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OSTEOPONTIN-REGULATED CHANGES IN THE MAST CELL POPULATION ASSOCIATED WITH BREAST CANCER

Background. The development of breast cancer (BCa) is largely determined by the characteristics of the tumor microenvironment (TME), which undergoes significant changes during the progression of the disease. Mast cells (MCs) are among the least studied components of the TME. **The aim** of the work was to investigate the relationship between the density of infiltration and the functional activity of MCs with indicators of osteopontin (OP) expression in BCa tissue. **Materials and Methods**. The study was conducted on the postoperative material of 15 patients with fibroadenoma and 78 patients with stage I—II BCa. MCs in the tissue of benign and malignant breast tumors were detected by a histochemical method using toluidine blue. The functional activity of MCs was calculated by the degranulation index. The OP expression in tumor tissue was assessed by the immunohistochemical method. **Results**. The density of MCs infiltration and their functional activity are associated with such indicators of BCa malignancy as tumor size, lymph node involvement, tumor grade, molecular subtype, proliferative activity, and PR- and HER2/neu-expression status. A high expression of OP in the stromal component of BCa is associated with the growth of the tumoral MCs population, metastatic lesions in regional lymph nodes, and a low differentiation grade of the tumors. In addition, OP is involved in the regulation of MCs in the tissue of the luminal B and basal molecular BCa subtypes. The level of OP expression in the parenchymal component of BCa is associated with the number of infiltrated MCs in the presence of metastatic lesions of regional lymph nodes. **Conclusions**. The identified relationship of OP expression level with the topology and functional activity of MCs in BCa tissue, depending on the clinical status of patients, indicates the prospects for their use in predicting the aggressiveness of the tumor process.

Keywords: breast cancer, mast cells, osteopontin, prognosis.

Breast cancer (BCa) is the most frequently diagnosed malignant neoplasm in women worldwide. According to the data from the International Agency for Research on Cancer (GLOBOCAN), the incidence of BCa in 2022 has reached almost 2.3 million new cases, surpassing lung cancer by 2.5 times [1]. In addition, there is a stable upward trend (by 0.2% per year) in the incidence of invasive BCa in young women (<50 years old) [2—4]. Despite the obvious progress in the treatment of BCa, the

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mortality rate among patients with this pathology is still the highest. It reaches 12.7 cases per 100,000 female population, which defines it as an urgent medical and biological problem.

It has been proven that the development of BCa is largely determined by the characteristics of the tumor microenvironment (TME), which undergoes significant changes during the disease progression. It has been shown that individual components of the TME, including tumor-infiltrating lymphocytes, tumor-associated macrophages, and fibroblasts can modulate the growth and migration of tumor cells, thereby playing an important role in the BCa metastasis [5, 6]. Among the least studied components of the TME, the role of which in BCa is still debatable, one could mention mast cells (MCs). The data on the relationship between MCs infiltration and tumor progression are contradictory [7], which is probably related to the functional heterogeneity of MCs. It is known that MCs are capable of inducing/stimulating angiogenesis and lymphangiogenesis by secreting VEGF and FGF-2 as well as stimulating the invasive activity of the malignantly transformed cells through the action of gelatinases (MMP-2 and MMP-9). It has been reported that MCs can secrete histamine and IL-10 into the TME and inhibit the activity of T cells and natural killer cells resulting in suppression of the immune response. In contrast, several works note the antitumor role of this type of immune cells. In particular, MCs inhibit the growth of tumor cells and induce their apoptotic death by releasing IL-1, IL-4, IL-6, IL-8, CCL7, CCL13, and TGF-β [8]. In addition, histamine released by MCs can stimulate vascular endothelial cells to produce prostacyclin, which promotes necrosis of transformed cells and inhibits metastasis [9].

Over the past two decades, increasing attention has been paid not only to the prognostic role of MCs in cancer of various histogenesis but also to the search for regulators of their functional activity. The regulatory role of many ILs and immunoglobulins, which control the secretion and degranulation of MCs, has been widely described [10, 11]. However, in recent years, there have been isolated reports on the dynamic interaction between MCs and malignantly transformed cells of the TME and its acellular components, which play an important role in the progression of the disease and the development of resistance to therapy [12]. At the same time, the overall picture of the regulatory effects of TME elements on MCs in BCa is not fully understood. The results of our previous studies allow us to assert the role of osteopontin (OP) in the migration of MCs to the TME in prostate cancer [13]. To date, the role of MCs has also been described, which through the mediated action of OP is involved in malignant pleural effusion promotion [14]. However, there are few studies on the effect of this matricellular protein on MCs in the TME of BCa. The aim of the work was to investigate the relationship between the density of infiltration and functional activity of MCs and the indicators of OP expression in BCa tissue.

