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ROLE OF STROMAL MICROENVIRONMENT IN THE FORMATION OF INVASIVE, ANGIOGENIC, AND METASTATIC POTENTIAL OF ENDOMETRIOID CARCINOMA OF ENDOMETRIUM

The aim of the study was to determine the association of indicators of the progression of endometrioid carcinoma of the endometrium (ECE) with the type of stromal microenvironment, the counts of $CXCL12^+$ fibroblasts and $CD163^+$ macrophages, and the expression of the chemokine CXCL12 and its receptor CXCR4 in tumor cells. **Materials and Methods.** Histological preparations of ECE samples (n = 51) were analyzed. Expression of CXCL2 and CXCR4 antigens in tumor cells, the content of $CXCL12^+$ fibroblasts and $CD163^+$ macrophages, and the density of microvessels were determined by the immunohistochemical method. Results: Groups of ECE with desmoplastic and inflammatory stromal reactions were delineated. The majority (80.0%) of tumors with desmoplasia were of low differentiation grade, deeply invading the myometrium; 65.0% of patients with these tumors were at stage III of the disease. In ECE cases of stages I–II, 77.4% of ECE showed an inflammatory type of stroma. The high angiogenic and invasive potential of EC of stages I–II was associated with an inflammatory stromal type, high counts of $CD163^+$ macrophages and $CXCL12^+$ fibroblasts in the tumor microenvironment, high expression of the chemokine receptor CXCR4, and reduced expression of its ligand CXCL12 in tumor cells. In the majority of EC of stage III, the increase in angiogenic, invasive, and metastatic potential was accompanied by the presence of desmoplastic stroma, increased expression of CXCR4 in tumor cells, and a high count of $CXCL12^+$ fibroblasts. **Conclusions.** The obtained results showed that the morphological architecture of the stromal ECE component is related to the molecular features of its constituents and tumor cells. Their interaction modulates the phenotypic characteristics of ECE associated with the degree of malignancy.

Keywords: endometrioid endometrial carcinoma, desmoplastic, inflammatory stroma, $CXCL12^+$ fibroblasts, $CD163^+$ macrophages, microvessel density.

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The data of modern scientific research indicate that malignantly transformed cells are surrounded by a specific stromal microenvironment, which affects the biological properties of the tumor, in particular, its ability for invasive growth and metastasis [1—3]. The stromal microenvironment consists of fibroblasts, immune cells, adipocytes, a vascular system with endothelial cells and pericytes, and an extracellular matrix, the main components of which are structural and specialized proteins (collagen, elastin, fibrillin, fibronectin), growth factors (HGF, TGF-β, VEGF, FGF), cytokines, chemokines, including IL-6, CXCL12, and its receptor CXCR4. The latter are secreted upon para- and autocrine communication of tumor cells with components of the stromal microenvironment, in particular, tumor-associated fibroblasts, macrophages, a subpopulation of T-regulatory lymphocytes (T_{reg}), which to a certain extent determine the progression of malignant neoplasms.

The interaction of CXCL12 chemokine with its CXCR4 receptor activates a number of intracellular signaling pathways, including RAS/MAPK, PI3K/AKT-mTOR, NFκB, MAPK/ERK, resulting in inhibition of apoptosis and stimulation of the proliferative potential of tumor cells [2, 4—12].

According to the literature, tumor-associated fibroblasts contribute to the increase in the expression of fibrillar collagen protein, which causes fibrous changes in the stromal component of a malignant tumor, that is, desmoplasia, which is associated with the tumor aggressiveness in most malignancies, in particular, breast, colon, pancreatic, and endometrial cancer [12—16].

Along with this, it is known that inflammatory cells, which include macrophages, are essential in oncogenesis, playing a dualistic role in this process, that is, they can exert both antitumor and protumoral properties. It should be noted that tumor cells and their microenvironment, which is formed in the setting of chronic inflammation, secrete a certain spectrum of cytokines (chemokines), some of which polarize M1 macrophages exerting an antitumor effect and some — M2 macrophages with immunosuppressive properties. The functional features of M2 macrophages are related to the pathophysiological characteristics of the tumor and the activity of other components of the stromal microenvironment and the secretion of a spectrum of mediators, which contributes to tumor growth, invasion, angiogenesis, and metastasis [17—19].

