ISSN 0095-4527, Cytology and Genetics, 2024, Vol. 58, No. 3, pp. 214–224. © Allerton Press, Inc., 2024.
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# Histological Study of the Features of Angiogenesis Lewis Lung Carcinoma under the Influence of Mesenchymal Stem Cells of the Placenta

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Received February 1, 2024; revised February 15, 2024; accepted May 18, 2024

**Abstract**—Morphological features of angiogenesis processes in Lewis lung carcinoma (LLC) regrowth tumors were investigated and analyzed during their independent development and under the influence of cryopreserved mesenchymal stem cells of the human placenta (hP-MSC) under different administration conditions. In LLC tumors, an earlier (day 1) onset of angiogenesis was revealed, with pronounced extramedullary hematopoiesis and the formation of unstructured provascular structures. It was shown that, in general, hP-MSC provides a more structurally perfect angiogenesis in the tumor, with the formation on the 15th day of vascular formations surrounded by a cell wall formed from the cells of the tumor itself. Different morphological forms of neoangiogenesis were detected both in LLC tumors that develop independently, and with different methods of hP-MSC administration—with simultaneous inoculation with tumor cells and with systemic (intravenous) administration. However, more active neoangiogenesis in LLC tumors was noted with systemic intravenous administration of hP-MSC.

**Keywords**: tumor angiogenesis, vascularization, Lewis lung carcinoma (LLC), cryopreserved placental mesenchymal stem cells (hP-MSC)

**DOI:** 10.3103/S0095452724030095

## INTRODUCTION

The formation of the blood supply system in tumor tissues plays an important role in tumor growth and metastasis. Neoangiogenesis in tumors (as well as in embryogenesis and postnatal development) is usually divided into vasculogenesis, the formation of blood vessels de novo, and angiogenesis itself, the formation of new blood vessels from existing vessels.

Angiogenesis is responsible for the remodeling and expansion of the blood network surrounding the tumor and includes two different mechanisms: endothelial sprouting and invaginal/splitting microvascular growth (*intussusceptive growth*), while vasculogenesis involves the differentiation of blood vessels in situ from progenitor cells (Patan, 2004).

Neoangiogenesis in tumors usually depends on several factors produced both by the tumor cells and by their environment. This process includes the activation of several signaling pathways, which lead to the secretion of proangiogenic factors that stimulate the proliferation and migration of endothelial cells, as well as other ways of forming a circulatory channel in tumor tissues. In particular, vascular endothelial growth factor (VEGF) is considered one of the most important factors in vasculogenesis and angiogenesis, the wide range of which is aimed not only at endothelial cells but also at several other cell types. In addition, this factor is represented by a whole family of related proteins (VEGF-A, VEGF-B, VEGF-C), and placental growth factor (PlGF) (Apte et al., 2019). All this largely explains its important and diverse role both in

ensuring physiological vascular homeostasis and in pathogenic effects (tumor growth and other pathologies).

Tumor angiogenesis is a serious problem in cancer treatment. The formation of new blood vessels can promote the spread of tumor cells and reduce the effectiveness of chemotherapy and radiation therapy. In addition, the peculiarities of tumor angiogenesis can lead to the development of abnormal blood vessels with imperfect organization, which leads to impaired circulation and leads to tissue damage and inflammatory processes. Therefore, it is extremely important to understand the features, factors, and mechanisms underlying both the development and inhibition of angiogenesis in tumors. One of the directions of research of such features is the study of the role of mesenchymal stem cells (MSC) in modulating angiogenesis in tumors.

MSC are capable of differentiating into different types of cells and also, depending on the microenvironment, secrete various factors that can have a paracrine effect on the processes of differentiation and functioning of other cells, in particular, on angiogenesis (Tao et al., 2016). They can enhance angiogenesis by releasing several angiogenic growth factors, such as bFGF, VEGF, TGF-β, PDGF, angiopoietin-1, placental growth factor (PGF), IL-6, and monocyte chemotactic protein-1 (MCP-1). On the other hand, MSC can also inhibit angiogenesis by secreting antiangiogenic factors, such as thrombospondins (TSP-1, TSP-2) and tissue inhibitors of metalloproteinases (TIMP1, 4) (Watt et al., 2013).

Now, the complex and ambiguous role of MSC in the pathophysiology of cancer is increasingly recognized due to their ability to limit or promote tumor progression, depending on the conditions, influencing the processes of proliferation/differentiation, intercellular interactions, activating pro- or anti-inflammatory effects, etc. (Slama et al., 2023). In our previous study, different effects of placental MSC on the growth, development, and metastasis of the Lewis carcinoma tumor were demonstrated, depending on the characteristics of the introduction of the MSC cells into the body and, therefore, on the environment, which affects the stem cells themselves, changing their potential (Stepanov et al., 2023).

Analysis of the specific effects of MSC in angiogenesis may provide new insights into the mechanisms of tumor progression and contribute to the development of innovative cancer treatments.

The purpose of the conducted experimental scientific work was to study the influence of mesenchymal stem cells from the human placenta and the method of their introduction on the features of histogenesis in the formation of the microcirculatory channel of the percutaneous Lewis lung carcinoma (LLC).

Lewis lung carcinoma is a tumor that can arise spontaneously as an epidermoid carcinoma in the lungs of C57BL mice. It was discovered by Dr. Margaret Lewis at the Wistar Institute in 1951 and became one of the first transplantable tumors (Bertram and Janik, 1980). It is now a representative model for the study of carcinogenesis under both in vivo and in vitro conditions.

