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# MORPHOLOGICAL CHARACTERISTICS AND EXPRESSION OF ADHESION MARKERS IN CELLS OF LOW DIFFERENTIATED ENDOMETRIAL CARCINOMA

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***The aim*** **of the study was to evaluate the morphological features of endometrioid carcinoma of the endometrium (ECE) of low differentiation grade with different invasive potential and to characterize their molecular phenotype by the expression of a number of adhesion markers.** ***Materials and Methods*****: We have studied the samples of operation material of 37 patients with ECE of low differentiation grade with deep invasion (> ½ myometrium), n = 26, and with invasion < ½ myometrium, n = 11, with the use of morphological and immunohistochemical methods, and flow cytometry.** ***Results*****: In the morphological study of tumors with deep invasion in the myometrium, we have detected pronounced structural heterogeneity, which became the basis for the discretion of two groups of tumors with different characteristics of morphological phenotypes. In the majority of cases, solid layers and glandular-like structures are detected, and the similarity of the tumor epithelium with the elements of the endometrium is completely lost. In such tumors high expression of adhesion molecules – E-cadherin, CD44, CD24, and β-catenin and low expression of the marker of mesenchymal tissues — vimentin were determined. Other tumors were characterized by morphological features of the epithelial-mesenchymal transition (EMT), with the decrease of the expression of E-cadherin, β-catenin, CD24, CD44, and a significant increase in vimentin expression in comparison with these indices in tumors without signs of EMT. In ECEs that invade < ½ myometrium, the morphological indices of malignancy were less pronounced, which was associated by the changes in the expression of the molecular markers.** ***Conclusion*****: This comprehensive study has established associations between the morphological heterogeneity of ECE and the expression of adhesion markers and vimentin, which is important for understanding the mechanisms of tumor cell migration.**

***Key Words*****: endometrioid endometrial carcinoma, cytoarchitectonics of tumors, invasion, E-cadherin, β-catenin, CD44, CD24, vimentin, proliferation index.**

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For decades now, researchers have been asked one of the most actual questions: why patients with equivalent clinical manifestations of the oncological pathology and an identical pathomorphologically verified diagnosis at the time of the initial examination, in most cases demonstrate significant differences in the further disease course.

This problem is also fully relevant for endometrial cancer (EC), the incidence of which increases annually [1]. Despite the fact that at diagnosis an endometrioid type EC of stages I–II is mainly determined (75.0–80.0% cases), in 17% of patients tumors with high proliferating activity are detected, characterized by deep invasion of the myometrium and early recurrence and metastasis.

According to the literature, the EC patients present variability in the clinical course of the disease, which may be associated with both morphological and molecular features of the tumor, causing the low effectiveness of antitumor therapy [2–4].

When studying the features of the biology of endometrioid carcinoma of the endometrium (ECE) by morphological structure, the presence of intratumoral heterogeneity of these tumors was determined [5]. According to the results of multicenter molecular genetic studies, among ECEs four molecular subtypes are identified, which differ in certain clinical manifestations, are associated with the survival of the patients and could be used potentially in the diagnosis of the pathological process [6–8]. However, the technology to identify such subtypes is complex and requires the use of high-cost equipment. Therefore, it is necessary to find more informative and effective markers of aggressive ECE forms, since their determination will facilitate the personalized treatment with the use of modern schemes of polychemotherapy [8].

It should be noted that the progression of malignant neoplasm, in particular its invasive growth, is the result not only of changes in the expression of tumor suppressor genes or oncogenes, but also of molecules that cause intercellular contacts [9, 10].

Loss of adhesive properties of tumor cells can contribute to their increased mobility and, as a consequence, penetration into surrounding tissues, blood and lymphatic nodes [11]. Recent studies have shown that the migration of neoplastic cells during tumor progression occurs through sequential molecular changes and complex morphological alterations, which are accompanied by destruction of the basement membrane and loss of polar orientation of tumor cells and their nuclei. In this case, due to changes in the expression of markers of the epithelial-mesenchymal transition (EMT) the epithelium can undergo mesenchymal-like changes [9–12]. In pathologically altered epithelial cells, along with the loss of expression of epithelial markers such as E-cadherin and β-catenin, expression of vimentin, a marker

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*Abbreviation used:* EC – endometrial cancer; ECE – endometrioid carcinoma of the endometrium; EMT – epithelial-mesenchymal transition; H&E – hematoxylin and eosin; IHC – immunohistochemical; PI – proliferation index.

of connective tissue cells, is observed. EMT activation can be influenced by other adhesive molecules, including CD44 and CD24, which are also involved in a variety of signaling pathways that stimulate tumor cell growth and motility [13, 14].