Materials and Methods

The study was conducted on the postoperative material of 15 patients with fibroadenoma (FAd) and 78 patients with stage I—II BCa (detailed clinical characteristics are given in Table 1), who were treated at the Municipal Non-Commercial Enterprise "Kyiv City Oncology Center" in 2019—2022. All patients were examined using generally accepted clinical and laboratory methods following the Standards of diagnosis and treatment of cancer patients, approved by the Order of the Ministry of Health of Ukraine No. 396 dated 06/30/2015 (registration number GS 2015—396). No patient received neoadjuvant treatment. All donors provided their consent to participate in the scientific research.

Histochemical study. MCs in the tissue of benign and malignant mammary gland neoplasms were assessed by a histochemical method using toluidine blue (Sigma–Aldrich, USA). The histological slides were examined with an AxioScope A1 light microscope (Carl Zeiss, Germany).

The number of MCs was evaluated in 20 fields of view at ×200 magnification and presented as M ± m per unit area of 1 mm^2, where M is the arithmetic mean, and m is the standard error of the mean. MCs localization was considered intratumoral in cases when they were identified in the parenchymal component of the tumor or were located at the border of the stromal and parenchymal components of the tumor tissue. The stromal localization of MCs was considered when they were located in the stromal component of the TME of BCa and did not have visual contact with tumor cells. The degree of MCs degranulation was determined at ×1000 magnifica-

tion. MCs degranulation index (DI) was calculated according to the Lindner formula [15]:

$$ID = (A\times0 + B\times1 + C\times2 + D\times3)/n,$$

where A is inactive MCs (granules are densely located in the cytoplasm, the nucleus is not visualized), B – weakly degranulated MCs (the nucleus is well visualized, granules are located inside the cell and do not extend beyond the cytoplasmic membrane), C — moderately degranulated MCs (granules partially extend beyond boundaries of intact cytoplasm), D — strongly degranulated MCs (completely degranulated MCs with a ruptured cytoplasmic membrane), and n — a total number of analyzed cells. The obtained DI values were presented in conventional units (c.u.).

Immunohistochemical study. Determination of the OP, estrogen receptors (ER), progesterone (PR), human epidermal growth factor (Her2/neu), and Ki-67 expression in tumor tissue was performed on paraffin sections with a thickness of 5 μm. The monoclonal antibodies specific for OP (clone 441, dilution 1:300, Thermo Scientific, USA), ER (clone 1D5, dilution 1:100, DakoCytomation, Denmark), PR (clone PgR636, dilution 1:100, DakoCytomation, Denmark), Her2/neu (clone e2400, dilution 1:100, Thermo Scientific, USA), and Ki67 (clone MIB1, dilution 1:100, DakoCytomation, Denmark) were used as primary antibodies.

The Master Polymer Plus Detection System (Peroxidase) reagent kit (Incl. DAB Chromogen) (Master diagnostica, Spain) was used to visualize the reaction results following the manufacturer's recommendations; the sections were stained with Meyer's hematoxylin (Thermo Scientific Richard-Allan, USA).

Analysis of the results of expression in tumor cells was performed using the method of counting immunopositive cells on an AxioScope A1 light microscope (Carl Zeiss, Germany) with a magnification of ×400. The expression was evaluated using the H-Score method, calculating its level according to the formula:

$$S = 0\times N_0\,(\%) + 1\times N_1\,(\%) + 2\times N_2\,(\%) + 3\times N_3\,(\%),$$

where S is the "H-Score" indicator; N_0 — the number of cells with no expression; N_1, N_2, and N_3 — with low, medium, and high expression, respectively. The final result of the calculation was expressed in points: from 1 to 100 points as low, from 101 to 200 as medium, and from 201 to 300 as high level of expression [16, 17].

To evaluate the OP expression in the stroma of breast neoplasms, 10 photomicrographs per sample were made at a magnification of ×400. Microphoto analysis was performed using the ImageJ software package (LOCI, University of Wisconsin, USA) and the IHC profiler plugin [18]. Quantitative stromal expression was calculated using the optical density score (ODS) formula:

$$ODS = P_0(\%)\times1 + P_1(\%)\times2 + P_2(\%)\times3 + P_3(\%)\times4,$$

where ODS is the optical density score; P_0 — the number of pixels with missing expression; P_1, P_2, and P_3 — with low, medium, and high expression,

Table 1. **Clinical and pathological characteristics of patients with BCa**

Characteristics	Number of patients	
	n	%
Total number of patients	78	100
Average age, years	53.7 ± 8.2	
Age fluctuation, years	24—84	
Reproductive status		
Menstrual cycle preserved	18	23.0
Menopause	60	77.0
Clinical stage		
I	21	26.9
II	57	73.1
Lymph node involvement (category N)		
N0	44	56.4
N1	34	43.6
Histological type		
Infiltrative ductal adenocarcinoma	55	70.5
Infiltrative lobular adenocarcinoma	23	29.5
Tumor differentiation grade		
G1 (high)	5	6.4
G2 (moderate)	66	84.6
G3 (low)	7	9.0
Molecular subtype		
Luminal A	33	42.3
Luminal B	24	30.8
Triple-negative (Basal-like)	11	14.1
HER2/neu-positive	10	12.8

