, complicating the determination of the stage of tumor progression in the conditions of clinical study and making practically impossible the establishment of correlation between tumor progression and angiogenesis. Confirmations of the existence (or absence) of the relations between progression of tumor and its neovascularization could be obtained only in experimental studies based on the use of tumor models of similar genesis, but characterized by different stage of progression.

That's why the aim of the present research was to study the relation between angiogenic potential of tumor and abundance of tumor growth using the model of Lewis lung carcinoma (LLC) with different degree of resistance to cisplatin (cis-DDP).

In the study, parental LLC, and its two variants (LLC-9 и LLC-19), obtained at 9th and 19th stage of experimental LLC progression toward formation of the resistance to cis-DDP *in vivo* were used. The changes of angiogenic potential upon tumor progression were evaluated by the level of production of VEGF by the primary cultures of LLC, LLC-9 and LLC-19 *in vitro*, and also by the dynamics of alteration of circulating VEGF during the growth of tumors *in vivo*.

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Abbreviation used: cis-DDP – cis-diamminedichloroplatinum; LLC – Lewis lung carcinoma.

MATERIALS AND METHODS

Experimental animals and tumor strains. The investigation was carried out in C57Bl/6 female mice weighing 20–23 g aged 2 to 2.5 months from the vivarium of R.E. Kavetsky Institute of experimental pathology, oncology and radiobiology of National Academy of Sciences of Ukraine (IEPOR). All investigations with animals were performed according to rules prepared animal by the Bioethical Committee of IEPOR.

As an experimental tumor model, Lewis lung carcinoma (LLC, National Bank of Cell Lines and Tumor Strains, IEPOR) and its two variants (LLC-9, LLC-19) developed upon 9th and 19th cycle of experimental LLC progression towards the formation of drug resistance to action of cis-DDP *in vivo*, were used. Experimental design of tumor progression was as follows. On the 1st cycle of formation of drug resistance to action of cis-DDP the tumor cell suspension (1×10^6 / 0.1 ml of Haenk's solution) which was obtained from LLC primary tumor tissues by routine procedure of trypsinization was inoculated intramusculary into mice. After tumor cell inoculation mice were divided into experimental and control groups (not less than 8 animals in each). Mice of experimental group recieved the course of chemotherapy with cis-DDP (5 injections at the total dose of 6.0 mg/kg of body weight every second day starting from 7th day after tumor cell inoculation). The tumor relapsed after chemotherapy was used to prepare the tumor cell suspension for inoculation into mice with the aim to carry out the following cycle of tumor progression.

The 2nd and following cycles were identical to the 1st one. A total of 27 cycles of LLC passage in mice and retreatment of tumor bearing mice with cis-DDP were performed. At every cycle of experimental progression the kinetics of primary tumor growth and its metastatic spreading were studied by routine methods.

For evaluation of the sensitivity of LLC, LLC-9 and LLC-19 to action of cis-DDP in vivo indexes of inhibition of tumor growth (GI) and metastasis (MI) were calculated using following formulas:

$$GI = 100 \times \frac{V_0 - V}{V_0} \quad (1)$$

$$MI = 100 \times \frac{N_0 - N}{N_0} \quad (2)$$

where $\boldsymbol{V_0}$, $\boldsymbol{N_0}$ — mean values of tumor volume and number of metastases (correspondingly) in control groups and $\boldsymbol{V}$, $\boldsymbol{N}$ — mean values of tumor volume and number of metastases (correspondingly) in experimental groups. The estimations were carried out on 7th day after chemotherapy completion.