## MATERIALS AND METHODS

**Animals.** Animal studies were conducted at the Institute of Biology and Medicine of Taras Shevchenko National University of Kyiv (Kyiv, Ukraine) on 1-week-old female C57Bl/6 mice with a weight of 1–21 g. Mice were kept in shared cages under standard controlled conditions with a 12-h light/dark cycle and provided with standard rodent chow and water.

**LLC cells.** The LLC strain was kindly provided by the National Bank of Cell Lines and Tumor Strains of the Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology (National Academy of Sciences of Ukraine). LLC cells were maintained in DMEM medium supplemented with 1% FBS, 1% glutamine, and 1% antimycotic antibiotic (Thermo Fisher Scientific, United States) at 37°C in a 95% humidified atmosphere with 5% $CO_2$.

**Production of mesenchymal stromal cells from cryopreserved human placenta (hP-MSC).** Full-term placenta (physiological delivery after a clinically normal pregnancy) was used to obtain hP-MSC, which was obtained from donors under the age of 36 at 39–41 weeks of pregnancy in the Kyiv City Maternity Hospital no. 3 according to written informed consent. The amnion was removed, and a fragment of the chorionic plate and chorionic villi with a weight of 8 g (thickness of 3–7 mm) was cut off with scissors. Placental tissue was then cryopreserved in Hanks' solution (Sigma, St. Louis, MO, United States) containing 5% DMSO (Sigma, St. Louis, MO, United States). Samples were frozen in cryogenic vials in a freezer with a controlled cooling rate and crystal formation (IceCube, Australia). When the temperature reached −14°C, the cooling process in the freezer was stopped, and the samples were transferred into liquid nitrogen (−196°C) for long-term storage. Thawing was carried out in a water bath at +38–40°C until the appearance of a liquid phase in the sample (0°C), and then the sample was gradually washed from DMSO by slowly adding Hanks' solution to the tissue. The thawed tissue was crushed, and the fragments were transferred to culture vials with a ready-made growth medium based on MEM alpha modification (Sigma-Adrich, United States) with the addition of 15% fetal bovine serum (Sigma-Adrich, United States) to obtain cell growth

from the explants. hP-MSC were cultured at 37°C and 5% $CO_2$ during four passages. The medium was changed every 3 days. Cells were characterized by the ability to differentiate in osteogenic, adipogenic, and chondrogenic directions and the expression of surface markers following the minimum ISCT criteria (Dominici, 2006).

**Inoculation of LLC and hP-MSC cells.** To study the effect of hP-MSC on the features of angiogenesis, the animals were divided into three experimental groups ($n = 10$ in each group). Mice were injected intramuscularly (in/m) (into the vastus lateralis muscle) $5 \times 10^5$ LLC cells in 100 μL of physiological solution (Sodium chloride, 9 mg/mL, Yuriia-Pharm LLC (Ukraine). Mice of the second group (LLC+hP-MSC group) were simultaneously injected with $5 \times 10^5$ LLC cells and $5 \times 10^5$ hP-MSC in 100 μL of physiological solution into the vastus lateralis muscle. Mice of the third group (LLC + hP-MSC (iv) group) received an injection of $5 \times 10^5$ of LLC cells in 100 μL of physiological solution into the vastus lateralis muscle, then $5 \times 10^5$ mL hP-MSC were injected intravenously into the tail vein on day 7 of LLC development.

**Histological analysis of samples.** Morphological analysis was performed on the tenth, 15th, and 23rd days of LLC tumor development. The obtained changes in tumor growth and histological features of angiogenesis in experimental mice with LLC + hP-MSC and LLC + hP-MSC (iv) were compared with the results of the control group (animals injected with LLC). After measuring the volume and weight of the tumor, the samples were fixed in neutral formalin and sealed in paraffin according to the standard method; histological sections with a thickness of 5 μm were stained with hematoxylin and eosin (H&E, Alfarus, Ukraine). Sections were analyzed using an Olympus BX41 microscope equipped with an Olympus C-5050 Zoom camera (Olympus Europe GmbH, Japan) and Olympus DP 80 FT 3.2 software (Olympus Imaging Corp., Japan). Quantitative morphometric analysis of vessel density and distribution was assessed on digital images of sections using ImageJ software (NIH, United States). The ratio of the area of the lumen of the vascular structure per 1 $\mu m^2$ tumor tissue in visual fields was calculated.

**Statistical analysis.** Statistical processing of the obtained results was carried out using Statistica 6.0 (StatSoft, United States) and OriginLab (OriginLab Corporation, United States) programs. The normality of data distribution was determined by the Kolmogorov-Smirnov test. Parametric (Student's t-test for two samples) and nonparametric (Mann–Whitney U-test for independent groups) methods were used to assess the reliability of the detected changes, and the difference was considered significant at $p < 0.05$. The obtained results were presented as M ± SD (mean@standard deviation).

## RESULTS AND DISCUSSION

Cells of the LLC line injected into the vastus lateralis muscle of mice infiltrate muscle fibers for 1–3 days, gradually forming a tumor node. On the tenth day, a tumor with a diameter of up to 5 mm was formed, the cells of which already have signs of anaplasia (pronounced polymorphism, anisocytosis, hyperchromicity of the nuclei, and high basophilicity of the cells in general, increased indicators of the nuclear-cytoplasmic ratio of 0.6–0.7) and the mitotic index (5–7 mitotic figures per visual field at magnification ×400) as well as abnormal mitoses and pyknotic nuclei (Fig. 1a).