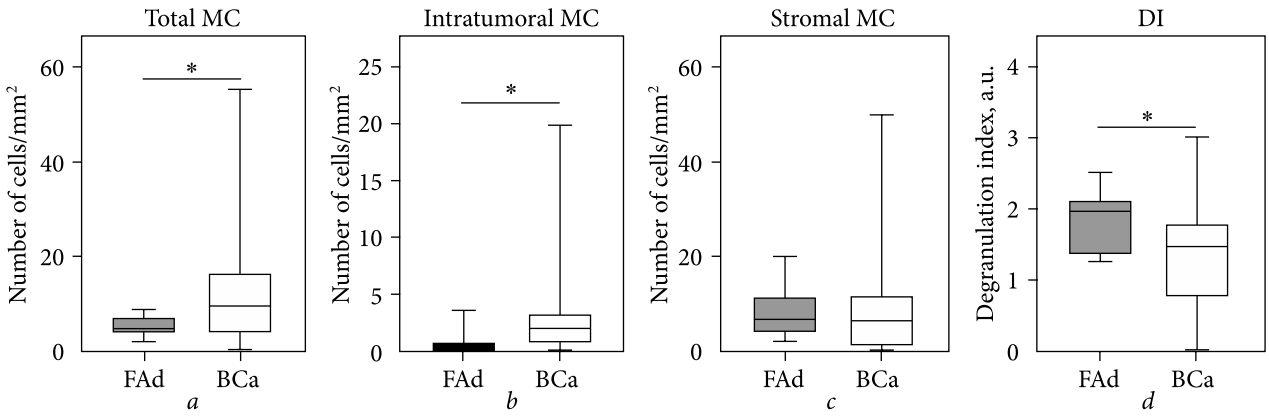

Fig. 1. Indicators of MCs infiltration (total — *a*, intratumoral — *b*, and stromal — *c*) and degranulation (*d*) in the tissues of benign and malignant breast neoplasms. *Note*: *The difference is significant, $p < 0.05$

respectively. The optical density score varied from 1 to 4 units.

Statistical analysis. Processing of the obtained data was performed using the software package GraphPad Prism v. 8.00 (GraphPad Software Inc., USA) A quantitative comparison of two independent groups was performed using the Mann — Whitney U-test, and Kruskal — Wallis test was used to compare three or more groups. The analysis data were presented in the form of M ± m, where M is the arithmetic mean, and m is the standard error of the mean. The critical level of the statistical significance was taken as equal to 0.05.

Results

Features of infiltration and functional activity of MCs in BCa tissue. At the first stage of the research, we conducted a comparative analysis of the features of infiltration and functional activity of MCs in benign and malignant neoplasms of the mammary gland (Figs. 1 and 2). It was shown that BCa tissue is characterized by a 2.0 times higher ($p = 0.0491$) level of MCs infiltration compared to FAd tissue. It should be noted that MCs in BCa tissue were 5.9 times ($p = 0.0004$) more often localized in the intratumoral region compared to FAd. No significant difference in MCs infiltration rates in the stromal component of benign and malignant neoplasms of the mammary gland was found. The MCs functional activity analysis made it possible to establish that the level of their degranulation in FAd tissue is 34% ($p = 0.0368$) higher compared to BCa tissue.

Subsequently, we studied the relationship between infiltration (Figs. 3—4) and functional activity (Table 2) of MCs and the clinical and pathological characteristics of BCa. It was revealed that T1 category BCa is characterized by 1.9 times ($p = 0.037$) higher rates of intratumoral MCs infiltration compared to T2 category tumors. The relationship between the number of MCs in BCa tissue and lymph node involvement was revealed. Significantly higher indicators of stromal infiltration (by 45.1%, $p = 0.017$) were recorded in the tumor tissue of patients with metastatic lesions in regional lymph nodes.

We found that a characteristic feature of G3 BCa is a low overall level of MCs infiltration ($p = 0.049$) compared to highly differentiated neoplasms. At the same time, there was observed a decrease in the number of MCs in the intratumoral zone of neoplasms ($p = 0.021$) against the background of an increase in their level in the stromal component of tumors ($p = 0.042$).

Also, the triple-negative and luminal B molecular BCa subtypes were characterized by significantly higher levels of MCs infiltration, as well as an increase in their number in the intratumoral and stromal components of the tumor compared to the tissue of neoplasms of the luminal A and HER2/neu positive subtypes.