The sensitivity of LLC, LLC-9 and LLC-19 cells to action of cis-DDP in vitro were determined using tumor cells obtained by trypsinization of primary tumor tissues removed on 28–29th day after tumor cell inoculation (primary cultures). IC_{50} (the concentration of cytostatic agent resulted in 50% cellular death) calculated from data obtained in short-term cytotoxic test, was used as a value of sensitivity of tumor cells to action of cis-DDP. For that purpose, 10^5 viable cells/well in 0,1 ml RPMI 1640 medium (Sigma, USA) supplemented with 2 mM glutamine, 10% fetal bovine serum (Sigma, USA) and 40 μg/ml gentamicine were plated in triplicate into 96-well plates (Nunclon, Denmark). After 3 h of pre-incubation of tumor cells at 37 °C in humidified atmosphere containing 5% CO_2, 0.1 ml medium containing cis-DDP in progressively reducing concentration ranging from 0.5 mg/ml to 0.0005 mg/ml was added in each well. After 24 h of tumor cell exposition to cis-DDP or without it (control), the number of viable cells/well was evaluated by MTT-test [12].

The level of VEGF production by primary cultures of LLC, LLC-9 and LLC-19 was determined after their 24 h incubation *in vitro* in RPMI 1640 complete medium under standard conditions. Tumor cells at the density of 1×10^5 viable cells/well in 0.2 ml complete medium were plated into 96-well plate and 24 h later the aliquotes of medium were collected and the number of tumor cells was evaluated by measurement of viable cells/well by MTT-test (expressed in units of extinction). The VEGF concentration in medium samples was measured by enzyme-linked immunosorbent assay (ELISA) mouse VEGF kits (R & D, GBn) according to the manufacturer's protocol. The level of VEGF production was recalculated per cell and represented in relative units according to following formula:

$$VEGF = VEGF_P / N_{ex}$$

where $VEGF_P$ — the total VEGF level in each medium sample (pg/ml), N_{ex} — the number of viable cells/well in units of extinction.

During LLC, LLC-9 and LLC-19 growth the level of circulating VEGF was determined using ELISA mouse VEGF kits (R & D) according to the manufacturer's protocol.

The dynamic changes of the level of circulating VEGF in tumor bearing mice during tumor growth was analyzed by Weibul's model:

$$VEGF = VEGF_0 \times \exp(a \times (t - t_{lag})^b), \quad (3)$$

where the parameter α reflects the maximal level of VEGF production (by tumor cells and/or other cells of host organism); the parameter β reflects the kinetics of VEGF level inhibition as a result of either decreasing of the number of VEGF-secreted cells or the activation of antiangiogenic processes. The parameter t_{lag} expresses a delay in the level of circulating VEGF; $VEGF_0$ is the level of circulating VEGF in intact animals.

The parameters of the model for all variants of LLC were determined from the best fit of mathematical model (3) to experimental data using nonlinear regression analysis.

The growth kinetics of primary tumors as well as total number and volume of lung metastases in tumor bearing mice were evaluated by routine methods. The portion of metastases in vascular phase was calculated considering that diameter of metastases in vascular phase is greater than 1.0 mm.

The statistical analysis of the results was performed using descriptive methods, *t*-test, correlation analysis and nonlinear regressive analysis.

RESULTS AND DISCUSSION

Sensitivity of LLC, LLC-9 and LLC-19 to the action of cis-DDP. As one may see in Table 1, experimental progression of LLC toward formation of the resistance to the action of cis-DDP was accompanied by progressing statistically significant decrease of antitumor and antimetastatic action of cis-DDP. In particular, administration of cis-DDP to mice bearing parental LLC strain resulted in the decrease of the volume of primary tumor and the number of lung metastases by 46 and 65% respectively, compared to control group. The response of LLC-9 and LLC-19 to administration of the drug was weaker compared to that of LLC: for LLC-9, the percent of tumor growth inhibition and the level of metastasis was 26 and 46%, respectively, being 1.8-fold ($p < 0.05$) and 1.4-fold ($p < 0.05$) lower than those in the case of parental LLC. In the case of LLC-19, antitumor and antimetastatic activity of cis-DDP was extremely low: the percent of primary tumor growth inhibition did not exceed 3%, whilst the number of metastases decreased only by 11% compared to respective indexes in LLC-19-bearing mice that did not receive cis-DDP treatment.

