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Ultrastructural features of primary cancer and its metastases

Características ultraestructurales del cáncer primario y sus metástasis

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SUMMARY

Mortality from metastatic lesions accounts for a significant proportion of oncological practice, and the issues of tumor chemoresistance and micrometastasis formation with the development of early recurrence are also important, which requires research and solution of these problems. **Methods:** *The study used sectional material from the Pathology Department for ultrastructural examination of primary tumors and metastases, employing fixation, embedding, sectioning, staining, and electron microscopy imaging techniques.* **Results***: The study revealed signs of tumor cell diversity within the same population, which may result from active mutagenesis within and the influence of microenvironmental cells from the outside. Metastatic invasion is characterized by increased fibroblast reactivity, but their functional role cannot be interpreted unambiguously.* **Conclusion:** *Angiolysis processes are manifested with angiogenesis processes, during which low-differentiated and functionally inferior vessels are formed, which favors metastatic migration processes. The blood supply to metastatic nodes is also carried out through pseudo vessels (intercellular gaps), which provoke the development of chronic hypoxia. This, in turn, serves as a factor in increasing tumors' resistance to treatment and reversibly enhances angiogenesis.*

Keywords: *Oncology, electron microscopy, morphology, tumor microenvironment, chemoresistance.*

RESUMEN

La mortalidad por lesiones metastásicas representa una proporción significativa de la práctica oncológica, y los problemas de quimiorresistencia tumoral y formación de micrometástasis con el desarrollo de recidiva precoz también son importantes, lo que requiere la investigación y solución de estos problemas. **Métodos:** *Para ello se realizó una evaluación morfológica y comparación de las células tumorales malignas primarias y sus células metastásicas mediante microscopía electrónica.* **Resultados***: El estudio reveló signos de diversidad de células tumorales dentro de una misma población, lo que puede ser consecuencia de una mutagénesis activa en el interior y de la influencia de células*

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microambientales procedentes del exterior. La invasión metastásica se caracteriza por una mayor reactividad de los fibroblastos, pero su papel funcional no puede interpretarse sin ambigüedades. **Conclusiones:** *Los procesos de angiolisis se manifiestan con procesos de angiogénesis, durante los cuales se forman vasos poco diferenciados y funcionalmente inferiores, lo que sirve para favorecer los procesos de migración metastásica. El aporte sanguíneo a los nódulos metastásicos se realiza también a través de pseudovasos (lagunas intercelulares), que provocan el desarrollo de hipoxia crónica, que a su vez sirve como factor de aumento de la resistencia de los tumores al tratamiento, y potencia de forma reversible la angiogénesis.*

Palabras clave: *Oncología, microscopía electrónica, morfología, microambiente tumoral, quimiorresistencia.*

INTRODUCTION

According to the Resolution of the Verkhovna Rada of Ukraine, No. 862-IX on the Recommendations of the Parliamentary Hearings on the Topic: "Organisation of the Fight Against Cancer in Ukraine. Problems and Ways to Solve Them" (1) mortality from malignant tumors ranks second in the overall mortality structure. It is second only to the share of mortality from cardiovascular diseases. In 2020, the National Cancer reported that approximately 113 000 cases of malignant tumors were registered in Ukraine (2). Researchers suggest that this figure may be even higher. Still, the coronavirus pandemic has led to a decrease in the detection and registration of new cases. Another huge obstacle to the provision of quality healthcare services to the population was the war conflict between Russia and Ukraine, so the issue of coverage and protection of the population in the context of cancer care is now acute (3). Globally, the incidence of malignant tumors depends on socioeconomic conditions, preventive measures taken at the national level, early diagnosis, and appropriate treatment in highly specialized institutions.

The war environment has directly disrupted the functioning of the healthcare system, resulting in significant damage to infrastructure, disruptions in supply chains, and mass population displacements. This has severely hindered the ability to provide comprehensive screening, diagnostics, treatment, and long-term monitoring for cancer patients. The ongoing conflict has led to considerable challenges in accessing healthcare services, particularly for individuals affected by cancer. The destruction of medical facilities, displacement of healthcare professionals, and interruptions in supply chains have exacerbated existing difficulties in cancer care delivery. The conflict has also disrupted routine healthcare operations, including cancer screening programs, diagnostic services, and access to essential treatments. As a result, the provision of quality cancer care has become increasingly difficult, underscoring the urgent need for comprehensive strategies to address the challenges posed by the conflict and ensure access to essential healthcare services for cancer patients.

Metastasis, or the spread of cancer cells from the primary source to specific organs, is one of the key characteristics of cancer aggressiveness. The continuous development of research on malignant tumors and the emergence of new paradigms in studying metastases have revealed some features of this process. Naleskina et al. (4) published a study in which they discussed the fact that the progress of a tumor cell on its way to the target site occurs in the course of communication with the cellular microenvironment under the influence of genetic and epigenetic factors, which gives metastatic cells plastic and mobile properties. As such, Pyaskovskaya et al. (5) demonstrated a close relationship between the tumor substrate and endothelial cells with a change in metastatic potential using Lewis lung carcinoma as an example. Establishing the mechanisms of the metastatic process is crucial for finding therapeutic opportunities for successful interventions. Klein (6) focuses on the fact that specialists are currently facing an increase in late recurrence of malignant tumors, as modern antiproliferative treatments are unable to eliminate early metastatic tumors that develop during the clinically latent period. Suhail et al. (7) agree that metastasis is a multidisciplinary problem and that understanding the entire cascade of mechanisms causes specific diagnostic difficulties. This requires the use of various research methods. Still, currently, computerized experimental and computational methods are often unsuccessful due to the inability to systematize all metastasis

studies, as researchers believe each case is unique in its pathophysiology.