A significantly higher MCs level (by 35.5%, $p = 0.031$) was identified in BCa tissue positive for HER2/neu expression compared to this indicator of HER2/neu - negative neoplasms. It should be noted that the increase in the infiltration of BCa by MCs was noted both in the intratumoral (by 44.1%, $p = 0.019$) and stromal (by 30.6%, $p = 0.009$) components of the tumors. Tumor tissue with a high proliferative activity (Ki-67 expression > 14%) was

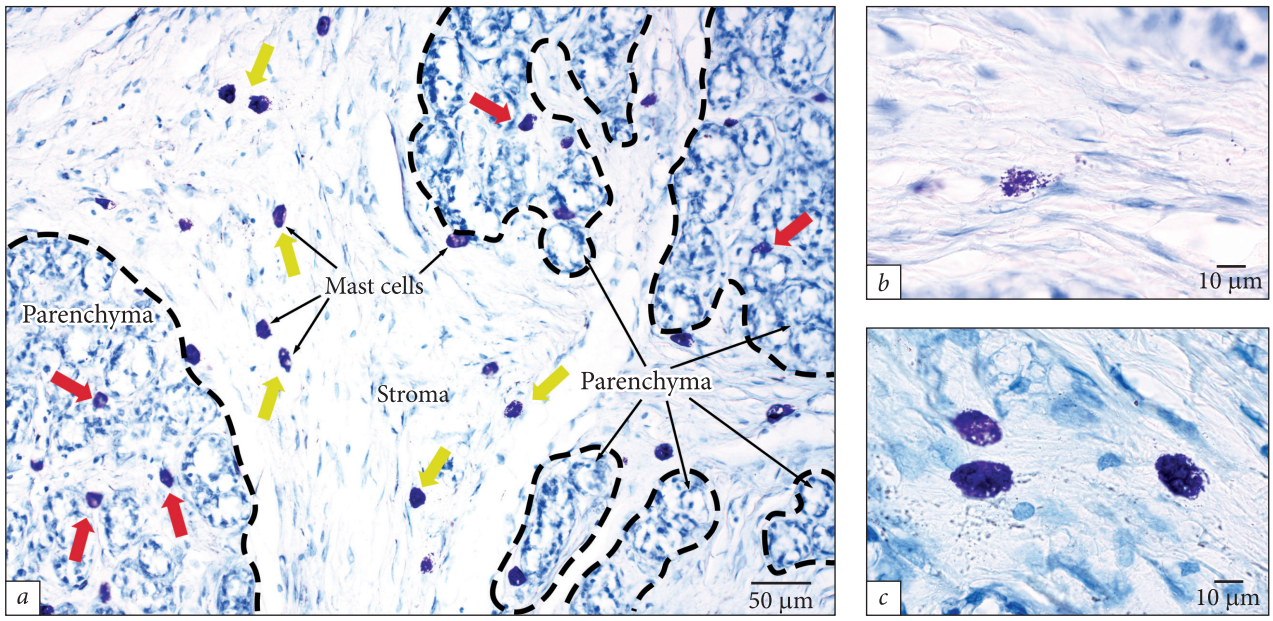

Fig. 2. MCs in parenchyma (red arrows) and stroma (yellow arrows) of BCa (*a*), as well as degranulated (*b*) and non-degranulated (*c*) MCs. Staining with toluidine blue