Table 1. The sensitivity of LLC, LLC-9 and LLC-19 to action of cis-DDP *in vivo*

Tumor variant	Group of mice	Tumor size (mm)	Number of lung metastases
LLC	control	14.0 ± 0.3	24.3 ± 2.1
	cis-DDP	7.6 ± 0.5*	8.6 ± 0.9*
LLC-9	control	14.5 ± 0.6	20.2 ± 1.4
	cis-DDP	10.7 ± 0.6*	10.8 ± 0.6*
LLC-19	control	13.5 ± 0.5	27.5 ± 2.7
	cis-DDP	11.5 ± 0.8	20.4 ± 1.3

*$p < 0.01$ vs corresponding value in control.

Similar results were obtained upon analysis of the sensitivity of LLC, LLC-9 and LLC-19 cells to the action of cis-DDP *in vitro* (Table 2). The sensitivity of the primary culture of parental LLC to cis-DDP action evaluated by IC_{50} value in short-term cytotoxic test *in vitro*, was equal to 0.004 ± 0.0003 mg/ml, whilst that of the primary culture of LLC-9 was practically twice higher ($p < 0.05$) than that of LLC; for the primary culture of LLC-19, IC_{50} was nearly 4-fold ($p < 0.05$) and 2-fold ($p < 0.05$) higher than those for LLC and LLC-9 cells, respectively.

Table 2. The sensitivity of LLC, LLC-9 and LLC-19 to action of cis-DDP *in vitro*

Tumor variant	IC50 (mg/ml)
LLC	0.004 ± 0.0003
LLC-9	0.0077 ± 0.0005*
LLC-19	0.0156 ± 0.0008*, **

*, **$p < 0.05$ vs LLC and LLC-9 correspondingly.

Production of VEGF by LLC, LLC-9 and LLC-19 cells in vitro. It has been revealed that at different stages of LLC progression, the ability of tumor cells to produce VEGF, evaluated by the level of production of the factor by the primary cultures during the first day of their incubation *in vitro,* varied significantly, and these changes possess non-monotonous character. The formation of drug resistance of LLC was characterized by nearly double ($p < 0.05$) increase of VEGF production by LLC-9 cells with the next decrease of this index toward the level typical for parental LLC cells (Fig. 1).

The level of circulating VEGF in animals bearing LLC, LLC-9 and LLC-19. In the process of progression of LLC toward formation of drug resistance to cis-DDP, significant differences in the dynamics of alteration of circulating VEGF level were observed (Fig. 2, Table 3). In animals bearing LLC-9 and LLC-19 at early stages of tumor growth, the sharp elevation of the level of circulating VEGF has been registered; at the same time the dynamics of alteration of circulating VEGF level in LLC-bearing mice was characterized by significant latent period (t_{lag} = 17.0 ± 0.3 days, Table 3). The increase of VEGF level in blood serum of LLC-bearing animals coincided with exponential phase of tumor growth. In the case of LLC-9 and LLC-19, significant elevation of the level of circulating factor has been observed at the latent phase of tumor growth when the formation of tumor nodule from separate tumor cells occurs. It was interesting to note that the dynamics of alteration of circulating VEGF level (at the periods larger than t_{lag}) in blood serum of LLC-bearing animals practically did not differ from that in LLC-19-bearing mice: there was no statistically significant differences in the parameters α and β (see Table 3).

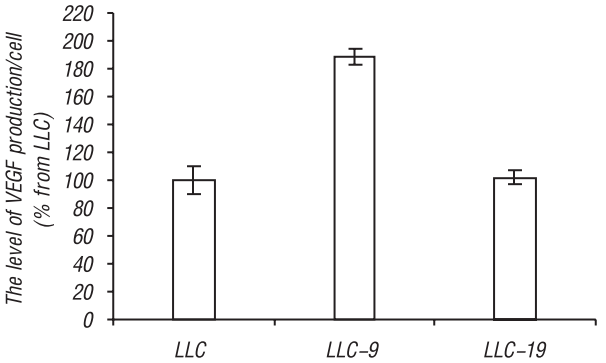

Fig. 1. The level of VEGF production by primary cultures of LLC, LLC-9 and LLC-19 *in vitro* (values are expressed in percentages from corresponding value for LLC)