Nutrition of the tumor tissue at this stage of development is carried out by single vessels existing in the adjacent tissues, around which a primary tumor node is formed. New vascular formations in the peripheral part of the tumor during this period were hardly registered. However, the newly formed vascular structures appeared closer to the central part of the tumor; the area where the formation of new "vessels" was noted composed of approximately 5% of the section area of the tumor node. Localization of vascularization processes in the central part of the tumor node is explained by tissue hypoxia, which is a known trigger factor of this process (Rajendran and Krohn, 2005). At this stage of angiogenesis, the presence of endothelial cells and other components characteristic of the histological structure of the vessel wall was not observed in such structures (Fig. 1a), which indicates that the formation of new vessels does not occur due to the growth of the vascular network already existing in the surrounding tissues but mainly by inducing the formation of new vascular structures (vascularization) involving various molecular and cellular mechanisms. At the same time, such structures were extremely disordered and had the appearance of channels, the walls of which are formed by undifferentiated cells of the tumor. Due to this, there was a pronounced diffusion of erythrocytes in the adjacent areas (Fig. 1a).

When hP-MSC were injected simultaneously with LLC cells directly into muscle tissue (LLC + hP-MSC model) and through hP-MSC injection into the tail vein (LLC + hP-MSC iv model) the histological morphology of the forming tumor was similar to an independent LLC tumor, and pronounced cellular and nuclear polymorphism was noted.

However, with the combined introduction of LLC + hP-MSC cells on the tenth day of development, a characteristic difference was a significantly lower level (by 90%, $p < 0.05$) of the formation of vascular structures, in particular, according to their number and the total area of lumens on tumor sections, in most visual fields, microscopic analysis revealed the absence of provascular structures (Figs. 1b, 1e). Individual small vascular formations with single erythrocytes made up less than 1% of the total visible area. The blood supply of the node occurred, probably,

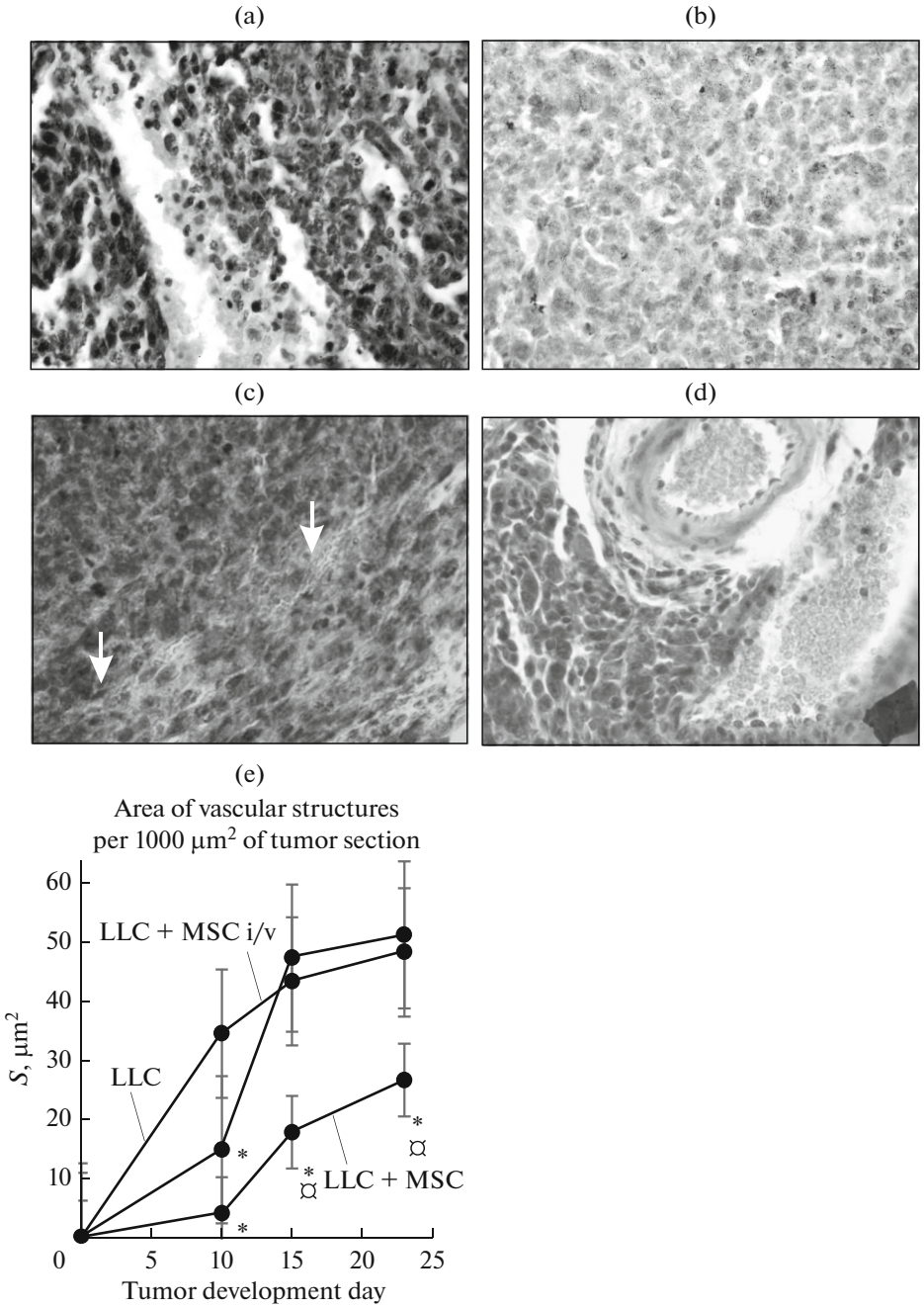


**Fig. 1.** Histological organization of blood supply in LLC tumor models on the tenth day after inoculation. (a) Low-differentiated proangiogenic formations in the LLC tumor; (b) the absence of vascular structures in most of the LLC+hP-MSC tumor node; (c) the beginning of the development of provascular structures (arrows) in the LLC tumor upon systemic administration of hP-MSC (i/v); (d) vessels with a typical histological structure (arteriole and vein) on the periphery of the tumor; H&E, ×400; (e) graph of the dependence of the area of lumens of newly formed vascular structures in the central part of tumors on the day of development in different models of interaction of LLC with hP-MSC. *$p < 0.05$ in comparison with group one (individual development LLC); (¤) $p < 0.05$ in comparison with group three (development of LLC with systemic administration of hP-MSC (i/v).

mainly due to the preexisting and sprouting vessels of the surrounding tissues, which were found in single quantities on the periphery of the LLC + hPMSC tumor but were well expressed (Fig. 1d).