Changes in the ratio of components of the tumor microenvironment modulate the stromal signature and play an important role in the mechanisms of immune avoidance by tumor cells, contributing to the growth of the angiogenic, invasive, and metastatic potential of a malignant neoplasm [2].

To date, the question of the significance of the association between the stromal reaction and the biological features of endometrioid carcinoma of the endometrium (ECE) and its influence on tumor aggressiveness remains poorly studied. Wei et al. [20] demonstrated that desmoplastic stroma correlates with the advanced stage of endometrial cancer (III and IV according to FIGO) and lymphovascular invasion. The inflammatory stromal reaction is associated with the early stage of the disease and its more favorable course but is not associated with the tumor differentiation grade and lymphovascular invasion. The authors emphasize that further studies of the role of the stromal component of ECE in the development of metastases are required.

Espinosa et al. [21] found that desmoplastic stroma in ECE was associated with the expression of SPARC and MMP11 markers on fibroblasts, while inflammatory stroma — with the expression of CD163, CD16, and CD32 on macrophages. The researchers showed a relationship between the inflammatory stromal reaction and the expression of markers of the macrophage lineage (CD163, CD16, and CD32) and ECE of low differentiation grade and the presence of lymphovascular invasion and PIK3CA mutations in tumor cells. In addition, in 83.0% of

ECE patients with metastases, the expression of CD163, CD16, and CD32 was observed both in the primary tumor and in the metastatic lymph nodes. Nevertheless, no relationship between the content of fibroblasts expressing SPARC and MMP11 and the clinical and pathological characteristics of ECE was found.

Therefore, the aim of the work was to determine the association of ECE progression indicators with the type of stromal microenvironment, the content of $CXCL12^+$ fibroblasts, $CD163^+$ macrophages, and $CXCL12^+$ and $CXCR4^+$ tumor cells.

Materials and Methods

The work was performed on samples of surgical material of 51 patients with ECE of stages I—II and III according to FIGO. The average age of the patients was 60.3 ± 1.4 years. All patients did not receive preoperative therapy and were treated in the Department of Oncology and Gynecology (headed by Prof. V.S. Svintsitskyi) of the National Cancer Institute of the Ministry of Health of Ukraine in the period from 2014 to 2019. All patients provided informed consent on the use of their biological material for scientific research. According to the conclusion of the Commission on Bioethics of IEPOR, all necessary ethical standards were complied in accordance with the requirements of international rules of the Helsinki Declaration 2008.

Morphological diagnosis, tumor differentiation grade, and the assessment of cytomorphological characteristics of the stromal microenvironment were determined using histological samples stained with hematoxylin and eosin, according to WHO criteria [22].

Immunohistochemical (IHC) detection of biomolecular markers was performed on parallel, deparaffinized sections using primary MoAbs to CXCR4, (NP PA3-305, Thermo Fisher Scientific, USA), CXCL12, (79018, Thermo Fisher Scientific, USA), CD163 (Mob460-05, DiagnosticBioSystems, USA). The number of *de novo* microvessels was detected by the expression of the vascular endothelium marker CD31 (EP78, Diagnostic BioSystems, USA). The PolyVue HRP/DAB Detection System (Diagnostic BioSystems, USA) and the chromogen 3-diaminobenzidine tetrachloride (DAB) were used to detect antigen-bound antibodies. Cell nuclei were stained with Mayer's hematoxylin.

The results of the IHC reaction were evaluated by a semiquantitative method by counting 800—1000 tumor cells per preparation. The number of positively stained cells was determined as a percentage, i.e. with the labeling index (LI, %).

The number of tumor cells positive for CXCL12 expression and the counts of $CXCL12^+$ fibroblasts were determined on one histological preparation. CXCL12 expression was considered positive when the protein was localized on the membrane or in the cytoplasm, and its receptor CXCR4 — in the cytoplasm. The counts of $CXCL12^+$ fibroblasts and $CD163^+$ macrophages were determined as the number of cells per field of view (cells/f.v.) in 10 fields of view at a magnification of x400.