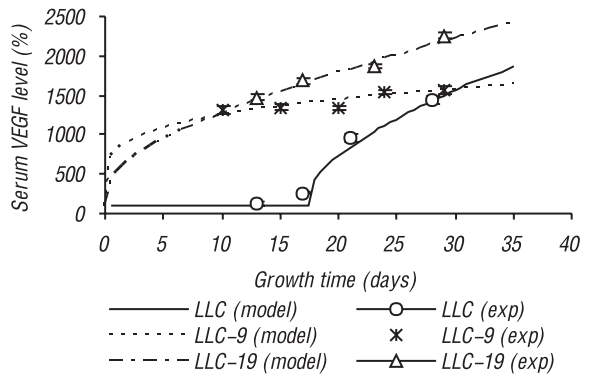

Fig. 2. The dynamic changes of the level of circulating VEGF during LLC, LLC-9 and LLC-19 growth *in vivo* (values are expressed in percentages from the level of VEGF from intact mice).

Table 3. Model parameters of the dynamic changes of circulating VEGF during LLC, LLC-9 and LLC-19 growth *in vivo*

Tumor variant	Model parameters		
	α (days-β)	β	tlag (days)
LLC	1.66 ± 0.15	0.2 ± 0.036	17.0 ± 0.3
LLC-9	2.1 ± 0.07*	0.08 ± 0.01*	0.0 ± 0.0*
LLC-19	1.68 ± 0.02**	0.18 ± 0.004**	0.1 ± 0.1*

*, **$p < 0.01$ vs LLC and LLC-R9 correspondingly.

During LLC-9 growth, the character of alteration of circulating VEGF level was significantly different from these for LLC and LLC-19, evidenced by statistically significant ($p < 0.01$) differences in the parameters α and β (see Table 3) between LLC and LLC-9, and also between LLC and LLC-19. At the initial stages of LLC-9 growth, vigorous (compared to two other LLC variants) elevation of the

level of circulating VEGF, reflected in α value that was by 25% higher in LLC-9 than those for LLC and LLC-19. This result is in good accordance with the data of comparative study of VEGF production by LLC cells with the different degree of drug resistance (see Fig. 1).

Contrary to the dynamics of VEGF level alteration in mice with LLC and LLC-19 characterized by constant progressing elevation of the level of circulating factor in blood serum, vigorous rise of VEGF level in blood serum of LLC-9-bearing mice that was observed at 1st day after tumor cell inoculation, markedly slowed down. Such deceleration was reflected in the parameter β, the value of which was by 60% lower ($p < 0.01$) for LLC-9 group than that for LLC- and LLC-19-bearing animals.

Relation between the level of circulating VEGF and indexes of growth and metastasis of LLC, LLC-9 and LLC-19. Analysis of correlation between the dynamics of change of the level of circulating VEGF and the dynamics of alteration of parameters of spread of tumor process has shown that independently on the