At the same time, in the model of hP-MSC administration via the central bloodstream (LLC + hP-MSC i/v) on the tenth day of development, an intermediate state of vasculogenesis was registered: new erythro-

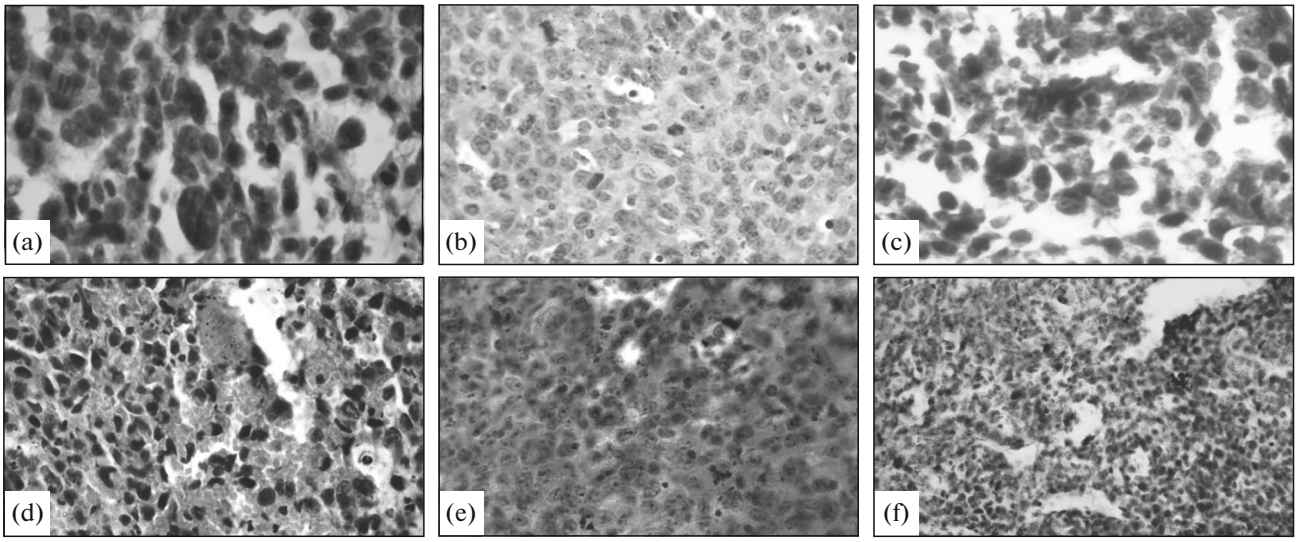


**Fig. 2.** Peculiarities of the histological organization of LLC tumors on the 15th day of development. Increased signs of anaplasia and cellular polymorphism in tumors: (a) LLC model, (b) LLC + hP-MSC; (c) LLC + hP-MSC i/v. Vascularization in the central areas, the 15th day of development: (d) LLC tumor—multiple unstructured provascular formations and extravasal clusters of erythrocytes; (e) LLC + hP-MSC—small newly formed vessels with the formation of a wall from tumor cells; (f) LLC + hP-MSC i/v—intermediate state. H&E staining. ×40.

cyte-containing structures started to form in tumors, but their number and the area of their lumens were significantly lower (by 57%, $p < 0.05$) in comparison with the individual development of the LLC tumor (Figs. 1b, 1e).

The most pronounced processes of tumor growth (including an increase in cellular polymorphism in all models (Figs. 2a, 2b, 2c) and vascularization in the central part of the tumors were noted on the 15th day after the inoculation of cells into the tissue and continued until the 23rd–25th day. However, foci of necrosis gradually appeared at this stage (the 15th day), the growth of which up to the 25th day of tumor development led to its disintegration. In individual LLC tumors, near the areas with necrotic changes, significant foci with extravasal erythrocyte mass were observed; as a result of the imperfect structure of "vessels" (Fig. 2d), such irregular erythrocyte-containing formations occupied most of the LLC tumor area. In both tumor models that interact with hP-MSC, this phenomenon was less expressed, especially hP-MSC in group two (Figs. 2d, 2f).

The phenomenon of erythrocyte hyperplasia we noted should be attributed to the so-called extramedullary hematopoiesis, the process of hematopoiesis outside the bone marrow, with the formation of myeloid and erythroid precursor cells and then differentiated blood cells. Under normal conditions, this process can occasionally occur in the liver and spleen due to certain bone marrow dysfunctions: anemia, myeloproliferative neoplasms, etc. (Zhou et al., 2014). At the same time, extramedullary hematopoiesis can sometimes occur in some solid tumors: carcinomas of the lungs and mammary gland, kidneys, etc. (Bao et al., 2018; Cho and Mandavilli, 2020). In such cases, it is not only a way to support the tumor's angiogenesis but it also plays an important role in disrupting antitumor immunity and reducing the effectiveness of immunotherapy at the expense of myeloid and erythroid precursor cells, formed as a result of tumor-induced extramedullary hematopoiesis (Chen et al., 2023).