To determine the microvessels density (MVD) in ECE, the number of vessels in 10 f.v. was counted (magnification x100, the size of one field of view was limited to a measuring square grid with a side of 1.25 mm). The obtained indicators were calculated according to the formula:

$$MVD = n : 1.56 \text{ vessels/mm}^2,$$

where n is the average number of vessels in one field of view; 1.56 mm^2 is the area of one field of view. Values of LI, cells/f.v., and MVD less than the median (Me) were considered low, and values greater than Me were considered high.

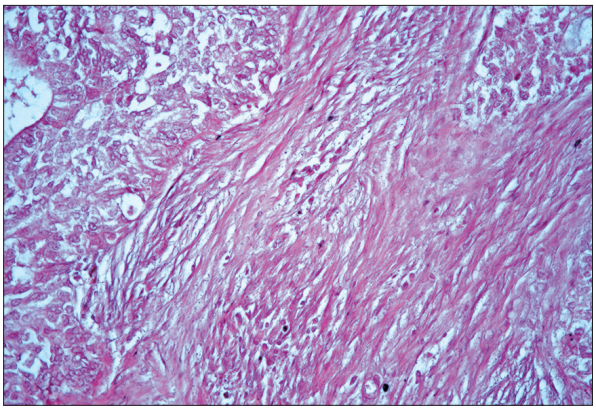
Statistical data processing was carried out using the STATISTICA 8.0 software package (StatSoft, Inc.) and non-parametric tests such as the Mann — Whitney test, Fisher (F-test), and correlation analysis (R — Spearman's rank correlation coefficient). $p < 0.05$ was taken as the level of statistical significance.

Results and Discussion

Examination of the samples of postoperative material from 51 patients with ECE for the morphological features of the stromal microenvironment according to the criteria of Espinosa et al. [21] revealed the tumors with desmoplastic and inflammatory stroma, which made up two study groups. Group I (n = 20) was composed of tumors with signs of desmoplastic stroma (Fig. 1). The characteristics of these tumors were the presence of stromal edema and immature (gray) or mature (pink) collagen. When both these features were revealed in at least one third of the histological sample, the presence of desmoplasia was evident. Group II (n = 31) with an inflammatory type of stroma was characterized by lymphocytic-histiocytic infiltration revealed at least in one third of the histological sample.

The results of the morphological study showed that the majority of tumors with a desmoplastic stromal variant were of low differentiation grade with deep invasion into the myometrium (80.0%), and in 65.0% of the cases, stage III of the disease was determined (Table 1).

77.4% of the ECE cases with an inflammatory type of stroma were of stage I—II and showed an



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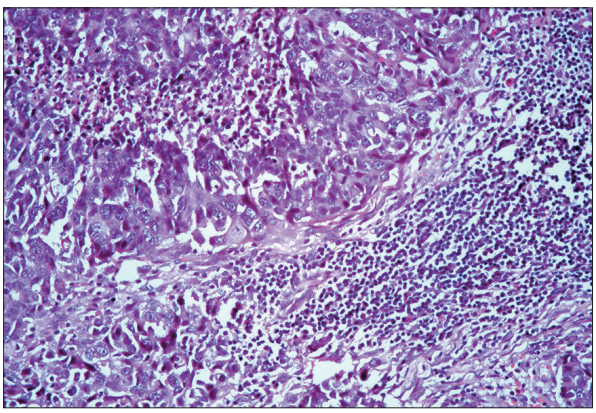

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Fig. 1. Microphoto of ECE with different stromal types: *a* — desmoplasia, *b* — inflammatory. H&E staining, ×400

Table 1. **Clinical and pathological characteristics of patients with ECE depending on the characteristics of the stromal component (n/%)**

Parameter	Morphological features of stroma			
	Desmoplastic, n = 20/100%		Inflammatory, n = 31/100%	
	Stage of the disease			
	I—II, n = 7/35.0	III, n = 13/65.0	I—II, n = 24/77.4	III, n = 7/22.6
Differentiation grade				
G2	2/10.0	2/10.0	13/41.9	1/3.2
G3	5/25.0	11/55.0$^{*,\#}$	11/35.5	6/19.4$^{*,\#}$
Invasion into the myometrium				
<1/2	3/15.0	1/5.0	12/38.7	1/3.2
>1/2	4/20.0	12/60.0**	12/38.7	6/19.4**