For example, one of the ways to study the metastatic process is electron microscopy, which can be used to examine the microstructure and morphology of cells and tissues at the nanometre level. Changes in cell morphology, nucleus structure, and other organelles that can determine the aggressiveness of cells and their increased ability to invade reveal cell morphology features and cell modification during metastasis. Thus, Arismendi-Morillo (8) emphasizes the practical importance of electron microscopy and the detection of mitochondria-associated endoplasmic reticulum, which plays a role in autophagy, cell death, and tumor cell signaling. Orel et al. (9) also used this imaging method to evaluate the effectiveness of therapeutic interventions, with considerable attention also paid to ultramicroscopic changes in mitochondrial morphology. Chechun et al. (10) investigated the heterogeneity of different types of tumors. They concluded that the clonal relationship between primary and metastatic populations of different histogenesis requires further study, as microenvironmental factors, the interaction between malignant tumors and stromal cells, immune cells, non-cellular matrix elements, and the impact on progression and metastasis, as well as signs of heterogeneity, determine the effect on progression and metastasis. Dittner-Moormann et al. (11) indicate that there are currently no unified histological criteria for prescribing and adjusting adjuvant therapy (for example, the treatment of retinoblastoma in pediatric practice), which suggests the lack of a clear view of the treatment tactics considering the microstructural features of the primary tumor and its metastases.

Therefore, the morphological evaluation of primary malignant tumor cells and their comparison with metastatic cells is promising and appropriate. As such, the research aims to determine the characteristics of primary and metastatic tumor cells and to identify possible application points for effective therapeutic measures. These data can be used to create a systematic approach to solving issues related to the management of cancer patients.

MATERIALS AND METHODS

Sectional material from the Pathology Department of Donetsk National Medical University, with a size of no more than 0.5-1 mm^3, was utilized for the ultrastructural examination of the primary tumor and its metastases. The following morphological substrates were included in the study: primary renal cancer with peritoneal metastasis; primary lung cancer with liver, renal, and peritoneal metastasis; primary undifferentiated gastric cancer with liver, pancreatic, liver, and lung metastasis; primary pancreatic cancer with liver metastasis.

Biopsies were previously stored in containers with a fixative solution (10 % formaldehyde). The organ fragments were prefixed with 2.5 % glutaraldehyde solution in phosphate buffer, followed by washing with the same buffer. The tissue was also fixed for two h in a thermos at ice temperature, dissolved in a 2 % solution of osmium tetrachloride in 0.1 M Millonig phosphate buffer at pH 7.36, and after fixation, was washed in the same phosphate buffer. After washing, tissue sections were dehydrated in increasing alcohol concentration, starting with 70 % ethanol in distilled water, followed by dehydration in concentrations with a difference of 10 % alcohol for 10 min each. The exposure was carried out three times in absolute ethanol for 10 min, then 5 min in propylene oxide, and the samples were kept in the catalyzed mixture for 24 h for resignification. The samples were then transferred to polypropylene molds and cured with fresh Araldite at 60°C for 24 h. The impregnated parts were filled with SPI-Pon™ 812 Epoxy Embedding Kit (USA).

The formed blocks were sharpened to a trapezoidal shape and fixed with a glass knife on a block holder to obtain semi-thin sections for staging in an ultramicrotome. Ultrathin sections with a 60-80 nm thickness were prepared using an ultramicrotome UMTP-6M (SELMI, Ukraine) and placed on a copper support grid (Mesh Regular Grid 200). The ultrathin sections were treated with a 1 % aqueous solution of potassium permanganate for 15 min at room temperature

and then rinsed twice with distilled water for 30 seconds. For contrast, a 2 % aqueous solution of uranyl acetate was used, which was treated for 10-15 min at room temperature under minimal light, after which the grids were rinsed once with distilled water. At room temperature, a 1 % lead citrate solution was used for 15-20 min. Samples were viewed and photographed using a TESLA-BS-613 transmission electron microscope (USA) with standard circuits using accelerating voltages of 60 kW and 90 kW and primary magnifications from 1 500 to 20 000. The electron micrographs were obtained using a digital image visualization system SEO-SCAN and the corresponding software. The images of the electron micrographs were interpreted according to the relevant electron microscopy guidelines, and the final diagnosis was made after a comprehensive examination. The data obtained were subjected to descriptive and comparative analysis to clarify the research objective. The images presented in this article were slightly adjusted for brightness and contrast.

Electron microscopy stands out as a preferred methodology for studying cellular structures and processes due to its unparalleled ability to visualize specimens at the nanometer scale, revealing intricate details inaccessible through other imaging techniques. The rationale for choosing electron microscopy over alternative methods, such as light microscopy and other imaging techniques, is multifaceted and stems from its unique advantages. Electron microscopy offers significantly higher resolution compared to light microscopy. While light microscopy can visualize structures at the cellular and subcellular level, it is limited by the diffraction of light, which prevents the observation of details smaller than approximately 200 nanometers. In contrast, electron microscopy utilizes a beam of electrons instead of photons, allowing for much higher-resolution imaging at the nanometer scale. This unprecedented clarity enables researchers to visualize ultrastructural features of cells and tissues, such as organelles, membranes, and macromolecular complexes. Moreover, electron microscopy provides superior contrast and definition, particularly in samples with high electron density. Unlike light microscopy, which relies on staining techniques to enhance contrast, electron microscopy exploits the differential interactions of electrons with specimen components to generate contrast. This inherent contrast mechanism allows for the visualization of subtle structural features and facilitates the identification of cellular organelles and morphological abnormalities.