Our previous studies have shown that ECE progression (increased proliferative potential, low differentiation grade, and deep tumor invasion into the myometrium) can occur along with both decreased expression of E-cadherin, β-catenin and glycoprotein CD24, and high vimentin expression, or without expression of vimentin, and high expression levels of E-cadherin, β-catenin and CD24 [15, 16].

Invasion of neoplastic cells into adjacent tissues is observed with the progression of many solid tumors, including ECE. It is accompanied by the migration of cell groups bound together by tight adhesive contacts (for example, via accumulation of E-cadherin on the surface of cell membranes). In this variant of migration, the invasion of the tumor into the adjacent tissues and metastasis occurs by a "united front" and, according to some authors, is even more effective than the migration of individual cells [9, 10].

Therefore, the aim of the study was to evaluate the morphological features of ECEs of low differentiation grade and with different invasive potential and to characterize their molecular phenotype by the expression of a number of adhesion markers.

## MATERIALS AND METHODS

The samples of surgical material of 37 patients with ECE of low differentiation grade and the stages I–II by International Federation of Gynaecology and Obstetrics, treated at the Department of Oncogynecology of the National Institute of Cancer of the Ministry of Health of Ukraine (Head Prof. V.S. Svintsitskiy) from 2014 to 2018, were studied. The average age of patients was 62.4 ± 2.5 years. All patients did not receive preoperative therapy and provided their informed consent on the use of their biological material for scientific research.

Diagnosis was verified by examination of histological preparations stained with hematoxylin and eosin (H&E). The tumor differentiation grade was determined according to WHO criteria (2014) when studying the features of their cytoarchitectonics [17].

Immunohistochemical (IHC) detection of biomolecular marker expression was performed on deparaffinized sections of endometrial tumors using the following MoAbs: anti-E-cadherin (clone NHC-38); anti-β-catenin (clone β-catenin-1) "DakoCytomation, Denmark"; CD44 (clone 196-3C11), CD24 (clone Ab-1) "Thermo Fisher Scientific", USA; anti-vimentin (clone V9) "Diagnostic BioSystems", Netherlands. PolyVue detection system "DakoCytomation", Denmark was used to visualize the proteins.

The results of the IHC reaction were evaluated by semi-quantitative method, by counting the number of stained cells in the tumor tissue — labeling index (%). If the marker expression in the tumor was lower than the median value (< Me) it was considered low, if higher (> Me) — high. The Me values of the markers in the studied tumors were as follows: for E-cadherin detection — 43.3%, β-catenin — 91.0%, vimentin — 27.0%, CD44 — 9.3%, and CD24 — 47.1%.

Laser flow cytometry was used to determine the proliferation index (PI, %, the number of cells in $S+G_2/M$ phases of the mitotic cycle) [18]. The study was performed on an EPICS-XL flow cytometer (Beckman Coulter, USA). The Me value for PI was 29.1%.

Statistical analysis was performed using Statistica 8.0 software package (StatSoft, Inc.), non-parametric Mann — Whitney *U* Test criteria and chi-square test, Fisher's exact test. At assessment of the differences between the groups, the value $p < 0.05$ was considered significant.

## RESULTS AND DISCUSSION

An analysis of such an index of the tumor progression as the level of tumor invasion into the myometrium showed that in 29.7% of cases of low-differentiated EC invasion < ½ myometrium was observed, however, most (70.3%) of the investigated neoplasms invaded > ½ myometrium.

In the morphological study of tumors with deep invasion in the myometrium, we have detected pronounced structural heterogeneity, which became the basis for the discretion of two groups of tumors with different characteristics of morphological phenotypes.