Notes: $^{*}p < 0.05$ compared to G2 tumors, $^{**}p < 0.05$ compared to tumors with invasion <1/2 in the myometrium, $^{\#}p < 0.05$ compared to ECE of stages I—II (F-test).

almost equal frequency of differentiation grade (G2 in 45.1%, G3 in 54.9%) and the depth of tumor invasion into the myometrium (<1/2 in 41.9%, >1/2 in 58.1%).

The IHC study has shown that ECE samples with desmoplastic stroma were characterized by significantly higher counts of $CXCL12^+$ fibroblasts and lower counts of $CD163^+$ macrophages compared to ECE with the inflammatory type of stroma (Table 2).

Also, a correlation was established (R = 0.74, $p < 0.05$) between the number of $CXCL12^+$ fibroblasts and $CXCR4^+$ tumor cells in ECE with desmoplasia.

We have studied the expression of biomolecular markers in tumor cells and the content of the microenvironment components in ECE with inflammatory and desmoplastic stroma depending on some clinicopathological characteristics of the tumor, namely its differentiation grade and the depth of invasion in the myometrium. We found that in ECE with an inflammatory stroma of low differentiation grade and deep invasion, the percentage of tumor cells with CXCL12 expression decreased, while with CXCR4 — increased, compared to ECE of the moderate differentiation grade and invasion of the myometrium <1/2 (Table 3).

In addition, the lowest content of $CD163^+$ macrophages was observed in G3 tumors with invasion <1/2 myometrium. In G2 tumors with deep and shallow invasion, the number of $CD163^+$ macrophages was almost the same and did not differ from that in G3 carcinomas with invasion >1/2. At the same time, a higher content of $CXCL12^+$ fibroblasts was observed in ECE with deep invasion, both in G2 and G3 tumors. It should be noted that the higher percentage of tumor cells with CXCR4 expression, the lower percentage with CXCL12 expression and a high count of $CXCL12^+$ fibroblasts in G3 carcinomas with invasion >1/2 of the myometrium were accompanied by a higher MVD compared to G2 neoplasms with invasion <1/2 of the myometrium ($p < 0.05$) (Table 3).

In ECE with desmoplasia, the percentage of tumor cells expressing the chemokine receptor CXCR4 was almost the same in tumors of different differentiation grades and different depths of tumor invasion into the myometrium. It is important to note that with this type of stromal microenvironment, an increase in the counts of $CD163^+$ macrophages, $CXCL12^+$ fibroblasts, and MVD was observed in poorly differentiated carcinomas with deep invasion compared to G2 tumors with invasion <1/2 of the myometrium (Table 4).

Therefore, with a decrease in the tumor differentiation grade and an increase in the ECE invasiveness, there was observed a decrease in the counts of $CXCL12^+$ tumor cells and an increase in $CXCL12^+$ fibroblasts and MVD, regardless of the stromal type. In contrast, the nature of changes in the counts of $CXCR4^+$ tumor cells and $CD163^+$ macrophages with increasing degree of malignancy was different depending on the type of stroma. In ECE with desmoplastic stroma, the percentage of $CXCR4^+$ tumor cells did not change with changing the tumor differentiation

Table 2. **Expression of biomarkers in ECE with different stromal types**

Type of stroma	Expression of markers in ECE cells, LI, %, M ± m		Count of stromal cells, cells/f.v.		MVD/mm^2
	CXCR4	CXCL12	$CD163^+$ macrophages	$CXCL12^+$ fibroblasts	
Inflammatory	44.3 ± 4.7	29.5 ± 3.3	38.6 ± 3.1*	9.5 ± 1.4*	39.2 ± 6.4
Desmoplastic	46.4 ± 5.3	24.9 ± 3.6	26.0 ± 4.6	25.4 ± 2.0	36.2 ± 6.2

Note: *$p < 0.05$ compared to the desmoplastic stroma.

grade and depth of invasion, but the content of $CD163^+$ macrophages increased in deeply invading G3 carcinomas, compared to the indicators in G2 tumors with invasion < 1/2 myometrium. In ECE with inflammatory stroma, the percentage of $CXCR4^+$ tumor cells increased and the content of $CD163^+$ macrophages did not change with decreasing the tumor differentiation grade and increasing the invasiveness.