Ethics approval for this study was obtained from the Institutional Review Board (IRB) of Donetsk National Medical University, ensuring that the research adheres to established ethical guidelines and safeguards the rights and welfare of human participants involved in the study. The IRB reviewed the study protocol, including the methods, procedures, and potential risks to participants. It granted approval based on compliance with ethical principles outlined in international standards and local regulations. Informed consent was obtained from all participants or their legal guardians, providing them with comprehensive information about the study objectives, procedures, potential risks, and benefits. Participants were assured of confidentiality, voluntary participation, and their right to withdraw from the study at any time without consequences. Measures were implemented to protect sensitive personal data and ensure the anonymity of participants in research publications and presentations.

RESULTS

The electron microscopic examination of primary cancer and its metastases revealed that both primary cancer and its metastases have distinct tissue and cellular polymorphism. At the same time, primary cancer is most often characterized by the presence of tumor cell growth zones with varying degrees of atypicality. At the same time, in metastatic nodes, especially at the early stage of their growth, the cellular composition is monomorphic, with distinct signs of malignancy: more pronounced nuclear polymorphism and a relative increase in nuclear polychromatism compared to the primary tumor. Figure 1 shows the presence of a large number of ribosomes in the narrow rim of the cytoplasm, indicating high synthetic activity, unlike in the primary tumor. The nuclei of tumor cells in metastases are distinguished by a more

pronounced uneven distribution of chromatin, often with an increased number of nucleoli in the nucleus. Different degrees of chromatin compactification can be interpreted ambiguously, as it can indicate both the functional activity of the nucleus and the response to nuclear damage. In the case presented in Figure 1, there is a greater tendency to believe that chromatin marginalization with karyopyknosis occurs due to the cell necrosis process.

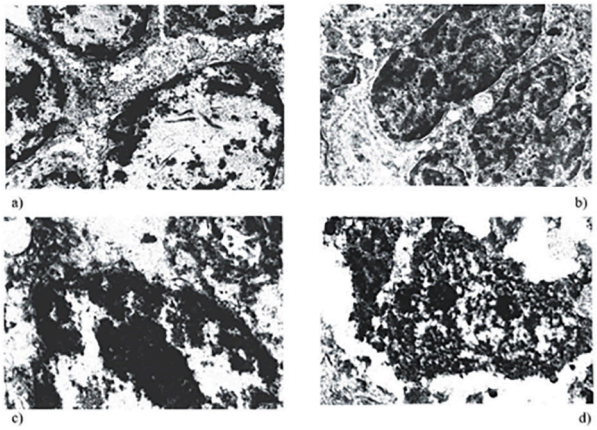

Figure 1. Metastatic progression in lung cancer: electron microscopic analysis.
Note: a) Primary kidney cancer; magnification x6 000; b) Same observation. Peritoneal metastasis; magnification x7 300; c) Tumor cell of kidney metastasis of lung cancer; magnification x10 000; d) Tumor cell of liver metastasis of lung cancer; magnification x7 300. Source: compiled by the authors.

The degree of differentiation in different metastases sometimes varies. For example, in primary undifferentiated lung cancer, one of the metastases was dominated by duct-like structures (Figure 2), which can be interpreted as a manifestation of glandular cell differentiation; in other metastases, tumor cells were arranged chaotically, without signs of tissue differentiation. Given that both cases of metastasis originated from the same source but had different signs of differentiation, this may be evidence of interaction with the tumor microenvironment and the acquisition of new properties in the course of such intercellular communication. It is also worth noting the similarity between metastatic cells of kidney, lung, and liver tumors, presented earlier in the context of uneven distribution of chromatin in the nucleus and its compacting, which gives them signs of similarity, regardless of the type of primary tumor.

In metastatic tumor cells, nuclei with more curved contours and deep invaginations predominate, and the cytoplasm contains a large number of ribosomes with the formation of foci of compaction near the endoplasmic reticulum, as a sign of active protein synthesis processes. Figure 3 shows two tumor cells (although chromatin

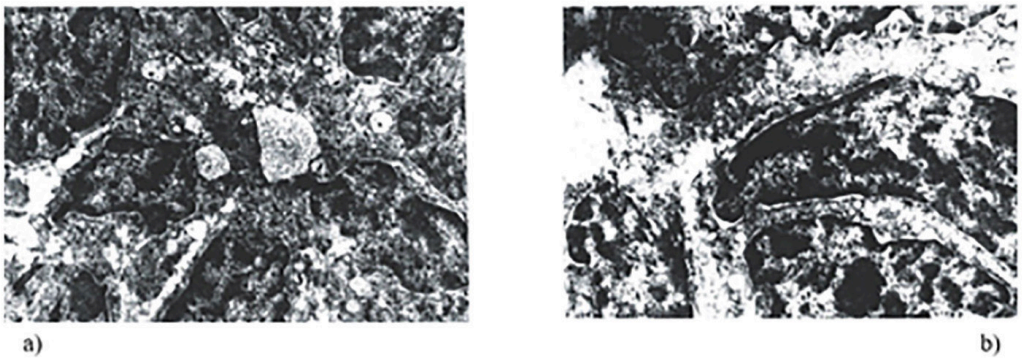

Figure 2. Metastatic Spread of Lung Cancer: Pleural and Liver Involvement
Note: a) Metastasis of undifferentiated lung cancer to the pleura; magnification x7 300, b) The same observation. Liver metastasis; magnification x7 300. Source: compiled by the authors.