From the 15th day, in the angiogenesis of tumors, the beginning of the formation of endothelium-like structures was noted: partial encirclement of the vessel by flattened cells. The cross-section of vessels in this case also most often had a flattened or pronounced spindle-like shape (Fig. 3). However, in the model of individual growth of LLC (group one), they were very rare and were also insufficiently functional in terms of ensuring the role of the vascular wall, as a result of which extravasation of erythrocytes occurred in adjacent areas (Fig. 3a).

In both ways of interaction of LLC with hP-MSC, both with simultaneous introduction (group two) and with the i/v introduction of stem cells (group three) such vascular structures made up the bulk of all registered "vessels," and their wall was more clearly and densely formed (Figs. 3b, 3c), than in a self-growing LLC tumor. Due to this, extravasation of erythrocytes was significantly reduced in these models, and blood supply to the central part of the tumor was better than

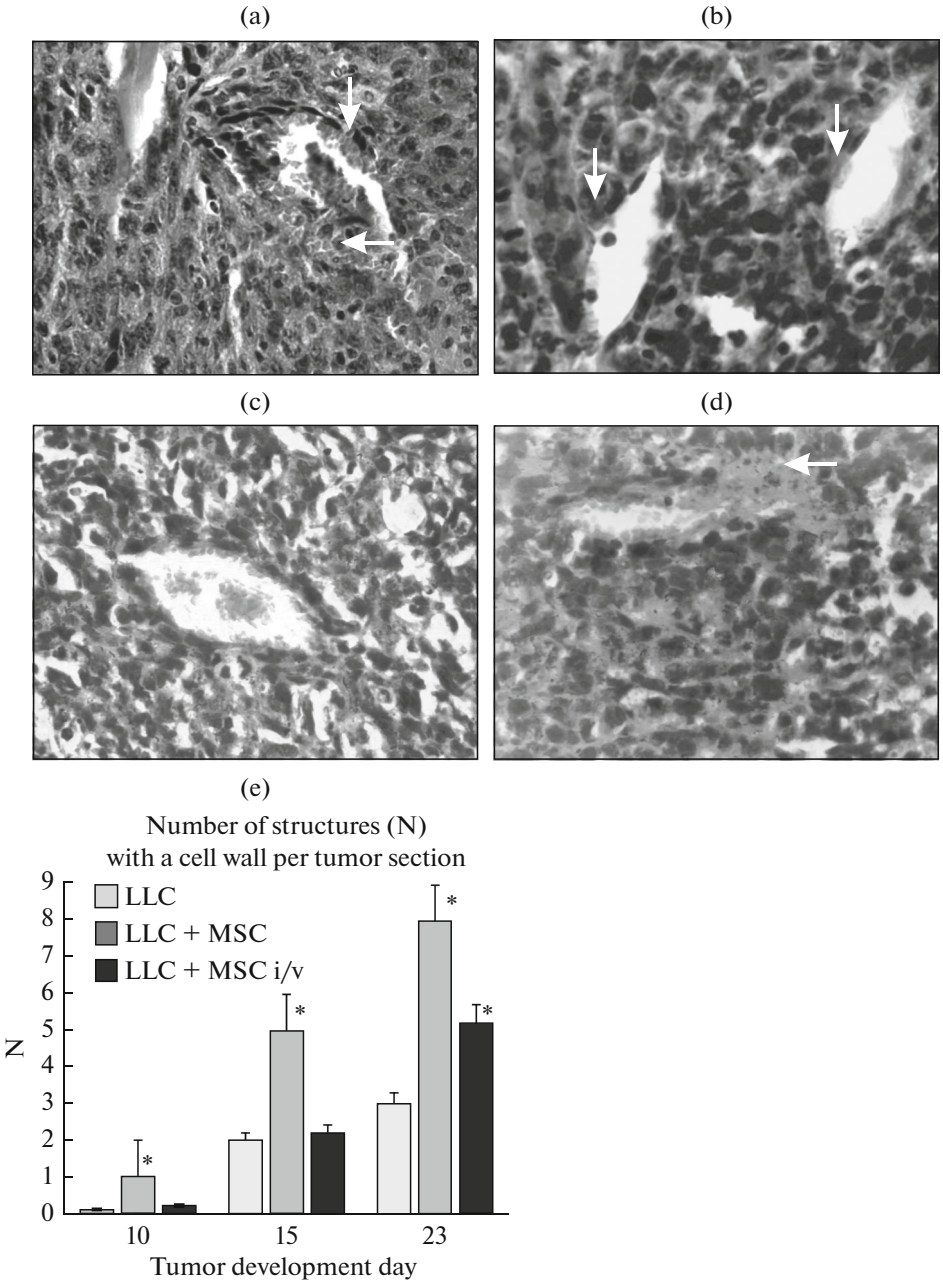


**Fig. 3.** Differentiation of endothelial-like cells in provascular structures, LLC model, day 15, ×400 "Vessel" with the initial formation of a wall of endothelial-like cells (white arrow), partial extravasation of erythrocytes (pink arrow). (a) LLC model, (b) LLC + hP-MSC; (c, d) LLC + hPMSC i/v.; histogram of the frequency of formation of provascular structures with a primary vascular wall. *$p < 0.05$ compared to group one (individual development of LLC).

in self-developed LLC. At the same time (according to a visual microscopic assessment) the vessel was formed mainly by the tumor cells (Figs. 3b, 3c, 3d), which later became flatter, which may indicate their differentiation in the endothelial direction.