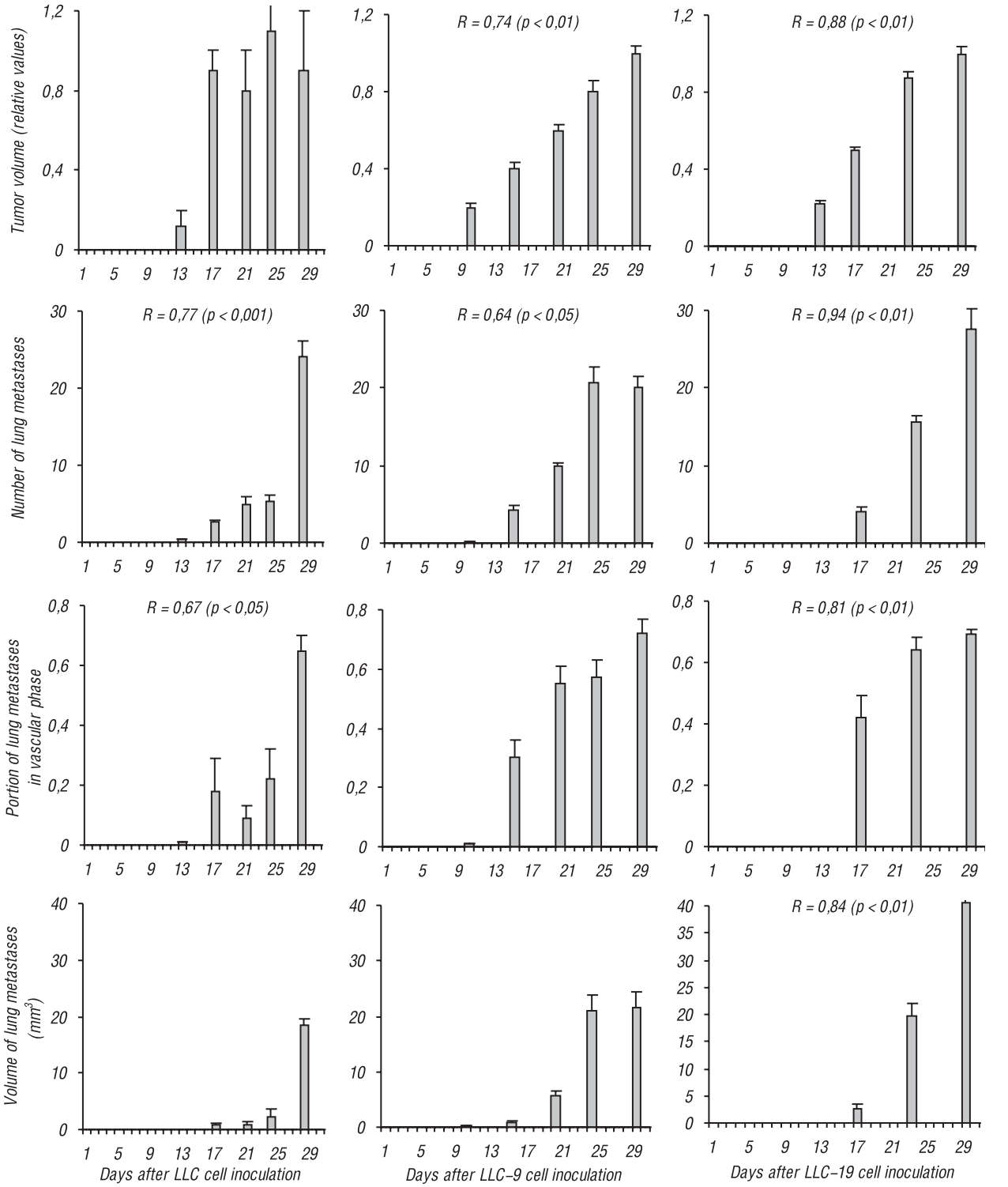

Fig. 3. The correlations between values of tumor growth and metastatic spreading of LLC, LLC-9 LLC-19 *in vivo* and the dynamic changes of the level of circulating VEGF in tumor bearing mice

degree of progression (resistance) of Lewis lung carcinoma, the changes of VEGF levels in blood serum of tumor bearing animals statistically significantly correlated with increase of the number of lung metastases (Fig. 3). The presence of correlation between the dynamics of VEGF alteration and the dynamics of change of other indexes of tumor process significantly depended on the degree of tumor resistance. In particular, during the growth of LLC, statistically significant correlation between VEGF level and the number of metastases in vascular phase has been observed, but VEGF increase did not correlate nor with the increase of the volume of primary tumors, nor with the changes in the volume of lung metastases. Contrary to LLC, for LLC-9 there has been found a distinct correlation between the dynamics of change of the level of circulating VEGF and the dynamics of change of the volume of primary tumors ($R = 0.74$, $p < 0.01$). It is necessary to note that sharp increase of the level of circulating VEGF at early stage of LLC-9 stipulated a higher rate of the primary tumor growth as well as the shift of metastasis for earlier terms compared to that in parental LLC model. However, at the terminal stage of LLC-9 growth the maximal number of lung metastases was by 20% lower ($p < 0.05$), than that in LLC-bearing mice.