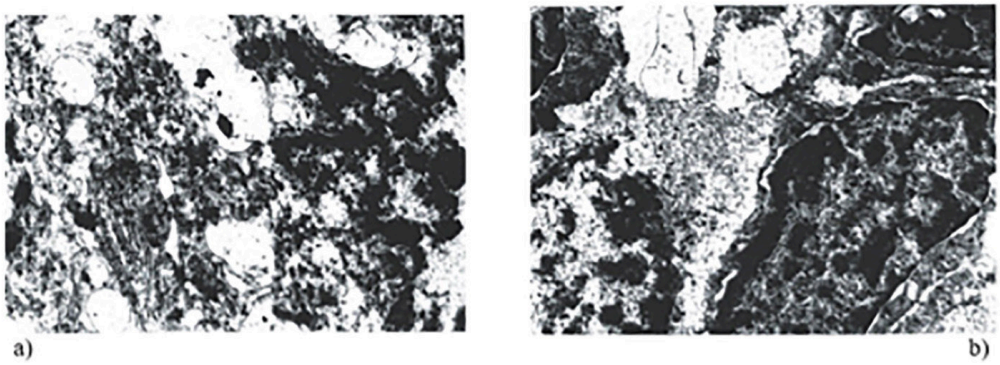

Figure 3. Metastasis of Lung Cancer: Kidney and Peritoneal Involvement.
Note: a) Tumor cell of lung cancer metastasis in the kidney; magnification x10 000; b) The same observation. Peritoneal metastasis; magnification x7 300. Source: compiled by the authors.

distribution is still uneven) with different amounts of chromatin in the nucleus and different electron densities of the cytoplasm.

In the cytoplasm of metastatic node tumor cells, relatively more free ribosomes are detected than in primary cancer tumor cells, which are arranged in the form of rosettes or chains and are not connected to the endoplasmic reticulum (Figure 4), and the cytoplasm is relatively dense. This pattern is a sign of functional cellular quiescence. In primary cancer, tumor cell growth zones are more common, with dense intercellular formations with cytoplasmic membrane compaction, i.e., structures resembling desmosomes, predominating in the area of intercellular contacts. At the same time, metastases are dominated by tumor cells with a tortuous cytoplasmic surface and virtually no desmosomes or other intercellular structures. This difference may indicate that, although desmosomes are considered a weak type of intercellular interaction, the primary tumor is more organized. At the same time, metastatic cells have greater autonomy, which increases their invasiveness and vulnerability. In metastatic nodes, there are more lysosomes in the cytoplasm of tumor cells than in the primary tumor. Still, lysosomal activity differs in different metastases: some have, and others have significantly less. Since lysosomes are markers of the catabolic activity of the cell, it can be assumed that in the

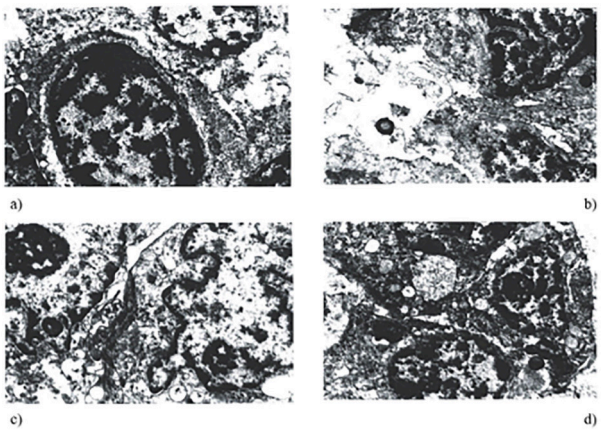

Figure 4. Metastatic Spread of Gastric Cancer: Liver and Pancreatic Involvement".
Note: a) Tumor cell of undifferentiated gastric cancer metastasis in the liver; magnification x7 300; b) Primary pancreatic cancer; magnification x7 300; c) The same observation. Liver metastasis; magnification x7 300; d) Tumor cell of gastric cancer metastasis in the pancreas; magnification x50 909. Source: compiled by the authors.

case of gastric cancer metastasis to the pancreas, shown in Figure 4, this tumor conglomerate has a high degree of aggressiveness, which is an adverse prognostic marker.

It has been previously noted that

immunomorphological reactions are usually poorly developed in the tumor nodes of metastasized primary cancer and its metastases. Electron microscopic examination shows that the primary tumor node of metastatic cancer has virtually no cellular immune responses, and in metastases, especially early metastases, lymphocytes are found among tumor cells in contact with them; the latter show signs of damage. For example, Figure 5 shows an immune lymphocyte in contact with a tumor cell and shows signs of lysosome activation and cytoplasmic lysis in a tumor cell. Figure 5 demonstrates the destruction of a metastatic cell after contact with a lymphocyte: cytoplasmic lysis and lysosomal activation are also evident. This may be a manifestation of a cell clone proliferating in the metastasis to which the immune system is not tolerant, or it may be a manifestation of local cellular immune responses since no such reactions were noted in the images of primary tumors. Several cancer cells in the primary tumor and its metastases show signs of necrosis in the growth zone even outside the contact with immunocompetent cells. This may be a consequence of exposure to humoral immune factors or the result of circulatory failure due to vascular growth retardation.