In the model of simultaneous introduction of LLC and hP-MSC (group two), such vascular structures in the central part of tumors from the 15th day of development made up the majority of newly formed vessels. With systemic intravenous administration of hP-MSC, their number was lower but exceeded the number of similar vessels in the LLC model (Fig. 3e). According to the structural organization of such vessels, the hP-MSC system administration model also occupies an intermediate position between individual growth of LLC and simultaneous inoculation of LLC+ +hP-MSC; there were blood vessels in it with a formed cell wall (Fig. 3c) but not fully organized, as a

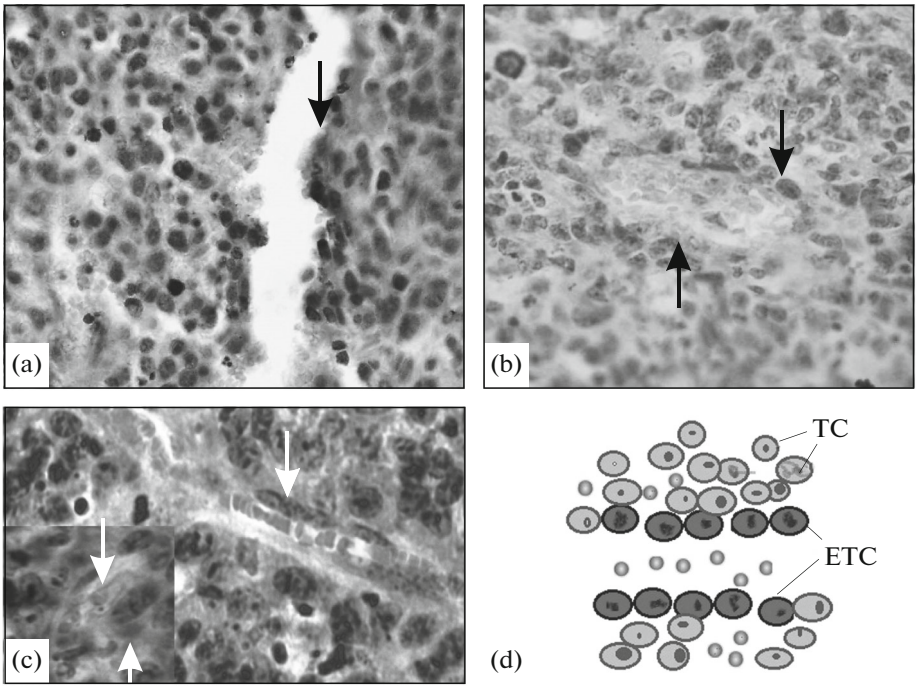


**Fig. 4.** Morphological manifestation of vasculogenic mimicry in models of the development of an inoculated tumor of LLC. The concentration of tumor cells around vascular structures (arrows) to form a vascular wall in the central part of LLC tumors, 15–23 days of development: (a) poorly organized channel structures in LLC (group one); vessels surrounded by tumor cells: (b) LLC + hPMSC (group two); (c) LLC + hP-MSC iv (group three ). H&E, ×400; (d) scheme of vessel formation by the mechanism of vasculogenic mimicry. (TC) Tumor cells; (ETC) endothelial-like tumor cells.

result of which the release of erythrocytes into the tumor tissue was noted (Fig. 3d).

From this moment of the development of LLC tumors (day 15), more pronounced processes of angiogenesis and vascularization were observed, with different mechanisms of tumor blood vessel formation but with certain differences in the various studied models.

Thus, in an inoculated LLC tumor that developed independently, we observed the predominance of the so-called vasculogenic mimicry processes, in which channel-like structures were formed in the tumor tissue for the movement of blood, the walls of which were formed from tumor cells (Folberg and Maniotis, 2004). The same mechanism was observed in the interaction of LLC cells with hP-MSC. However, if these channel-like structures were extremely incomplete in the case of the independent development of LLC, then we already observed morphological signs of differentiation of tumor cells into endothelial-like cells under the influence of hP-MSC, which allowed the forming vessels to hold blood cells effectively and direct their movement (Fig. 4). At the same time, the largest number of such vascular structures was observed in the model of simultaneous inoculation of LLC cells with hP-MSC (Fig. 3e).

At the periphery of tumors of all studied development models, we observed several methods of tumor angiogenesis due to the growth and increase of existing differentiated vessels. At the same time, we again noted a more active process of vessel growth in the presence of hP-MSC.

An increase in the diameter of the vessels surrounding the tumor can be considered as one of the simplest mechanisms of angiogenesis. In individually growing tumors, this process began earlier, already on the tenth day, but later (for 15–23 days) it became more active in tumors exposed to the action of hP-MSC and, especially, with their systemic (intravenous administration) (Fig. 1e); peripheral vessels in this case had a larger lumen diameter, and the number of dilated vessels was also higher. In such vessels, both individual tumor cells and tumor emboli, representing a way of realizing the metastatic potential of this tumor were often noted (Fig. 5).

In addition to increasing the diameter of existing vessels, the increase in blood flow in tumors is also achieved due to an increase in their number. In this case, we observed two methods of angiogenesis on the periphery of tumors.