The counts of CXCR4-expressing tumor cells, $CD163^+$ macrophages, and MVD in tumors with an inflammatory stromal type, low differentiation grade, and deep invasion into the myometrium were higher than these indices in ECE with desmoplastic stroma (by 1.3, 1.4, and 1.3 times respectively). On the other hand, in ECE with desmoplasia, low differentiation grade, and deep invasion into the myometrium, a 1.4-fold increase in $CXCL12^+$ fibroblast counts was observed as compared to ECE with inflammatory stroma.

In EC with both the desmoid and inflammatory types of stroma and metastases, we detected an insignificant tendency to the increased counts of $CXCR4^+$ tumor cells compared to ECE cases without metastases. In ECE cases without metastases and with desmoplastic or inflammatory stroma, a significantly higher percentage of CXCL12-positive tumor cells was observed compared to ECE with metastases. The lowest percentage of tumor cells expressing CXCL12 was observed in ECE with the inflammatory type of stroma and metastases. At the same time, in such tumors, the largest MVD was observed

Table 3. **Expression of biomarkers in ECE with inflammatory stroma depending on clinical and pathological characteristics**

Clinical and pathological characteristics of ECE	Expression of markers in ECE cells, LI, %, M ± m		Count of stromal cells, cells/f.v.		MVD/mm^2
	CXCR4	CXCL12	$CD163^+$ macrophages	$CXCL12^+$ fibroblasts	
G2 < 1/2	29.4 ± 3.7*	44.9 ± 4.3*	42.2 ± 3.6	7.4 ± 0.9	33.7 ± 4.1*
G2 > 1/2	49.7 ± 10.3	21.9 ± 4.9	40.1 ± 4.9	11.1 ± 4.2	46.1 ± 5.2
G3 < 1/2	41.7 ± 10.4	33.6 ± 8.7	25.9 ± 4.8	6.6 ± 1.6	20.3 ± 2.1
G3 > 1/2	56.3 ± 10.5	19.6 ± 4.1	41.9 ± 7.8	12.3 ± 1.5	55.2 ± 5.6

Note: *$p < 0.05$ compared to G3 tumors that deeply invaded the myometrium.

Table 4. **Expression of biomarkers in ECE with desmoplastic stroma depending on clinical and pathological characteristics**

Clinical and pathological characteristics of ECE	Expression of markers in ECE cells, LI, %, M ± m		Count of stromal cells, cells/f.v.		MVD/mm^2
	CXCR4	CXCL12	$CD163^+$ macrophages	$CXCL12^+$ fibroblasts	
G2 < 1/2	41.6 ± 9.5	46.8 ± 9.6*	19.0 ± 4.2*	11.7 ± 2.7*	28.3 ± 2.5*
G2 > 1/2	33.5 ± 6.3	19.5 ± 3.7	24.4 ± 6.0	15.4 ± 3.4	37.6 ± 3.8
G3 < 1/2	39.2 ± 8.2	30.3 ± 0.7	14.4 ± 6.4	22.7 ± 2.7	34.5 ± 3.2
G3 > 1/2	44.3 ± 6.5	16.5 ± 4.2	29.3 ± 5.7	29.8 ± 2.3	42.8 ± 4.9

Note: *$p < 0.05$ compared to G3 tumors that deeply invaded the myometrium.

compared to the ECE cases without metastases and cases with desmoplasia without and with metastatic lesions (Table 5).

It should be noted that the inflammatory type of stroma in ECE was characterized by the highest MVD (in G3 tumors with deep invasion) and that this type of stroma was observed most often (in 77.4% of cases) in patients with stages I—II. Despite the favorable ECE course, in 7.0% of patients, even at stage I, within 0.5—3 years after treatment, relapses of the disease occur [23]. Therefore, it seems reasonable to investigate the expression of biomolecular markers in ECE of the initial stages (I—II) depending on the tumor differentiation grade and the depth of tumor invasion into the myometrium.