In the majority of cases, solid layers and glandular-like structures are detected, and the similarity of the tumor epithelium with the elements of the endometrium is completely lost. Tumor cells are drastically anaplastic, often polygonal in shape with hyperchromic polymorphic nuclei. The glands are characterized by a disturbance of architectonics inherent to the endometrium, are of various sizes and shapes, often convoluted, papillary protrusions into the lumen are observed, and are closely spaced. Organic architectonics of cells in such glands is lost due to their active proliferation. Thus, tumor cells with marked signs of atypia and polymorphism are characterized by a multilayered disordered arrangement with the formation of large accumulations in some glands (Fig. 1).

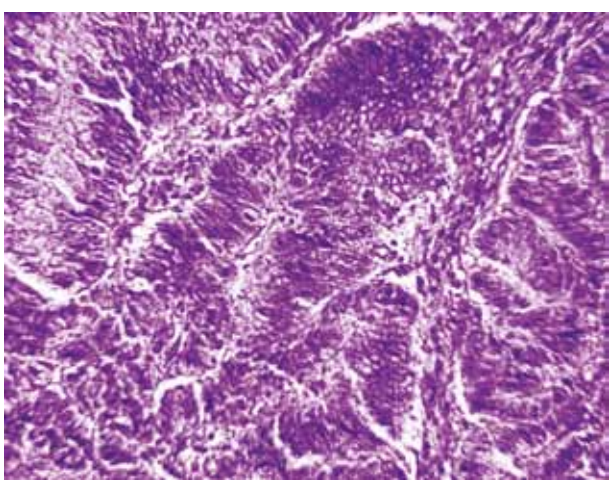

**Fig. 1.** ECE. Tumor cells in the glands show atypical features, are polymorphic in size and shape, and have a multilayered location with accumulation in some glands. H&E, ×400

In most glands, tumor cells are of large enough sizes, with a well-pronounced eosinophilic cytoplasm, enlarged, sometimes bubble nuclei, with compactly located heterochromatin. A characteristic feature of these tumors is a large number of pathological mitoses, often asymmetrical, and multipolar. Along with this, sometimes glands contain the cells of small size, more monomorphic, with small rounded or oval-shaped monotonous colored hyperchromic nuclei, often with several nucleoli. Tumor cell growth is observed, both in the lumen of the gland with filling its entire space and in adjacent tissue. Mainly, microenvironment infiltration is performed by large groups of tumor cells, which is a morphological manifestation of collective migration. In some less differentiated areas of the stroma, fibroblasts, reticular and collagen fibers are found.

In other cases, in tumors of this group, along with the glandular component there are determined the different groupings of tumor cells in the form of solid foci and bands, which can also be considered as a consequence of their collective migration (Fig. 2).

In solid structures, large cells with a well-expressed eosinophilic cytoplasm and large nuclei with a varied chromatin texture, are observed. Cells in the band structures are of slightly smaller size with moderately sized hyperchromic nuclei. In some tumors there is an invasion of tumor cells in the form of tubular structures (Fig. 3, 4).

There were also observed areas of sharply anaplastic cancer cells, which form glandular-like structures with atypical papillary lesions from polymorphic cells that were similar by their cytoarchitectonics to those of serous EC (Fig. 5).

According to the data of IHC studies, these tumors was characterized by high expression of adhesive molecules of E-cadherin (46.7 ± 3.3%), CD44 (28.1 ± 1.8%), CD24 (49.1 ± 2.4%), β-catenin (84.1 ± 3.1%), and low expression of vimentin (17.8 ± 1.9%) were detected. The PI in such tumors was high and amounted to 37.7 ± 1.8%.