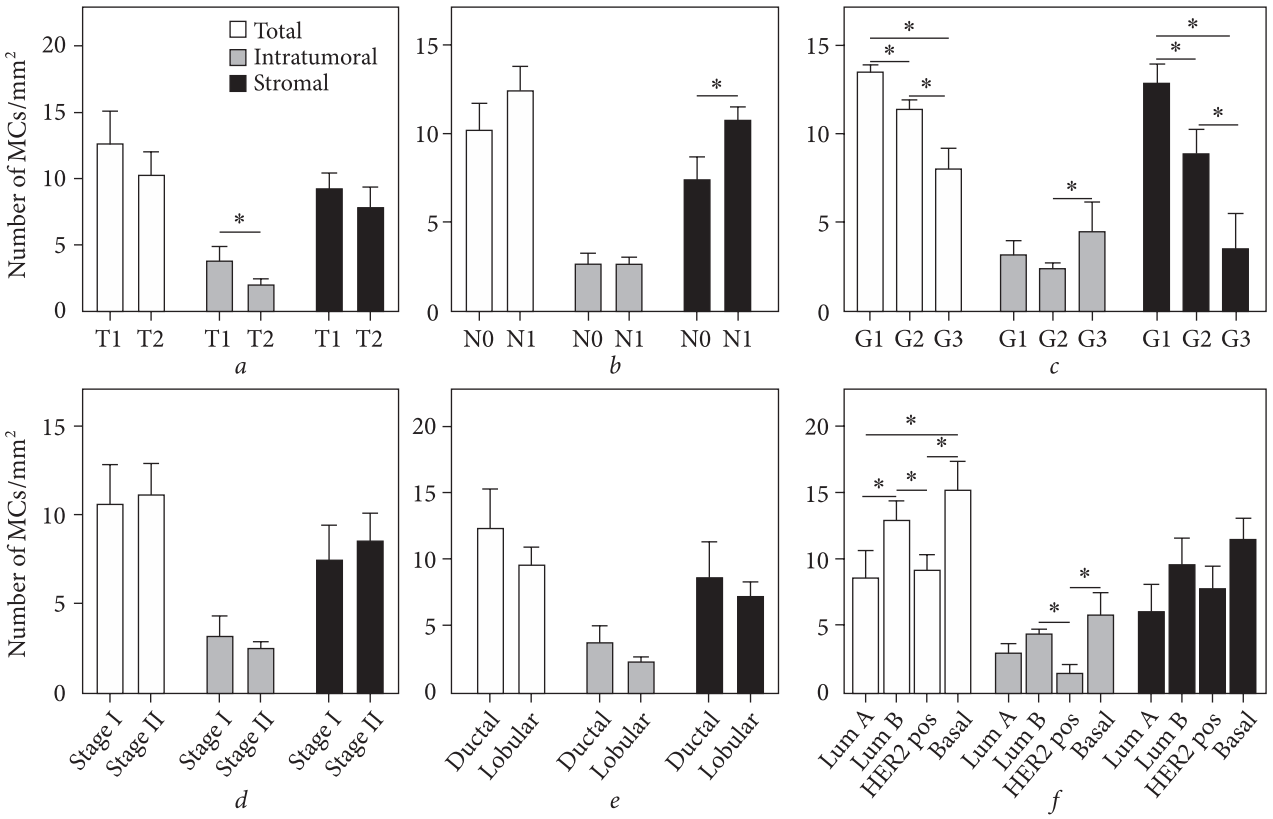

Fig. 3. Relationship between MCs infiltration and the clinical characteristics of BCa: *a* — tumor size (T), *b* — lymph node involvement (N), *c* — tumor grade (G), *d* — clinical stage, *e* — histological type, *f* — molecular subtype. * The difference is significant, $p < 0.05$

characterized by a 5.7-fold increase in MCs infiltration ($p = 0.002$), while tumors with a low proliferative potential (Ki-67 expression < 14%) were characterized by a decrease in the number of MCs in the intratumoral (3.5 times, $p = 0.006$) and stromal (3.6 times, $p = 0.006$) zones. At the same time, no relationship between MCs infiltration and the expression status of ER and PR, as well as the stage of

the tumor process and the histological type of BCa was found (Fig. 4).

We established that the MCs DI was 31.6% ($p = 0.048$) lower in BCa tissue of stage I than in patients with BCa of stage II. T1 category BCa tissue was characterized by a 28.0% decrease in the level of degranulation ($p = 0.029$), compared to T2 category tumors (Table 2). The significantly higher indicators of the DI (by 1.23 times) of MCs were recorded in the tumor tissue of patients with metastatic lesions of regional lymph nodes. In addition, an inverse relationship between the tumor grade and the MCs degranulation was demonstrated. In the tumor tissue of PR-negative BCa, a 32.7% ($p = 0.038$) increase in the level of MCs degranulation was recorded, compared to neoplasms positive for PR expression. No significant difference in MCs degranulation rates in BCa tissue was found depending on the histological type, molecular subtype, and ER, HER2/neu, and Ki-67 expression status.

Table 2. **The relationship between the DI of MCs and the clinical characteristics of BCa**

Clinical stage	I	**1.152 ± 0.1324**
	II	**1.517 ± 0.1144**
Tumor size (T)	T1	**1.129 ± 0.156**
	T2	**1.447 ± 0.1206**
Lymph node involvement (N)	N0	**1.248 ± 0.1137**
	N1	**1.539 ± 0.1785**
Histological type	Ductal adenocarcinoma	1.313 ± 0.2030
	Lobular adenocarcinoma	1.331 ± 0.1093
Tumor grade (G)	G1	**1.252 ± 0.0778**
	G2	**1.495 ± 0.0985**
	G3	**1.737 ± 0.1601**
Molecular subtype	Luminal A	1.271 ± 0.1362
	Luminal B	1.552 ± 0.1945
	Her2/neu positive	1.264 ± 0.2657
	Basal/Triple negative	1.598 ± 0.1459
ER status	ER positive	1.375 ± 0.1125
	ER negative	1.364 ± 0.1923
PR status	PR positive	**1.237 ± 0.1151**
	PR negative	**1.642 ± 0.1667**
HER2/neu status	HER2/neu positive	1.306 ± 0.1865
	HER2/neu negative	1.371 ± 0.1135
Proliferative activity	Ki-67 < 14%	1.583 ± 0.3210
	Ki-67 > 14%	1.484 ± 0.306

Note: Significant difference ($p < 0.05$) is highlighted in bold.

The obtained data show that the density of MCs infiltration and their functional activity are associated with such indicators of BCa malignancy as tumor size, lymph node involvement, tumor grade, molecular subtype, proliferative activity, and PR- and HER2/neu-expression status.