High statistically significant correlation between the dynamics of change of VEGF level and the dynamics of change of the studied indexes of tumor spread (volume of primary tumor, the number of metastases, volume of lung metastases, the part of metastases at vascular phase) was registered only for LLC-19 - Lewis lung carcinoma with high degree of resistance to cis-DDP.

So, one may conclude that elevation of the level of circulating VEGF upon tumor growth may serve as a marker of metastasis independently of the degree of tumor's sensitivity to the action of cis-DDP. The direct correlation between the level of circulating VEGF and all studied indexes of the spread of tumor process (including volume of primary tumor and volume of lung metastases) is observed upon the growth of the tumor with high degree of drug resistance.

One should note that LLC progression toward formation of the resistance to cis-DDP was accompanied by non-monotonous alterations of both angiogenic and metastatic potential. There were absent the direct correlations between the level of VEGF production by tumor cells, the level of circulating VEGF at terminal stage of tumor growth and indexes of metastasis of Lewis lung carcinoma with different sensitivity to the action of cis-DDP (Fig. 4).

The most interesting finding of the present research was the decrease of metastatic potential of LLC revealed at early stages of formation of drug resistance (LLC-9). Despite unexpectedness of the obtained result, it may be caused by phenomenon of domination of metastatically active cells [13]. It is known that the process of domination of metastatically active cells in primary tumor is taking place before an appearance of distant metastases. Two biologic properties of cells (high proliferative activity and high migration ability) providing domination of tumor cell subpopulations are known; metastatically active cells are characterized by elevated migration activity upon the background of relatively low proliferative activity [14]. High rates of growth of primary tumors revealed at stage 9 of LLC progression, are decreasing the chance of metastatically active cells to overgrow non-metastatic subpopulations, thus cousing, possibly, the decrease of tumor's metastatic potential. At the same time, an increased level of VEGF production by LLC-9 cells at the background of its decreased metastatic potential is not corresponding to the statements that tumor angiogenesis promotes tumor progression, and that's why should be studied in additional experiments .