In contrast, some tumors with invasion of more than ½ myometrium were characterized by the presence of large areas with accumulation of monomorphic histiocyte-like cells (diamond-shaped, triangular shape) with hyperchromic nuclei, which can probably be regarded as a manifestation of EMT (Fig. 6, *a, b*). Also, in these tumors, atypical glandular structures, regions of disordered anaplastic tumor cells, as well as large accumulations of polymorphic cells with solid

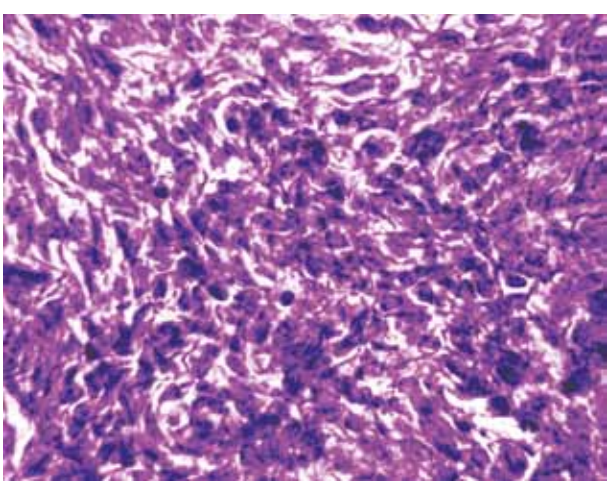

**Fig. 2.** ECE. Collective migration of disorderly proliferating tumor cells into the myometrium. H&E, ×200

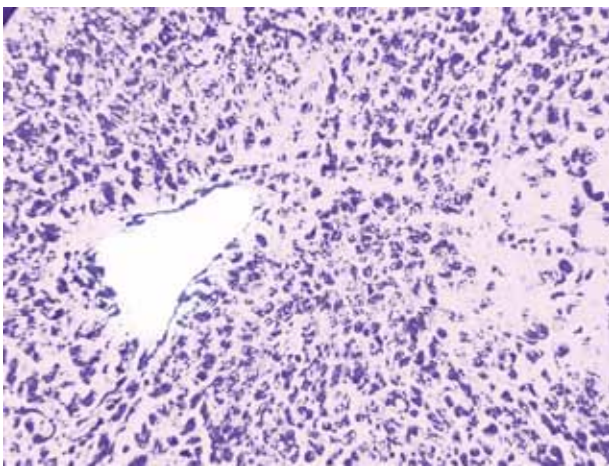

**Fig. 3.** ECE. Collective migration of tumor cells in the form of solid structures. H&E, ×400

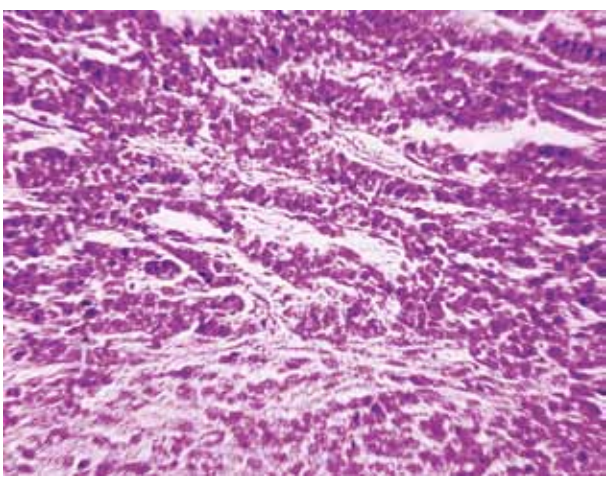

**Fig. 4.** ECE. Invasion of tumor cells in the form of tubular structures. H&E, ×400

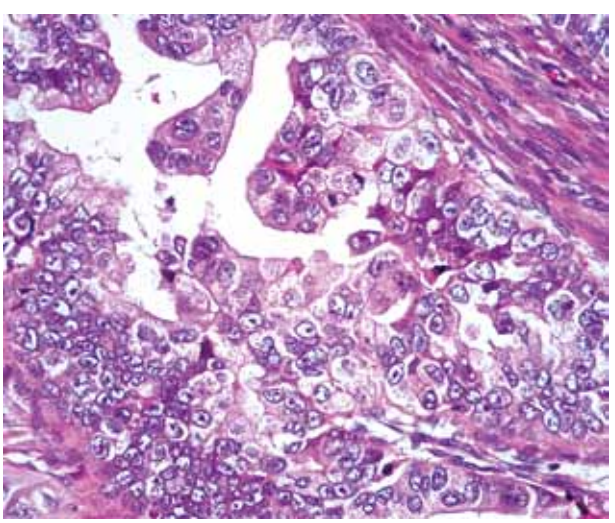

**Fig. 5.** ECE with papillary structures similar to serous-type endometrial cancer. H&E, ×200

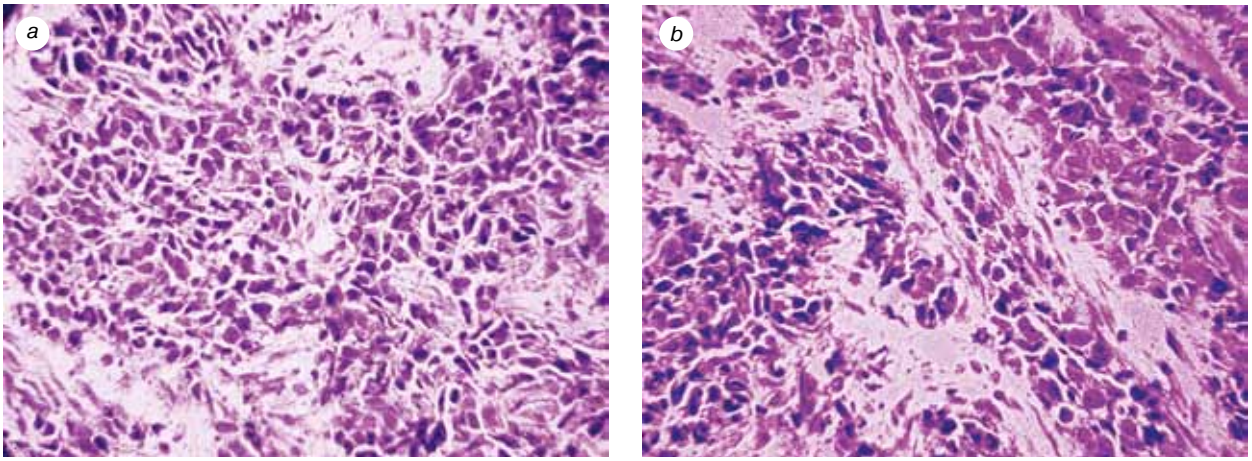


**Fig. 6.** ECE. Tumors with signs of epithelial-mesenchymal transition (*a* — complete, *b* — incomplete with the presence of epithelial cells — right, top). H&E, ×400

compact growth were observed. Significant variability of the number of cells invading the myometrium is determined: from single cells and their small clusters to unstructured groups (Fig. 6, 7).