The first is vascular endothelial sprouting, which can be stimulated by exposure to hypoxia, trauma, or angiogenic growth factors induced by oncogenic signals (Dudley, 2023). VEGF, which has a concentration-dependent activity that stimulates endothelial motility/proliferation, is considered the main among

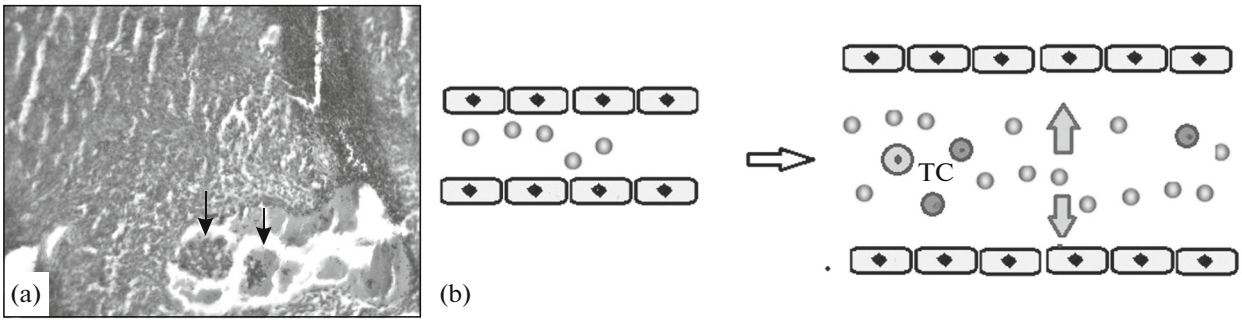


**Fig. 5.** Increase in the circulatory channel in the tumor due to the expansion of peripheral vessels. (a) Dilated vein on the periphery of the LLC+hP-MSC tumor iv (day 23 of development), with metastatic clusters of tumor cells in the lumen (arrow), H&E, ×400; (b) scheme of angiogenesis due to expansion of blood vessels on the periphery of the tumor. (TC) tumor cells.

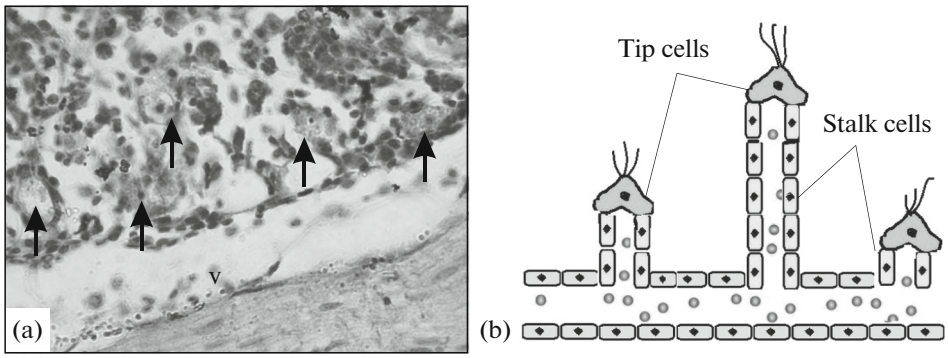


**Fig. 6.** Sprouting angiogenesis. (a) Venous-type vessel (V) and numerous small vessels (arrows) sprouting from existing vessels at the periphery of the tumor. LLC + hPMSC (15th day of development); H&E, ×400. (b) Scheme of sprouting angiogenesis (explanation in the text).

angiogenic factors (Wiszniak and Schwarz 2021). Individual cells were the first to be activated in the endothelium, becoming tip cells during the invasion, followed by stalk cells, which initially form immature and leaky vascular sprouts, which are later stabilized by a new extracellular matrix (ECM) and pericytes (Dudley and Griffioen, 2023). More active sprouting angiogenesis was observed in tumors, the cells of which were inoculated simultaneously with hP-MSC and developed under their direct influence (Fig. 6).

Another way of expansion of the vascular network, which we observed on the periphery of tumors, is intussusceptive or splitting angiogenesis (Mentzer and Konerding, 2014). It is mediated by the formation of an intraluminal column of endothelial cells on the opposite walls of the capillary or by the growth of a membrane from a group of progenitor cells in the middle of the lumen (Fig. 7). The vessel gradually divides along (splits) into two capillaries, thus forming two vessels, and, accordingly, widening the vascular bed (Fig. 7c).

## CONCLUSIONS

Summarizing the results of the morphological analysis of neoangiogenesis in LLC tumors and the influence of placental mesenchymal stem cells on it, we can make the following generalizations. The presence of hP-MSC cells had no significant effect on the morphological signs of neoplasia development and eukaryotic cells in all studied models maintain a high level of malignancy (anaplasia). Vascularization, due to the formation of its vascular structures, occurs mainly in the central part of tumors. The borders of the vessels were formed by the cells of the tumor itself. In the LLC model of the control group, the formation of its own, weakly organized and low-functioning vascular structures occurred earlier (on the tenth day) and was much more quantitatively expressed (their total area was approximately five to eight times, depending on the tumor site, larger compared to tumors in the presence of hPMSC, neoangiogenesis became pronounced in them on the 15th day). In LLC tumors, the process of extramedullary hematopoiesis was significantly expressed: the formation of blood cells outside the bone marrow. Vessels newly formed before day 15