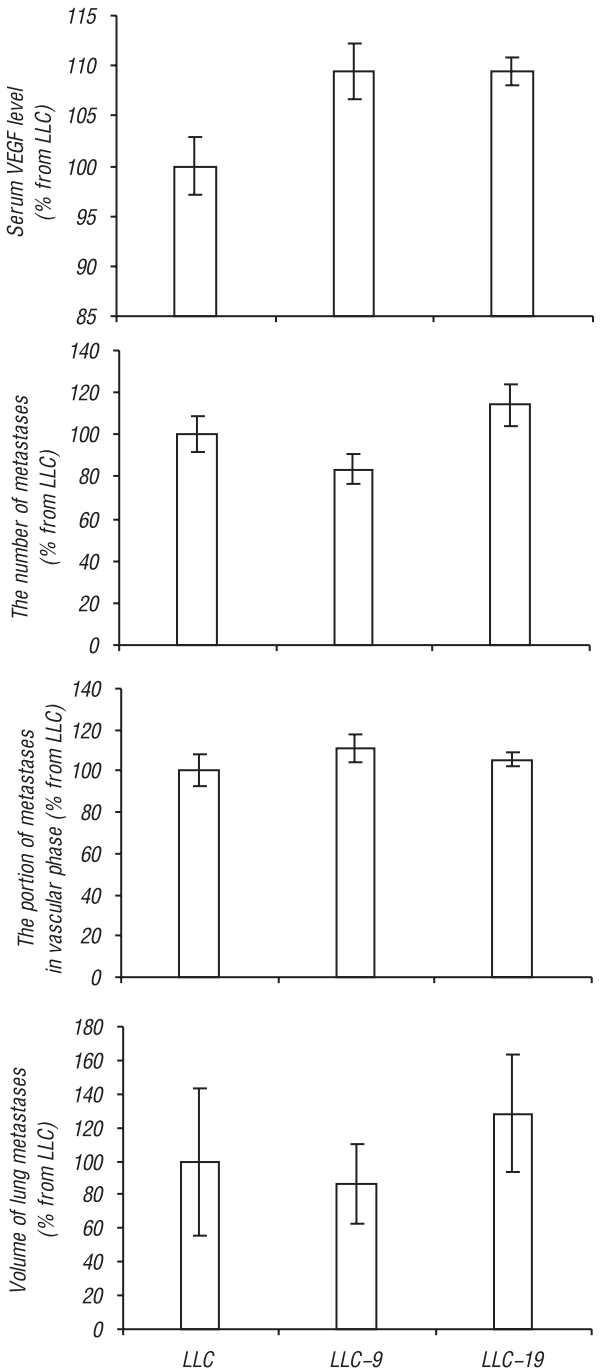

Fig. 4. The level of circulating VEGF and tumor growth characteristics at the 28–29 days of LLC, LLC-9 and LLC-19 growth

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REFERENCES

1. **Hanahan D, Folkman J.** Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996; **86**: 353–64.

2. **Folkman J, Shing Y.** Angiogenesis. J Biol Chem 1999; **267**: 10931–4.

3. **Tanigawa N, Amaya H, Matsumura M, Shimomatsuya T, Horiuchi T, Muraoka R, Iki M.** Extent of tumor vascularization correlates with prognosis and hematogenous metastasis in gastric carcinomas. Cancer Res 1996; **56**: 2671–6.

4. **Ugurel S, Rappl G, Tilgen W, Reinhold U.** Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. J Clin Oncol 2001; **19**: 577–83.

5. **Rasila KK, Burger RA, Smith H, Lee FC, Verschraegen C.** Angiogenesis in gynecological oncology-mechanism of tumor progression and therapeutic targets. Int J Gynecol Cancer 2005; **15**: 710–26.

6. **Zhao HC, Qin R, Chen XX, Sheng X, Wu JF, Wang DB, Chen GH.** Microvessel density is a prognostic marker of human gastric cancer. World J Gastroenterol 2006; **12**: 7598–603.

7. **Demirkesen C, Buyukpinarbasili N, Ramazanoglu R, Oguz O, Mandel NM, Kaner G.** The correlation of angiogenesis with metastasis in primary cutaneous melanoma: a comparative analysis of microvessel density, expression of vascular endothelial growth factor and basic fibroblastic growth factor. Pathology 2006; **38**: 132–7.

8. **Loges S, Clausen H, Reichelt U, Bubenheim M, Erbersdobler A, Schurr P, Yekebas E, Schuch G, Izbicki J, Pantel K, Bokemeyer C, Fiedler W.** Determination of microvessel density by quantitative real-time PCR in esophageal cancer: correlation with histologic methods, angiogenic growth factor expression, and lymph node metastasis. Clin Cancer Res 2007; **13**: 76–80.

9. **Hillen F, van de Winkel A, Creytens D, Vermeulen AH, Griffioen AW.** Proliferating endothelial cells, but not microvessel density, are a prognostic parameter in human cutaneous melanoma. Melanoma Res 2006; **16**: 453–7.

10. **Kurschat P, Eming S, Nashan D, Krieg T, Mauch C.** Early increase in serum levels of the angiogenesis-inhibitor endostatin and of basic fibroblast growth factor in melanoma patients during disease progression. Br J Dermatol 2007; **156**: 653–8.

11. **Coomber BL, Yu JL, Fathers KE, Plumb C, Rak JW.** Angiogenesis and the role of epigenetics in metastasis. Clin Exp Metastasis 2003; **20**: 215–27.

12. **Mosmann T.** Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assayas. J Immunol Methods 1983; **65**: 55–63.

13. **Kerbel RS.** Growth dominance of the metastatic cancer cells: cellular and molecular aspects. Adv Cancer Res 1990; **55**: 87–131.