The study established that there was no significant difference in the percentage of tumor cells with CXCL12 expression and the counts of $CD163^+$ macrophages and $CXCL12^+$ fibroblasts in G2 and G3 tumors. At the same time, in G3 tumors, a tendency to the increased percentage of $CXCR4^+$ tumor cells and MVD compared to G2 tumors was observed (Table 6).

Along with this, it was established that the content of $CXCR4^+$ tumor cells, $CD163^+$ mac-

Table 5. **Expression of biomarkers in ECE depending on the tumor process stage and the type of stromal component**

Stage of disease and type of ECE stroma	Expression of markers in ECE cells, LI, %, M ± m		Count of stromal cells, cells/f.v.		MVD/mm^2
	CXCR4	CXCL12	$CD163^+$ macrophages	$CXCL12^+$ fibroblasts	
Stage I—II (without metastases)					
Desmoid (n = 7)	38.9 ± 9.2	33.9 ± 5.7	26.1 ± 1.3	14.9 ± 2.5	34.7 ± 6.0
Inflammatory (n = 24)	36.7 ± 9.7	34.8 ± 3.2	30.7 ± 5.3	9.6 ± 1.7*	37.5 ± 5.9
Stage III (with metastases)					
Desmoid (n = 13)	48.7 ± 6.6	21.0 ± 4.2	25.9 ± 5.7	18.6 ± 3.6	38.4 ± 9.9
Inflammatory (n = 7)	45.0 ± 5.5	10.7 ± 3.2*,**	40.2 ± 3.3*	9.1 ± 1.3*	49.4 ± 3.2**

Note: *$p < 0.05$ compared to ECE with desmoid stroma and the same stage; **$p < 0.05$ compared to ECE of stage III and desmoid stroma, and ECE of stages I—II and desmoid or inflammatory type of stroma.

Table 6. **Expression of biomarkers in ECE with an inflammatory type of stroma in cases with stage I–II of the disease**

Parameter	Expression of markers in ECE cells, LI, %, M ± m		Count of stromal cells, cells/f.v.		MVD/mm^2
	CXCR4	CXCL12	$CD163^+$ macrophages	$CXCL12^+$ fibroblasts	
Differentiation grade					
G2	39.1 ± 5.7	31.4 ± 4.5	36.2 ± 5.3	8.3 ± 2.5	36.8 ± 6.2
G3	49.6 ± 7.4	27.4 ± 3.2	41.4 ± 2.6	10.6 ± 1.3	42.6 ± 8.5
Invasion into the myometrium					
< 1/2	31.6 ± 5.6	39.7 ± 4.2	34.9 ± 3.9	6.6 ± 3.6	30.7 ± 4.2
> 1/2	58.0 ± 7.5*	24.3 ± 2.8	44.5 ± 5.0*	13.1 ± 1.3*	47.5 ± 6.9*

Note: *$p < 0.05$ compared to ECE with invasion <1/2 into the myometrium.

rophages, and CXCL12$^+$ fibroblasts significantly increased in ECE with the deep invasion of the myometrium, while the percentage of CXCL12$^+$ tumor cells decreased compared to ECE, which infiltrated the myometrium shallowly. At the same time, the MVD in ECE of stages I—II with deep invasion was almost equal to MVD in ECE with the inflammatory stromal type and metastasis (see Table 5). Correlation analysis showed that in such tumors, a high content of CXCL12$^+$ fibroblasts (>Me = 8.6%) and CD163$^+$ macrophages (>Me = 37.7%) positively correlated with a high percentage of CXCR4$^+$ tumor cells (R = 0.63, $p < 0.05$). That is, the counts of tumor cells with CXCR4 expression significantly increased along with the rise of counts of CXCL12$^+$ fibroblasts and CD163$^+$ macrophages.