It should be noted that according to the literature malignantly transformed cells that migrate into the surrounding tissues, being separated from the main tumor, often acquire morphological phenotype and properties characteristic of mesenchymal cells [19–23].

The presence of EMT features in these tumors is also evidenced by the IHC data, namely, 6-fold lower expression of E-cadherin (7.6 ± 1.1%, $p < 0.01$) and significantly higher vimentin expression were observed (by 3.8 times, 66.9 ± 3.1%, $p < 0.01$) as compared with the tumors without EMT features. The expression of β-catenin and CD44 tended to decrease (72.6 ± 3.6% and 23.8 ± 2.7%, respectively), and the expression of CD24 protein was significantly reduced (almost twice) to 28.3 ± 2.1%, $p < 0.05$ compared with these indices in tumors without EMT features (Fig. 8). However, PI in these tumors remained high — 35.4 ± 3.7%.

When assessing the morphological features of ECE that invaded less than ½ myometrium, it was determined that, along with signs of marked cataplasia, small moderately differentiated areas with preserved atypical glandular, alveolar and tubular structures were observed. Tumor cells in such glands were characterized by a more or less ordered growth pattern, less pronounced signs of anaplasia. However, in some glands, cancer cells form multilayered growths, glandular-cribriform structures, and numerous glands are observed with complete solidification of the lumens (Fig. 9).