In the area of tumor cell necrosis, leukocytes with signs of macrophage activity are seen – for example, in Figure 6, a neutrophilic leukocyte with signs of pronounced phagocytic activity is visualized next to a lysed tumor cell. Beyond the growth limit of most metastases, activation of fibroblasts and fibroblasts is noted, and single plasma cells with signs of functional activity are seen. It is worth noting that in Figure 7, in the cytoplasm of the activated fibroblast, the granular endoplasmic reticulum tubules are dilated, forming cisternae, and collagen fibers are located outside the fibroblast, indicating an active interaction between the fibroblast and the tumor cell, and the nature of this interaction is most likely pro-tumor. A comparable scenario is

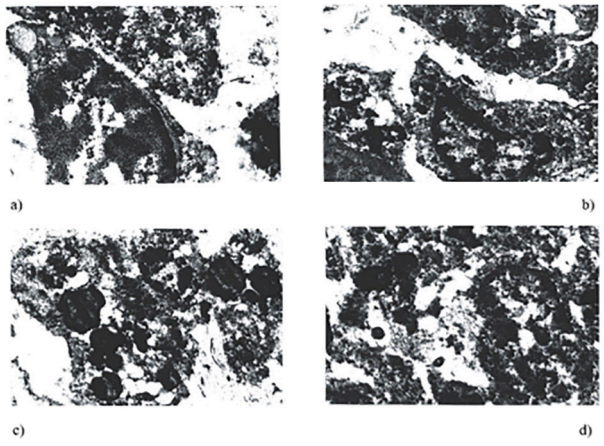

Figure 5. Metastatic Spread of Gastric Cancer: Involvement of Liver and Pancreas.
Note: a) Gastric cancer metastasis in the pancreas; magnification x10 000; b) Same observation. Cancer metastasis in the liver; magnification x6 000; c) Same observation. Cancer metastasis in the liver; magnification x10 000; d) Same observation. Cancer metastasis in the liver; magnification x7 300. Source: compiled by the authors.

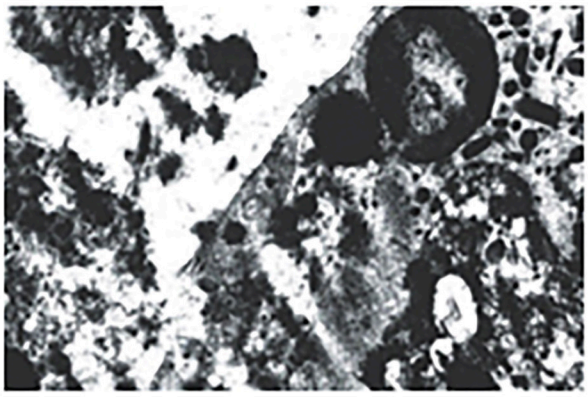

Figure 6. Same observation. Cancer metastasis in the lung; magnification x10 000.
Source: compiled by the authors.

observed, wherein a plasma cell is seen adjacent to the tumor cell, its cytoplasm entirely filled with a granular endoplasmic reticulum. In cases of intravascular growth of metastatic cells, endothelial cells have a distinct dystrophy.

Their desquamation is followed by destroying the vessel wall and releasing tumor cells outside

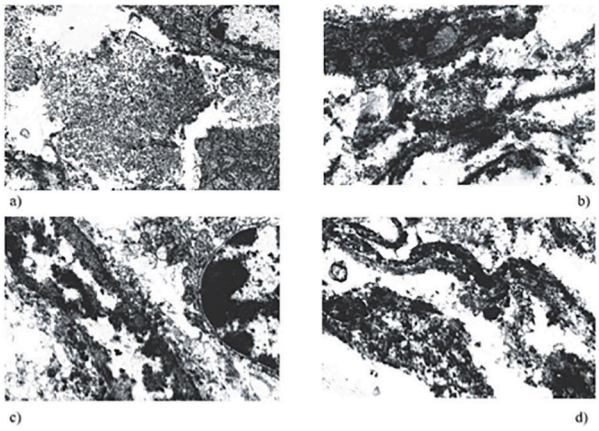

Figure 7. Peripheral Cellular Features in Gastric Cancer Metastasis and Lung Cancer Intravascular Growth.
Note: a) Activated fibroblast and plasma cell in the periphery of gastric cancer metastasis to the liver; magnification x6 000; b) Same observation. Activated fibroblast and plasma cell in the periphery of gastric cancer metastasis to the liver; magnification x12 000; c) Lung cancer metastasis in the kidney; magnification x10 000; d) Endothelial desquamation in a capillary during intravascular growth of lung cancer metastasis tumor cells; magnification x7 300. Source: compiled by the authors.

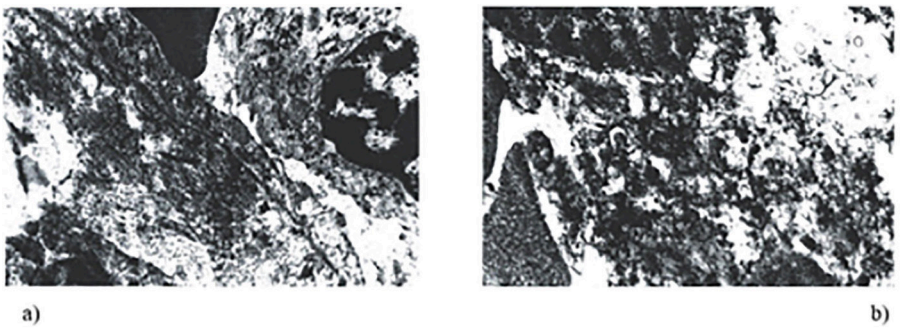

Figure 8. Intravascular Metastasis of Lung Cancer.
Note: a) Tumor cell of intravascular metastasis of lung cancer; magnification x8 800; b) Lung cancer metastasis in the pleura; magnification x10 000compiled by the authors.

the vessel (Figure 8). Gaps between tumor cells—pseudo-vessels containing red blood cells with altered configuration, which are most likely functionally unable to function—are also noticeable. This effect on blood cells is a factor in the development of hypoxia.