Therefore, our study allowed us to establish that the desmoplastic type of the stromal microenvironment of ECE, characterized by a high content of CXCL12$^+$ fibroblasts and the inflammatory stromal type with a high content of CD163$^+$ macrophages correlates with some indicators of tumor progression, in particular, low differentiation grade, deep invasion of tumor into the myometrium, and metastasis. We determined that in ECE of stages I and II and the inflammatory stromal type, high counts of CD163$^+$ macrophages, CXCL12$^+$ fibroblasts, a high percentage of CXCR4 expressing tumor cells, and reduced counts of tumor cells with CXCL12 expression are associated with a more aggressive course of the disease and the high angiogenic and invasive potential of the neoplasm.

The stromal microenvironment plays a significant role in the progression of a malignant neoplasm [2, 3]. Most studies have shown that tumor-associated fibroblasts and M2-macrophages are among the most common cells in the tumor microenvironment, and their dynamic relationship with each other and with tumor cells modulate the biology of a malignant neoplasm [12]. It should be noted that tumor-associated fibroblasts, macrophages, lymphocytes, including T-regulatory ones, as well as endothelial and epithelial cells expressing the chemokine CXCL12 and its receptor CXCR4 play a significant role in chemotaxis, invasion, angiogenesis, metastasis, and therapeutic resistance of many malignant tumors [4—7].

Also, it has been established that the presence of a fibroblastic (desmoplasia) stromal reaction in malignant tumors of various genesis is associated with the aggressiveness of the tumor process [20, 24, 25], while the development of tumors with an inflammatory stroma depends on the composition of cell populations and the ratio of immune cells. A high content of immunocompetent cells with CD8$^+$ cytotoxic properties is associated with a favorable course of the disease, while an increased content of CD163$^+$macrophages correlates with cancer aggressiveness in various nosological forms of malignant neoplasms [26—29].

We have established that in ECE cases of stages I—II, the inflammatory type of stroma was found nearly 3.5 times more often than the desmoid type. In contrast, the desmoid type was found triply more often than the stromal type in ECE cases with metastases. The majority (4/5) of ECE cases with desmoplasia were of low differentiation grade and deep invasion into the myometrium.

Along with this, the association of CXCL12$^+$ fibroblasts and CD163$^+$ macrophages with a certain architecture of the stromal microenvironment and features of the CXCL12 and CXCR4 expression in the epithelial component of ECE has been shown. We determined that the desmoplastic type of stroma in ECE is associated with a high content of CXCL12$^+$ fibroblasts, while the inflammatory type is associated with a high content of CD163$^+$ macrophages. Also, in the metastatic ECE cases with desmoplasia, low differentiation grade and deep invasion along with a high percentage of tumor cells with CXCR4 expression were found, which may be associated with the increased counts of CXCL12$^+$ fibroblasts. According to the current literature, the secretion of CXCL12 by tumor-associated fibroblasts can

induce the expression of CXCR4 [30]. In addition, the high percentage of $CXCR4^+$ tumor cells may be the result of exposure to their direct activators TGF-β and S18-2, which leads to increased migration ability of malignant cells [31, 32].

The obtained data on the high content of $CXCL12^+$ fibroblasts in the tumors of patients with metastases are consistent with the results of other researchers who showed that the interaction between tumor-associated fibroblasts and tumor cells can increase the metastatic potential of malignant cells, in particular, in tumors of the mammary gland and endometrium [33, 34].

The increased counts of $CD163^+$ macrophages in the tumor microenvironment of metastatic ECE cases with an inflammatory type of stroma and in ECE of stages I—II, low differentiation grade, and the deep invasion seem to result from increased counts of $CXCL12^+$ fibroblasts as far as the CXCL12 chemokine is a powerful chemoattractant for M2 macrophages [17].

It is important to note the role of CXCL12 and its receptor CXCR4 in increasing the neovascularization of endometrial tumors via stimulation of VEGF expression, which, in turn, can stimulate the expression of both CXCL12 and CXCR4 [8, 35]. Also, it was shown that VEGF secretion is promoted by $CXCL12^+$ fibroblasts and $CD163^+$ macrophages [36]. That is, the high rate of neovascularization (MVD), determined in the inflammatory stromal type in ECE cases of stage III and in ECE of stages I—II, low differentiation grade, and deep invasion in the myometrium may be due to the high content of $CXCL12^+$ fibroblasts and $CD163^+$ macrophages.