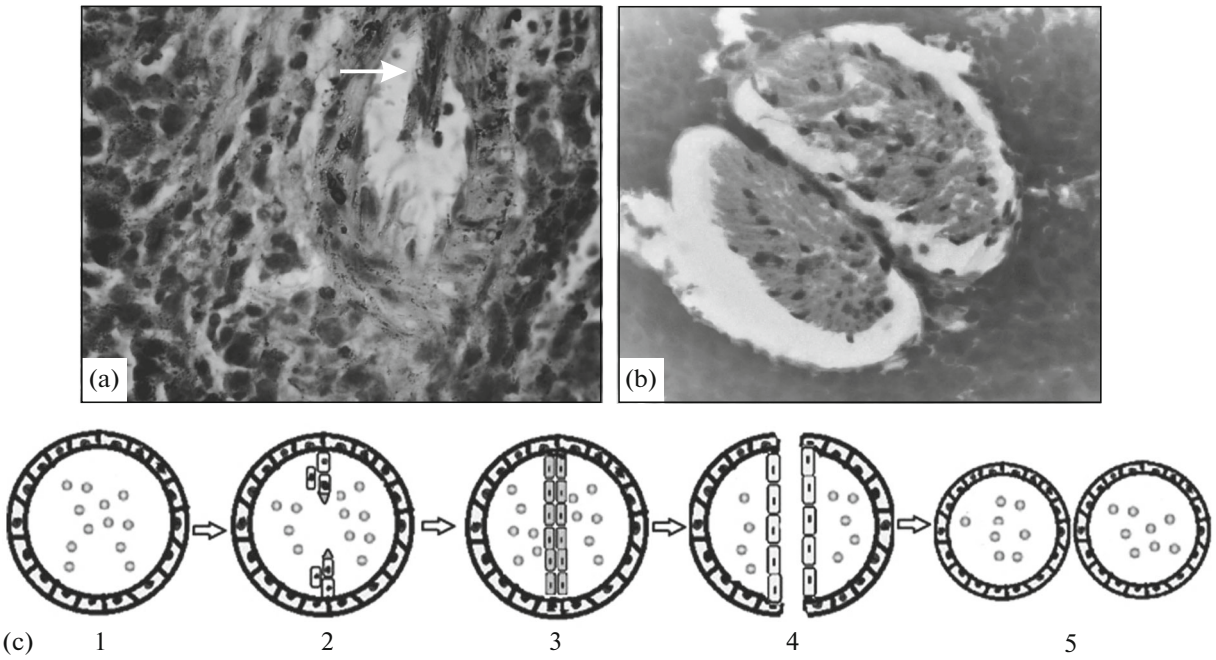


**Fig. 7.** Intussusceptive angiogenesis in tumors (15th day of development). (a) Formation of an endothelial membrane in a peripheral vessel of an individually growing LLC tumor (group one) (arrow); (b) complete formation of a membrane with splitting into two vessels in the tumor with simultaneous injection of hP-MSC cells (LLC + hP-MSC, group two); H&E, ×400. (c) scheme of intussusceptive angiogenesis: (1) initial vessel; (2–3) formation of a membrane (intraluminal "column") from progenitor endothelial cells in the vessel lumen; (4) longitudinal division of a vessel; (5) formation of two new capillaries.

in LLC tumors in the presence of hP-MSC had a more perfect and organized structure, they hardly had extravasation of erythrocytes (exit to the surrounding tissues) compared to the LLC tumor. The wall of such vessels was formed by tumor cells, which morphologically differentiated into endothelium-like cells. This similarity to endotheliocytes was more pronounced under the effect of systemically introduced hP-MSC compared to the effect of simultaneously inoculated stem cells and increases until the last stages of tumor development. During the final stages of development (days 15–23), tumors in the presence of hP-MSC (by both methods of administration) were better provided with differentiated (tissue) vessels sprouting from the surrounding tissues. Both types of vascular structures (differentiated peripheral and tumor) were used for vascular migration of tumor cells. It is obvious that such differences in the time and morphological features of vascular bed formation in various growth models of inoculated LLC tumors primarily depended on the secretory properties of mesenchymal stem cells. The transcriptome of hP-MSC derived from cryopreserved placental tissue was previously analyzed, and data was deposited in NIH depository number PRJNA921 741 (https://www.ncbi.nlm.nih.gov/bioproject/921741). Annotation of metabolic pathways of enriched categories in the KEGG database showed the activation of signaling pathways associated with inflammation in cryopreserved hP-MSC. The following cytokines had an increased level of expression in hP-MSC: *IL34*, *IL6*, *CXCL8*, *EDN1*, *IL17D*, *CSF2*, *IL32*, *IL11*, and *IFNA1* and a lower level of expression was demonstrated for *IL12B* and *IL18.* For chemokine genes, quite heterogeneous expression levels were demonstrated. The following chemokines were activated in hP-MSC: *CXCL12*, *CXCL6*, *CCL2*, and *CXCL1.* Also, in the expression profile of growth factors in hP-MSC, proinflammatory growth factors, such as *THBS1*, *EFNA5*, and *EREG*, were activated. At the same time, an anti-inflammatory factor *HGF* had a lower level of expression in cryocells, unlike *TGFB2* and *MFAP2,* which were overexpressed. Decreased expression of angiogenesis-related genes *ANGPTL1*, *ANGPTL2*, *PDGFB* and *PDGFD* was detected. y-hP-MSC can negatively affect the induction of blood vessel development (Navakauskiene et al., 2023). At the same time, the heterogeneous hP-MSC population contained cells of perivascular origin that expressed ERG, a transcription factor characteristic of endothelial cells (Shablii et al., 2019). Therefore, it is logical to assume that the different activity of angiogenesis and the predominance of its different methods depending on the presence and conditions of hP-MSC administration will depend on the gene expression profile of hP-MSC and the time of exposure to the microenvi-

ronment of tumor cells under different conditions of development.

## FUNDING

The research was carried out within the framework of the topic The Histological Effects of Metabolic and Proliferative Disorders and Methods of Their Correction 2020–2023, no. DR 0120U103507).

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All experiments on animals were conducted following the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (National Research Council (United States) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011) and following the provisions of domestic and international bioethical documents: IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, ETS 123, 1986) and legislative documents of Ukraine on animal experiments: General Ethical Principles of Animal Experiments, approved by the First National Congress on Bioethics (September 20, 2001). The study was approved by the Protocol of Bioethics Committee of Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine no. 36 of February 24, 2024.

## CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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