14. **Kolobov AV, Polezhaev AA, Solyanik GI.** The role of cell motility in metastatic cell dominance phenomenon: analysis by a mathematical model. J Theor Med 2000; **3**: 63–77.

ИЗМЕНЕНИЯ УРОВНЯ VEGF И ПОКАЗАТЕЛЕЙ РОСТА И МЕТАСТАЗИРОВАНИЯ КАРЦИНОМЫ ЛЬЮИС В ПРОЦЕССЕ ЕЕ ПРОГРЕССИИ В НАПРАВЛЕНИИ ФОРМИРОВАНИЯ РЕЗИСТЕНТНОСТИ К CIS-DDP

Цель*: изучить взаимосвязь между ангиогенным потенциалом опухоли и распространенностью опухолевого процесса на модели карциномы Льюис разной степени резистентности к действию cis-DDP.** ***Методы: **в работе использовали карциному легкого Льюис (LLC), а также два ее варианта (LLC-9 и LLC-19), полученные на 9-м и 19-м этапе экспериментальной прогрессии LLC в направлении формирования резистентности к cis-DDP *in vivo*. Ангиогенный потенциал LLC, LLC-9 и LLC-19 оценивали иммуноферментным методом по уровню продукции VEGF первичными культурами *in vitro*, а также по динамике изменений уровня циркулирующего VEGF в процессе роста LLC *in vivo*.** ***Результаты:*** **прогрессивное снижение чувствительности LLC-9 и LLC-19 к действию cis-DDP, подтверждавшееся *in vitro* (IC_{50} = 0,0077 ± 0,0005 мг/мл для LLC-9 и 0,0156 ± 0,0008 мг/мл для LLC-19 против 0,004 ± 0,0003 мг/мл для LLC, $p < 0,05$) и *in vivo* (индекс торможения цитостатиком роста LLC-9 и LLC-19 составлял 26 и 3% соответственно против 46% для LLC; метастазирования — 46 и 11% против 65% для LLC, $p < 0,05$), сопровождалось значительными изменениями ее ангиогенного потенциала. Уровень продукции VEGF *in vitro* первичной культурой LLC-9 был в 1,5 раза выше ($p < 0,05$) по сравнению с таковым первичной культурой LLC, тогда как уровень продукции VEGF первичной культурой LLC-19 практически не отличался от такового для LLC. Уровень циркулирующего VEGF резко повышался уже на ранних этапах роста LLC-9 и LLC-19 *in vivo*, тогда как у мышей с LLC динамика изменения его уровня характеризовалась длительным латентным периодом (t_{lag} = 17,0 ± 0,3 сут). Характер изменений уровня циркулирующего VEGF в процессе роста LLC ($p < 0,001$) коррелировал с количеством метастазов; у животных с LLC-9 — с объемом опухоли ($p < 0,01$) и количеством ($p < 0,05$) метастазов. Несмотря на то, что максимальный уровень циркулирующего VEGF у мышей с LLC-9 значительно ($p < 0,05$) выше, чем у мышей с LLC, максимальное количество метастазов в легких у мышей с LLC-9 достоверно ($p < 0,05$) меньше, чем у мышей с LLC. В отличие от LLC-9, в процессе роста LLC-19, сопровождавшегося повышением уровня метастатического поражения легких ($p < 0,05$), уровень циркулирующего VEGF коррелировал как с объемом опухоли ($p < 0.01$), так и с исследованными показателями метастазирования ($p < 0.01$).** ***Выводы*: прямую корреляционную взаимосвязь между уровнем циркулирующего VEGF и изученными показателями распространенности опухолевого процесса выявляют лишь для опухолей с высокой степенью лекарственной резистентности, тогда как повышение уровня циркулирующего VEGF в процессе роста опухоли можно рассматривать в качестве маркера имеющихся метастазов независимо от степени ее резистентности.**

Ключевые слова: **VEGF, карцинома легких Льюис, резистентность к cis-DDP.**