As known, a high estrogen content is an important factor regulating the expression of CXCR4 and CXCL12 [37]. It is possible that the increased counts of tumor cells with CXCR4 expression in low differentiation grade ECE with deep invasion are due to the high concentration of estradiol in the peripheral blood of patients with ECE [38]. Along with this, the chemokine CXCL12, expressed by epithelial tumor cells, regulates the expression of its receptor in an autocrine mode, which correlates with the lower malignant potential of the neoplasm. Indeed, the data of literature indicate that patients with colorectal, breast, and endometrial cancer, whose tumor cells express high levels of CXCR4 and CXCL12, have a more favorable prognosis of the disease. In contrast, tumor cells with high expression of CXCR4 and low expression of its ligand migrate to tissues with a high level of CXCL12 expression, which correlates with the aggressiveness of the tumor process [33, 39, 40].

To sum up, we have identified variants of the stromal component of ECE associated with its cellular composition: $CXCL12^+$ fibroblasts and M2 macrophages, the interaction of which with tumor cells modulates the phenotypic characteristics of tumor associated with its aggressiveness. The data obtained will allow for a more objective assessment of the ECE malignancy degree in patients with ECE of stages I—II and the improvement of the treatment strategy.

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РОЛЬ СТРОМАЛЬНОГО МІКРООТОЧЕННЯ У ФОРМУВАННІ ІНВАЗИВНОГО, АНГІОГЕННОГО І МЕТАСТАТИЧНОГО ПОТЕНЦІАЛУ В ЕНДОМЕТРІОЇДНІЙ КАРЦИНОМІ ЕНДОМЕТРІЮ

Мета дослідження полягала у визначенні асоціації між показниками прогресії ендометріоїдної карциноми ендометрію (ЕКЕ) і типом стромального мікрооточення, вмістом $CXCL12^+$- фібробластів і $CD163^+$-макрофагів та експресією хемокіну CXCL12 і його рецептора CXCR4 в пухлинних клітинах. **Об'єкти і методи.** Проаналізовано гістологічні препарати 81 хворої на рак ендометрію (РЕ), забарвлені гематоксиліном та еозином. Імуногістохімічним методом з використанням первинних МкАТ визначали антигени CXCL2 і CXCR4 у пухлинних клітинах, вміст $CXCL12^+$-фібробластів і $CD163^+$-макрофагів. Щільність мікросудин виявляли за допомогою CD31. **Результати.** Визначено групи новоутворень з десмопластичною та запальною стромальною реакцією. Більшість (80,0%) пухлин з десмоплазією були низького ступеня диференціювання, глибоко інвазували міометрій; у 65,0% хворих з такими пухлинами визначалась III стадія пухлинного процесу. Натомість, у хворих з I-II стадією пухлинного процесу у 77,4% ЕКЕ виявлено запальний тип строми. Визначено, що високий ангіогенний та інвазивний потенціал карцином ендометрію у хворих з I-II стадією пухлинного процесу асоціювався з запальним типом стромальної реакції, високим вмістом $CD163^+$-макрофагів і $CXCL12^+$- фібробластів у пухлинному мікрооточенні, високою експресією рецептора хемокіну CXCR4 та зниженою експресією його ліганда CXCL12 в пухлинних клітинах. У більшості карцином ендометрію хворих з III стадією захворювання зростання ангіогенного, інвазивного і метастатичного потенціалу супроводжувалось наявністю десмопластичної строми, підвищеною експресією CXCR4 у пухлинних клітинах і високим вмістом $CXCL12^+$-фібробластів. **Висновки.** Отримані результати показали, що морфологічна архітектоніка стромального компоненту ЕКЕ пов'язана з молекулярними особливостями його складових і пухлинних клітин. Їхня взаємодія модулює асоційовані зі ступенем злоякісності фенотипові характеристики ЕКЕ.

Ключові слова: ендометріоїдна карцинома ендометрія (ЕКЕ), десмопластична, запальна строма, $CXCL12^+$-фібробласти, $CD163^+$-макрофаги, щільність мікросудин.