OP as a modulator of migratory and functional activity of MCs in BCa tissue. Next, we studied the OP role in the homing and activation of MCs in BCa. Our previous studies have established that BCa tissue is characterized by significantly higher expression of this matricellular protein in tumor cells compared to FAd tissue samples [19]. Similar dynamics was observed for OP expression in tumor stroma (Fig. 5). Stromal expression of the *SPP1* gene product in BCa tissue was 25.9% ($p = 0.027$) higher compared to the same indicator in FAd tissue.

The dependence of the MCs number infiltrated into the BCa tissue on the levels of parenchymal and stromal expression of OP was revealed (Fig. 6). In neoplasms with high rates of OP expression in tumor cells, an increase in intratumoral MCs infiltration was observed (by 2.48 times, $p = 0.023$), while the number of MCs in the stroma of BCa decreased (by 2.86 times, $p = 0.017$). The BCa tumor tissue with a high level of OP expression in the stromal component was characterized by a significant increase (by 2.36 times, $p = 0.022$) in the total number of MCs, and the pattern of growth (by 3.32 times, $p = 0.014$) of their stromal subpopulation was followed (Fig. 6).

It was proven that high expression of OP, regardless of its topology, in tumor tissue is associated with levels of MCs degranulation (Fig. 7). An increase in the levels of parenchymal and stromal expression of OP was associated with a significant increase in the functional activity of MCs by 54.38% ($p = 0.033$) and 101% ($p = 0.042$), respectively.

At the subsequent stage of the study, we analyzed the relationship between OP expression indicators and MCs infiltration and the functional activity, depending on the clinical status of patients with BCa. It was shown that the increased expression of this matricellular protein in the parenchymal component of BCa is associated with an increase in the number of MCs in the intratumoral zone of neoplasms with a simultaneous decrease in their number in the stroma, which probably indicates the re-

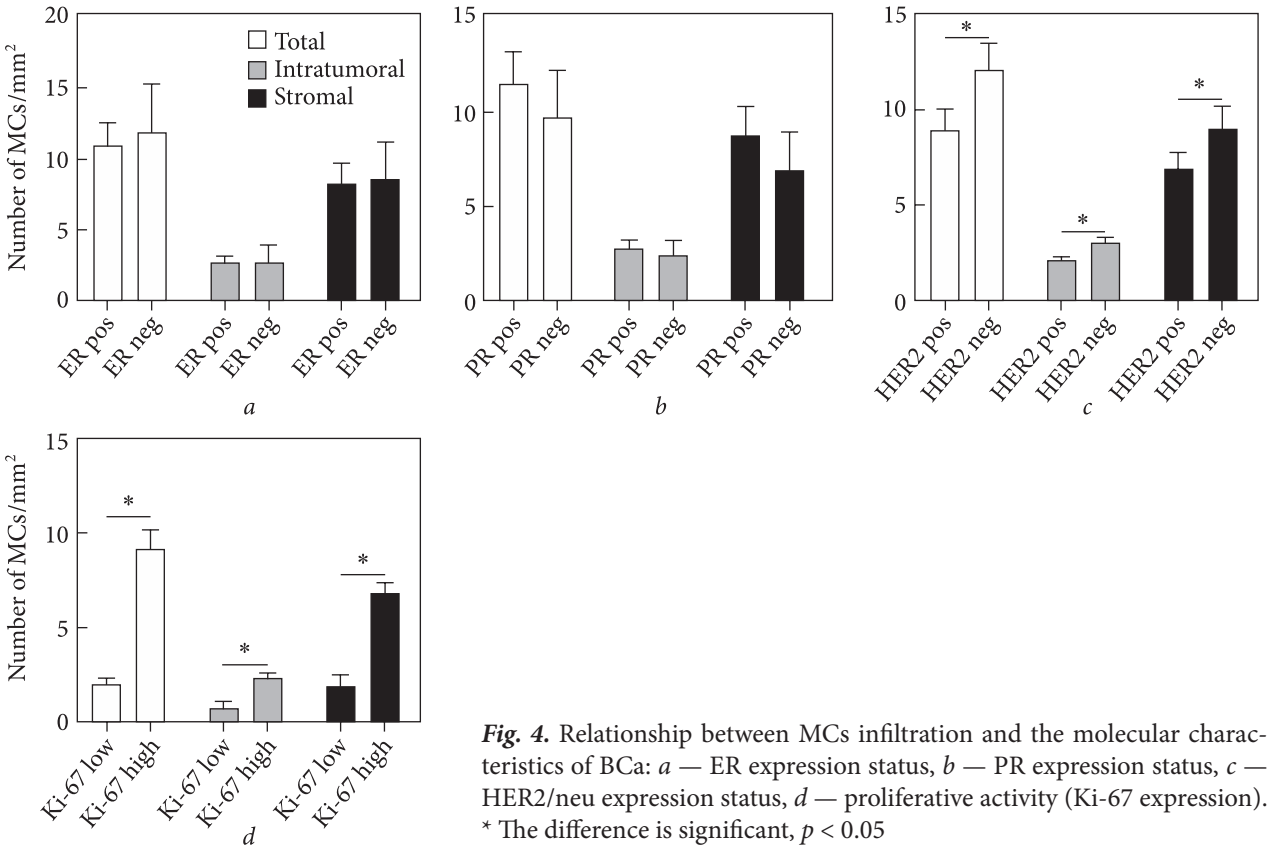

Fig. 4. Relationship between MCs infiltration and the molecular characteristics of BCa: *a* — ER expression status, *b* — PR expression status, *c* — HER2/neu expression status, *d* — proliferative activity (Ki-67 expression). * The difference is significant, $p < 0.05$