Growing tumor cells of metastases form complexes that often lack blood vessels, and electron microscopic examination reveals gaps (ducts) formed by tumor cells – the pseudo vessels mentioned earlier – that contain modified red blood cells (Figure 9) and other blood cells. The endothelium may show signs of lysis. The altered configuration of red blood cells along the walls of these channels, represented by tumor cells, is also evidence of pseudo-vessel involvement in transporting blood and nutrients. However, this type of nutrition is insufficient, which provokes the development of hypoxia and increased anaerobic glycolysis, a characteristic feature of carcinogenic transformation, increasing the metastatic potential of the tumor (12,13). Therefore, hypoxia should be considered a powerful trigger factor in improving the invasive

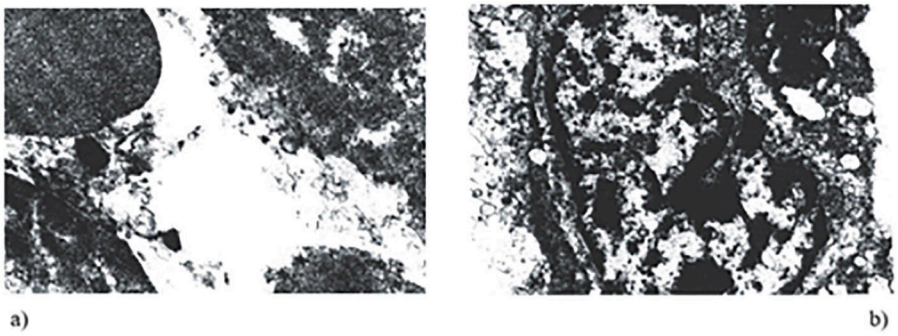

Figure 9. Lung Cancer Metastasis: Pancreatic Observation.
Note: a) The same observation. Lung cancer metastasis to the pancreas; magnification x10 000; b) Tumor cell of lung cancer metastasis in the kidney next to a blood vessel; magnification x7 300.
Source: compiled by the authors.

potential of the tumor.

Pre-existing vessels in the area of tumor cell proliferation are usually destroyed, with endothelial desquamation in the blood vessel surrounded by metastatic tumor cells, leading to further destruction of the vascular wall in the area of tumor growth (Figure 10). At the same time, active neoangiogenesis occurs, with the cytoplasm of endothelial cells containing a distinct endoplasmic reticulum and mitochondria in the absence of the basement membrane or with its thin structural organization (Figure 11). The peculiarity of angiogenesis in the area of metastasis growth is that newly formed low-differentiated vessels predominate, which are functionally defective because their endothelium has poorly expressed cytoplasmic differentiation.

Electron microscopy detected a distinct cellular polymorphism in the primary tumor and its metastases. At the same time, the degree of atypicality of tumor cells and their functional activity in daughter metastases is sometimes unequal. This can be regarded as a sign of their multiclonality. Tumor cells in metastatic nodes

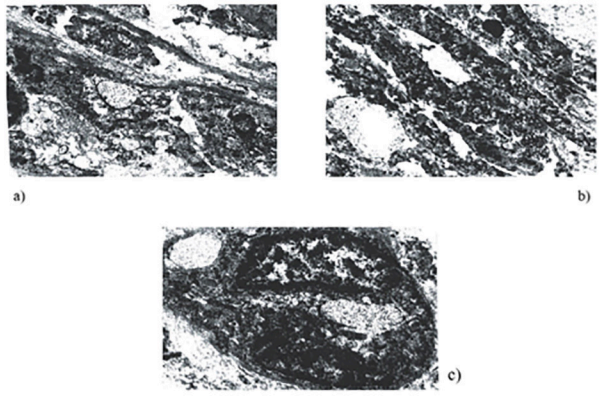

Figure 10. Metastatic Tumor Cells and Vascular Changes in Various Organs.
Note: a) Same observation. Tumor cell of lung cancer metastasis in the kidney next to a blood vessel; magnification x6 000; b) Lung cancer metastasis in the pancreas; magnification x6 000; c) Newly formed vessel of gastric cancer metastasis to the liver, lined with endothelium; magnification x7 300.
Source: compiled by the authors.

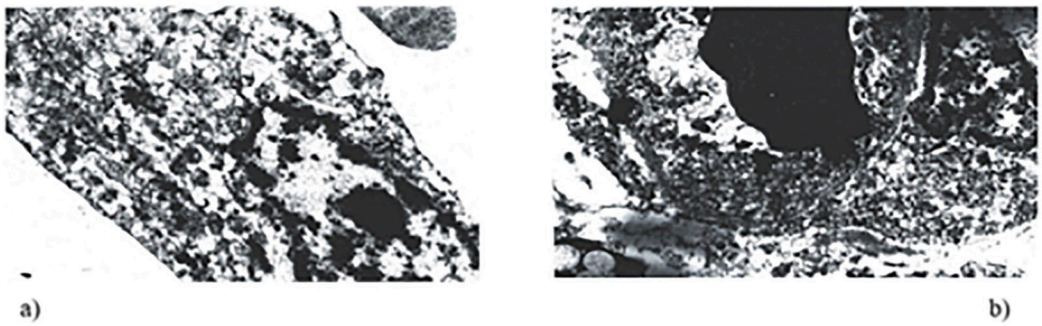

Figure 11. Neoangiogenesis in Lung Cancer Metastases.
Note: a) Endothelial cell of a newly formed vessel in a lung cancer metastasis in the kidney; magnification x10 000; b) Newly formed vessel in a lung cancer metastasis in the pancreas; magnification x6 000. Source: compiled by the authors.