The average expression values of the studied biomolecular markers in tumors that invaded less than ½ myometrium were as follows: the expression of E-cadherin and β-catenin was lower than the values of Me (37.4 ± 3.8% and 83.3 ± 5.4%, respectively) while high expression of vimentin, CD44 and CD24 was determined (33.8 ± 2.4%, 21.0 ± 1.8% and 57.0 ± 4.2%, respectively). Based on the data obtained, the molecular phenotype for the expression of the studied molecules in tumors with invasion < ½ myometrium was significantly different from those identified in ECE cells of tumors with deep invasion of the myometrium. These morphological and molecular features in tumors with invasion < ½ myometrium were associated with a significant decrease in proliferative potential (PI = 27.4 ± 3.2%) compared with ECE with deep invasion of the myometrium.

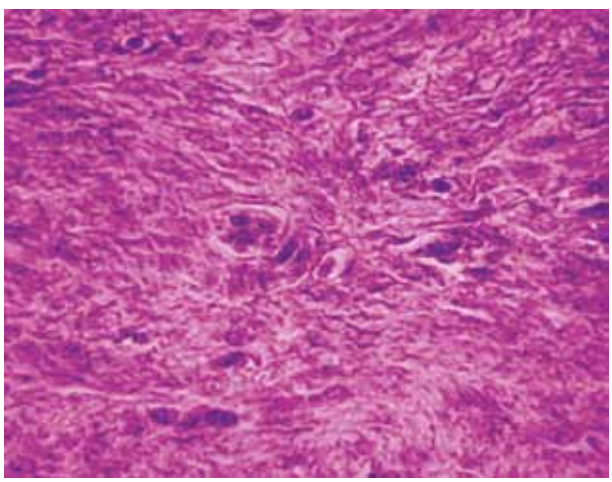

**Fig. 7.** ECE. Migration of small clusters of tumor cells into the myometrium. H&E, ×400

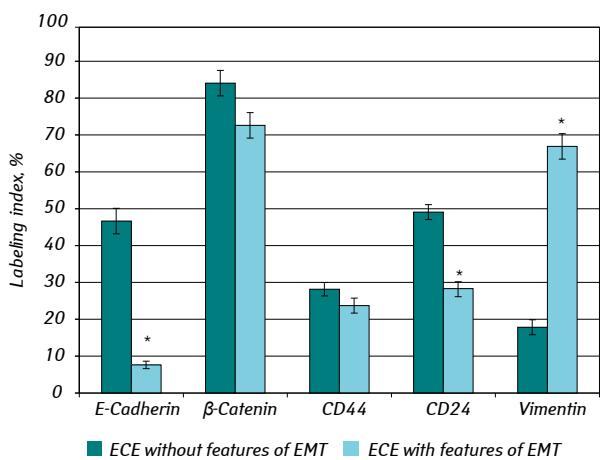


**Fig. 8.** Comparison of expression of adhesion markers and vimentin in ECE of low differentiation grade with deep invasion in myometrium between two groups of tumors with different character of morphological changes. *$p < 0.05$

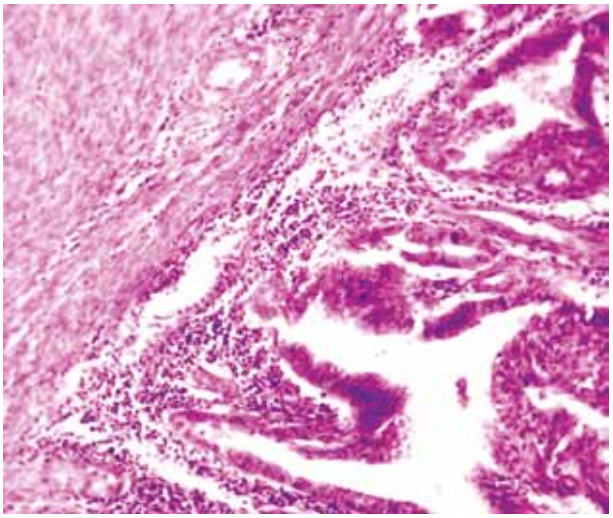

**Fig. 9.** ECE with initial tumor invasion into the myometrium. H&E, ×100

Analyzing the morphological changes in ECE of low differentiation grade with different levels of tumor cell invasion, it should be noted that in general the revealed morphological pictures reflect the active phase of neoplasm progression. However, it is not always possible to trace the gradual features of the initial changes in the invasive front, which have recently been vividly discussed in the literature. Thus, invasion is considered to be the first step in tumor metastasis that begins with local invasion of tumor cells when they budge from the main lesion and acquire properties of non-differentiated cells, able to move and migrate through the basement membrane into the microenvironment, ie, acquire aggressive phenotype [22]. Invasion, according to many studies, can be due to the movement of one such cell or the collective migration of several cells [23]. Today, clusters of such budding cells in the tumor stroma are considered as a tumor inside the tumor or "tumor budding" [24].