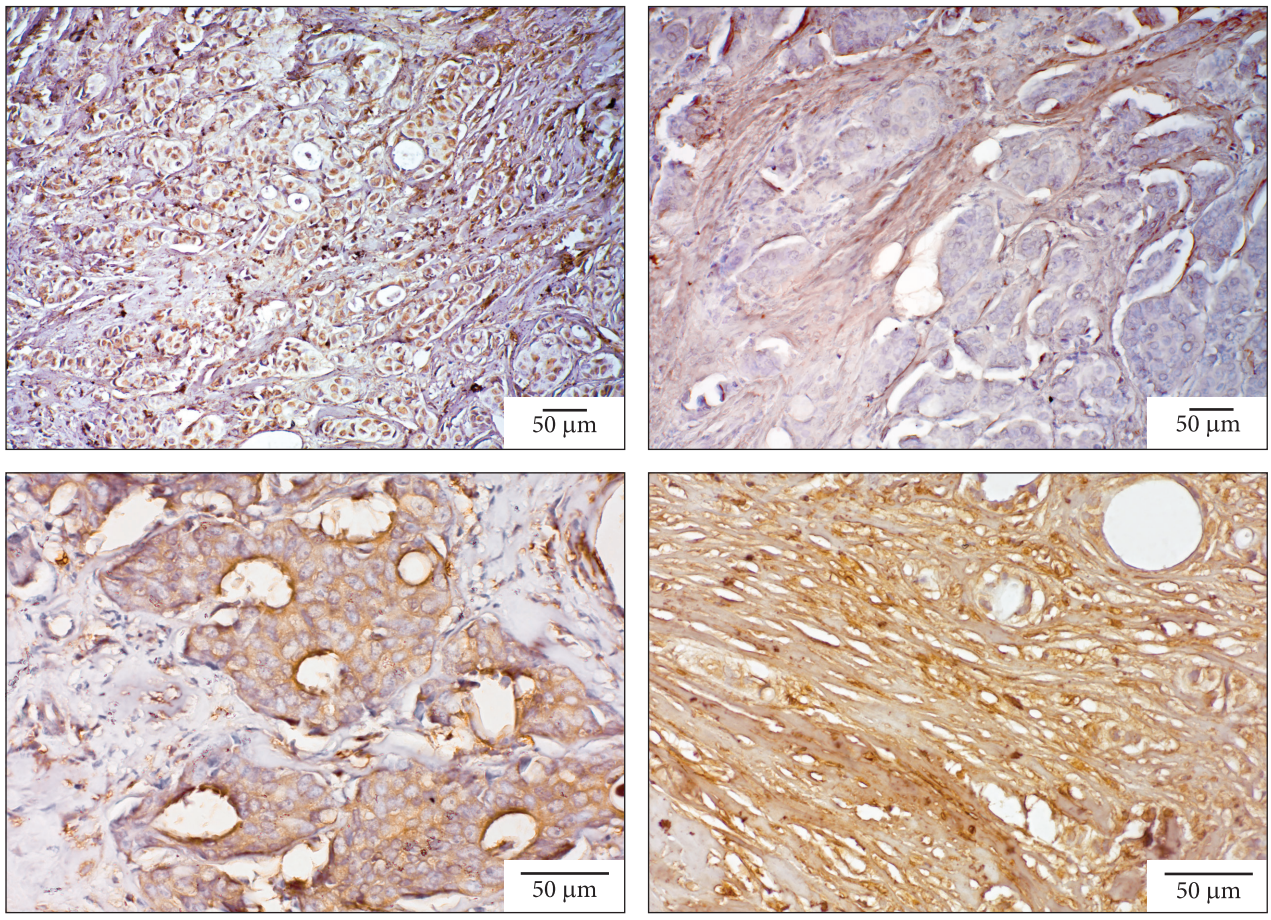

Fig. 5. OP expression in BCa tissue. Immunohistochemical label — 3,3-diaminobenzidine; staining with Mayer's hematoxylin