have relatively fewer intercellular contacts than in the primary tumor, which is a sign that in metastases, tumor cells are less mature and are more prone to migration, i.e., metastasis, than in the primary tumor node. In metastatic tumor cells, nuclear polymorphism is relatively more pronounced (with invaginations and irregular chromatin distribution, sometimes an increase in the number of nuclei is noted). The nature of the changes in tumor cells in daughter metastases is not the same, which is also a sign that they are multiclonal. Cellular immune reactions are more pronounced in some metastases than in the primary tumor. They are manifested by the antitumor activity of lymphocytes and even the activity of plasma cells and other leukocytes. Metastatic node growth is accompanied by stimulation of fibroplastic reactions. In the zone of metastatic cell growth, angiolytic processes are evident, manifested by destruction of the vessel wall during intravascular growth of the metastasis and destruction of the pre-vessels growing with tumor cells, as well as by expressive angiogenesis. The vascular bed of metastatic nodes is represented mainly by newly formed low-differentiated vessels, which are functionally inferior, as the endothelium has weakly expressed signs of cytodifferentiation, sometimes poorly expressed basement membrane. The wall of such vessels is highly permeable to blood elements and tumor cells, leading to metastasis development. Along with blood vessels, the blood supply to metastatic nodes is carried out by so-called pseudo vessels, i.e., through the gaps between tumor cells. This is because the newly formed vessels do not provide normal nutrition to tumor cells due to their functional inferiority. Since the pre-existing vessels in the metastasis growth zone die, the role of a possible impact on neoangiogenesis to prevent metastasis growth becomes apparent.

DISCUSSION

The electron microscopic evaluation of primary tumor cells plays a pivotal role in unraveling their migratory and invasive potential, offering crucial insights into cancer metastasis mechanisms. Understanding the ultrastructural alterations in tumor cells is essential for predicting the likelihood of metastasis and devising effective therapeutic strategies. By delving into the intricate cellular morphology and molecular features, electron microscopy enables us to discern subtle changes indicative of increased aggressiveness and metastatic propensity. Integrating these findings into prognostic assessments empowers clinicians to tailor treatment approaches, identify high-risk patients, and optimize therapeutic outcomes.

The issue of heterogeneity in oncological practice is widely discussed. This condition manifests as morphological differences between cells, protein and biomarker expression levels, and genetic profiles within a single tumor and its metastatic lesions (14). Such heterogeneity arises due to genomic instability, cellular differentiation, microenvironmental influences, and mutation accumulation, which leads to the formation of subclones of the genetic population.

The present study observed the same: metastases had significant cellular atypia and polymorphism compared to the primary tumor. Authors (15,16) investigated this issue in the context of malignant breast tumors by sequencing single-cell RNA from samples of primary tumors and metastases in the liver, peritoneum, ovaries, and lymph nodes. The study (17) showed that patients rarely receive effective targeted therapy for breast cancer in the early stages of the disease due to the relatively late detection of metastatic lesions. It is worth noting that understanding the mechanism of metastasis heterogeneity will contribute to the development of effective strategies to combat metastases at the stage of their colonization and growth, and genomic and transcriptomic analysis, single-cell gene expression analysis, experiments in animal models and pedigree analysis is promising in this area. Using the example of metastatic gastric cancer, Jiang et al. (18) also demonstrated the presence of malignant epithelial subclusters in the primary tumor, associated with the peculiarities of invasion and differentiation and a tendency to intraperitoneal metastases. The microenvironment cells demonstrated cellular heterogeneity and created pro-tumor and immunosuppressive conditions. In addition, the presence of depleted cytotoxic T-lymphocytes derived from the lymph nodes was recorded. To summarise, the interaction with stromal and immune cells can be an effective point of application in cancer treatment. Although this study did not use molecular research methods, it is possible to draw similar conclusions.

The study of Mashouri et al. (19) in the context of tumor exosomal formations is worth paying attention to. According to the authors, they are involved in the formation and progression of remodeling of the tumor microenvironment of cells (especially fibroblasts, infiltrating immune cells, and vascular endothelial cells), angiogenesis, invasion, metastasis, and drug resistance, as these formations initiate or inhibit various signaling pathways in recipient cells by transferring heterogeneous signaling molecules. Similar conclusions were obtained by Deepak et al. (20), where attention is paid to reprogrammed fibroblasts that exhibit similar properties. Such ultrastructures can be detected by high-resolution electron microscopy, and together with proteomic analysis methods, we can determine exosome composition. In this article, the presence of such structures was not determined, which requires better technical support and a more detailed focus in future studies. Regarding the possibility of using various research methods, one should refer to the study by Stoletov et al. (21), which argues that studying metastatic human cancer cells in animal models is a more powerful approach for accurate visualization and characterization of metastasis dynamics. Thus, *in vivo*, significant gene expression and metabolic activity differences are detected in both primary tumors and metastases. Still, the authors did not note morphological and functional differences between them.

Fu et al. (22) presented data on an important part of the tumor microenvironment – macrophages, which, despite their protective function, participate in tumor progression by producing chemoattractants and cytokines (including growth factors) that suppress the activity of immune cells and inactivate antitumor responses. They also serve as angiogenesis-promoting cells by producing pro-angiogenic factors and matrix metalloproteinases, which ensure the supply of oxygen and nutrients to solid tumor cells. Macrophages also play an important role in metastasis by promoting invasion, extravasation, intravasation, and colonization of tumor cells (23,24). Therefore, macrophage activity in this study may be questioned and maybe a topic for further investigation.