For some tumors, such as squamous cell carcinoma of the oral cavity, classification of invasion methods with several options has been proposed [25]. A link between the invasion method and metastasis features has been identified. Tumor budding has been shown to be the only independent predictor of regional metastasis in patients with stage I and II disease [26, 27]. In addition, these invasive structures have been found to be valuable prognostic markers of colorectal cancer [28, 29]. At the same time, a number of researchers have noted that detecting tumor budding at the embryonic stage during conventional pathohistological examination of sections stained with hemotoxilin and eosin is difficult because they can sometimes be similar to stromal cells of the tumor microenvironment [30, 31]. In addition, comparative studies of serial tumor sections in 3D and 2D reconstructions showed that only the 3D format really depicts the pattern of tumor cells budding that have not lost contact with the tumor mass and that have acquired invasive properties [25].

In the studied ECE material, we observed invasions of several tumor cells only in some cases, and mainly determined the pattern of collective migration in the form of multicellular entities, different structures involved in the process of tumor progression. Characteristic features of collective migration are indicated in the study of endometrial, breast, colon, melanoma, prostate cancer, etc. [13]. In addition, *in vitro* experiments have shown the benefits of collective migration of tumor cells compared with invasion of individual cells [32].

Summarizing the results of the study, it should be noted that there are associative relationships between the morphological characteristics of neoplasms and their IHC profile. At the same time, some differences in the morphofunctional features of tumors have some explanations. It is well known that in the process of EMT, when tumor cells lose their epithelial properties and become mesenchymal, there is a loss of expression of some cell adhesion molecules, in particular, E-cadherin, and an increased expression of mesenchymal markers — vimentin, N-cadherin [10, 14]. However, it has been established that EMT is not always equally pronounced, and is therefore characterized by varying degrees of manifestation of the transition of tumor cells from the epithelial to mesenchymal phenotype, that is, it is incomplete, partial [13]. Our studies are consistent with the literature on the unequal expression of adhesion markers in tumors with signs of EMT. Moreover, the investigated tumors are polymorphic enough by cytoarchitectonics and, according to existing reports, in the invasive component of tumors, subpopulations of cells with co-expression of both epithelial and mesenchymal markers, i.e. with the hybrid phenotype may be detected [33–35]. It should be noted that in the investigated tumors with signs of EMT, along with a significant decrease in the expression of E-cadherin and high levels of vimentin, low expression of CD24 was observed, which may also contribute to the reduction of adhesive intercellular connections. However, according to some authors, increased CD24 expression in some tumors could indicate a high malignancy of the tumor. In particular, overexpression of this protein in the ascitic fluid of ovarian carcinoma is associated with the acquisition of cancer stem cell characteristics and the aggressiveness of the tumor [36]. The above data evidence that the plasticity of epithelial phenotype markers is one of the factors that determines and modulates the morphological features of ECE, as well as such an indicator of progression as the character of tumor invasion into the myometrium.

It should be noted that the part of the neoplasms was characterized by hypo- and hyperploidy, which may indicate a high potential for malignancy of such tumors, since aneuploidy is a characteristic feature of such an aggressive form of malignant neoplasms of endometrium as serous cancer, which is characterized by an unfavorable course of the disease [2, 7].

Thus, the results of a comprehensive study evidence on association of heterogeneity of the ECE morphological phenotype with changes of the expression

of EMT markers, and substantiate their association with possible tumor cell migration pathways. In addition, an integrated assessment of the morphofunctional features of ECE allows us to objectively determine the nature of the neoplastic process, whose phenotypic features from the standpoint of evidence-based medicine, are predictors of the aggressiveness and invasive potential of this form of cancer.

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