Antiangiogenic therapy is a promising method for treating solid tumors and early neutralizing metastatic lesions (25). However, the low therapeutic efficacy of modern antiangiogenic drugs is caused by the high expression of angiogenic factors and inflammatory cytokines in the tumor microenvironment, as well as hypoxia (26). The present study demonstrated the presence of pseudo vessels and pressures as the main sources of nutrition for metastases, and even their functional failure only enhances neoangiogenesis. Al-Ostoot et al. (27) showed that the lack of blood supply led to tumor necrosis, which was confirmed in the present study. However, the influence of immune humoral factors was also questioned, which, in turn, requires the use of other research methods mentioned earlier. In addition to angiogenesis, an important place is given to inflammation, which

can be both acute and chronic, which, according to Zhao et al. (28), is of fundamental importance since the chronic course of inflammation contributes to tumor progression and treatment resistance. In contrast, the induction of acute inflammatory reactions often stimulates dendritic cell maturation and antigen presentation, leading to an antitumor immune response. In the present study, signs of acute inflammation were observed in metastatic tumor cells. Still, the presence of dendritic cells was not detected, and the immune response was insufficient to eliminate metastatic lesions, let alone the primary tumor.

As mentioned earlier, fibroblasts are a heterogeneous family of cells consisting of numerous subtypes that can alter immune cell fractions, promote or inhibit tumor growth, build pre-metastatic niches, or stabilize blood vessels. The pro- or anti-tumor phenotypes of fibroblasts show variability among different malignancies and in the context of primary and metastatic tumors (29,30). Knipper et al. (30) suggest that fibroblast inhibition, achievable through drugs such as those belonging to the group of angiotensin-converting enzyme inhibitors, may enhance treatment sensitivity. However, they also note this inhibition's potential to promote tumor growth simultaneously. The activated fibroblasts detected in this study are most likely to determine proinflammatory and tumor necrotic processes (31). Still, it is impossible to visually assess the entire cascade of functional processes and unambiguously determine the role of fibroblasts in the presented tumor samples. Wang et al. (32) demonstrated a hypothesis in their study on metastasis to liver tissue and microenvironment that there are factors created by the primary tumor that may predispose to the formation of metastatic niches from activated residual cells that contribute to the progression of the tumor process, which could explain the difference in the cellular state of the tumors themselves and their microenvironment. Despite advances in immunohistochemistry and molecular biology, in clinical practice, most diagnosis and evaluation of metastatic lesions still relies on light microscopy using histological sections (33), and according to Roskell and Buley (34), a skilled morphological assessment still provides a prognostic basis in the vast majority of practical cases. Electron microscopy has also offered a wide range of information. However, certain data still need to be clarified, especially when it comes to developing new treatments based on genetic and molecular interaction factors.

Thus, the results of this study have been confirmed by other authors using various diagnostic methods that neoangiogenesis, tumor cell microenvironment (especially macrophages and fibroblasts), heterogeneity, and multiclonality of primary and metastatic tumors are critical in the growth and spread of the tumor process. The impact of these links in the pathogenetic chain may allow for effective therapeutic interventions and increase the sensitivity of tumors to various drugs.

The study limitations include several important aspects that should be carefully considered when interpreting the results. Although the study used a sample of patients with different types of cancer and metastases, the sample size may be limited, affecting the results' overall representativeness. Although a variety of cancer types were included in the study, there may be selection bias in the sample, as it may not fully reflect the diversity of patients with different stages and disease characteristics. The study may have limited access to complete patient clinical data, such as disease stage information, previous treatments, and clinical outcomes. The study is based primarily on electron microscopy, which may limit the ability to gain a complete understanding of metastases at the cellular level. Other research methods can complement these results. To better understand the metastasis process, additional analyses, such as cellular and molecular biology, may be required to confirm the results and identify additional factors that influence the metastasis process.

CONCLUSIONS

The electron microscopy study revealed that primary and metastatic tumors have distinct cellular polymorphisms, which can have different degrees of atypicality and functional activity within the same population of tumor cells. This can be regarded as a sign of their

heterogeneity and active mutagenesis. Tumor cells of metastatic nodes are less prone to form strong intercellular contacts, which is a sign of immaturity and high migration capacity, which may be associated with the relationship with the cells of the tumor microenvironment. In tumor cells of metastases, nuclei are characterized by a high degree of polymorphism with the formation of invaginations and irregular chromatin distribution and an increase in the number of nuclei that varies between different cells – such changes also testify in favor of the heterogeneity of metastatic cells. In some metastases, immune responses are more pronounced compared to the immunoreactivity of the primary tumor, and the antitumor activity of lymphocytes was found to be combined with that of plasma cells, indicating active but heterogeneous defense responses. The stimulation of fibroblastic reactions accompanies the progression of metastatic invasion, but the functional role of fibroblasts cannot be considered unambiguous. The processes of angiolysis, manifested by the destruction of the vessel wall and destruction of the pre-vessels, go in parallel with the processes of distinct angiogenesis, characterised by the creation of low-differentiated and functionally inferior vessels since the endothelium has a weak basement membrane and signs of cytodifferentiation. This, in turn, enhances the migratory ability of metastatic tumor cells. Along with blood vessels, the blood supply to metastatic nodes is carried out through intercellular gaps (pseudo vessels). The state of chronic hypoxia caused by functional insufficiency of blood supply can also increase the resistance of tumors to treatment, as well as only intensify the processes of angiogenesis and progression of tumor processes. No significant difference or dependence on the location of the oncogenic process was found.

All of the above features of primary and metastatic tumors can be used as potential points of application for destroying the tumor process and enhancing the response to therapeutic measures. The data obtained can serve as a basis for refinement and further investigation by molecular and genetic diagnostic methods since electron microscopy does not fully assess functional interactions with cells in the microenvironment and within the tumor.

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