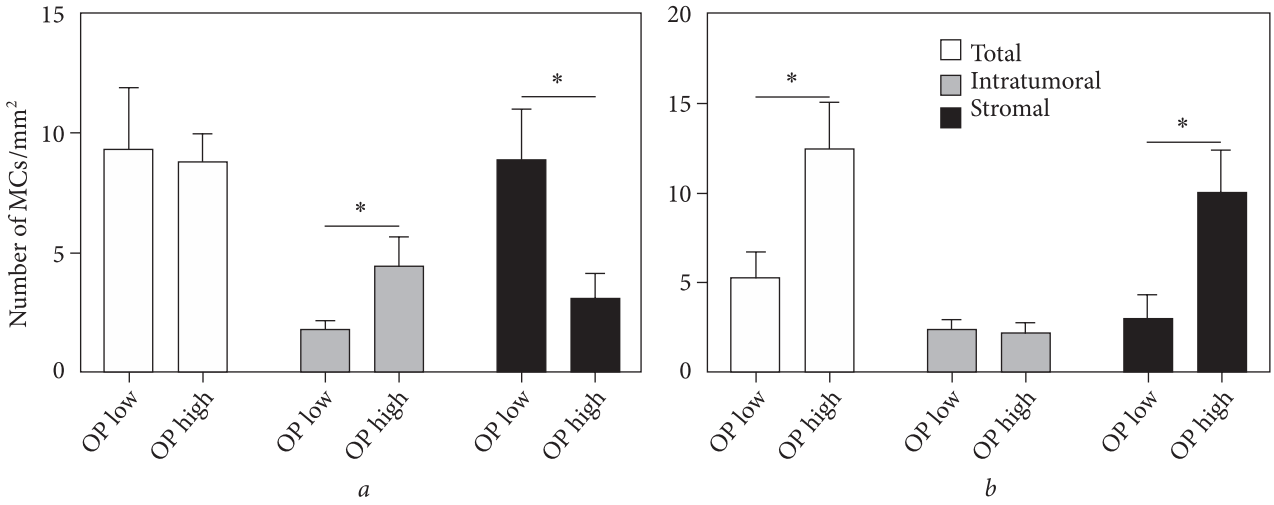

Fig. 6. Relationship between the topology of OP expression (parenchymal (*a*) and stromal (*b*)) and indicators of MCs infiltration of BCa tissue. * The difference is significant, $p < 0.05$

Table 3. **Relationship of the expression level of OP in the parenchymal component of BCa with the levels of MCs infiltration and degranulation in patients with different clinical status**

			MCs infiltration level, cells/mm²			DI, c.u.
			Total	Intratumoral	Stromal	
Stage	I	OP low	10.108 ± 1.902	2.709 ± 0.561	7.662 ± 0.599	1.309 ± 0.078
		OP high	11.007 ± 1.385	3.673 ± 1.344	7.304 ± 0.820	1.175 ± 0.387
	II	OP low	11.317 ± 0.724	2.740 ± 0.831	8.491 ± 0.346	1.229 ± 0.316
		OP high	10.592 ± 1.338	3.071 ± 0.530	8.569 ± 0.558	1.693 ± 0.295
Tumor size (T)	T1	OP low	6.024 ± 1.318	0.954 ± 0.539	6.596 ± 0.811	0.800 ± 0.219
		OP high	8.958 ± 1.726	3.857 ± 0.882	5.977 ± 0.749	0.667 ± 0.183
	T2	OP low	7.116 ± 1.599	1.792 ± 0.426	**1.303 ± 0.538**	1.250 ± 0.209
		OP high	4.886 ± 1.048	1.692 ± 0.205	**6.515 ± 0.992**	1.333 ± 0.264
Lymph node involvement (N)	N0	OP low	9.370 ± 1.103	1.248 ± 0.407	**8.099 ± 0.580**	**0.886 ± 0.223**
		OP high	11.071 ± 2.861	**3.996 ± 0.711**	**6.753 ± 0.413**	**1.614 ± 0.169**
	N1	OP low	11.193 ± 1.447	**1.480 ± 0.389**	**10.122 ± 0.876**	**1.105 ± 0.111**
		OP high	12.006 ± 1.590	**3.764 ± 0.272**	**8.219 ± 0.633**	**2.899 ± 0.213**
Tumor grade (G)	G1	OP low	12.288 ± 1.094	2.893 ± 0.529	9.679 ± 1.120	**0.314 ± 0.096**
		OP high	12.654 ± 0.711	3.409 ± 0.318	9.940 ± 0.835	**2.308 ± 0.478**
	G2	OP low	11.612 ± 0.993	2.443 ± 0.398	9.207 ± 1.277	1.437 ± 0.227
		OP high	11.108 ± 0.708	2.490 ± 0.442	8.682 ± 1.094	1.729 ± 0.205
	G3	OP low	7.723 ± 1.020	4.599 ± 0.507	3.083 ± 0.574	**1.180 ± 0.173**
		OP high	8.205 ± 0.746	4.424 ± 0.566	3.749 ± 0.499	**2.056 ± 0.301**
Histological type	Ductal	OP low	11.602 ± 1.539	3.571 ± 1.076	7.930 ± 0.519	1.556 ± 0.156
		OP high	12.516 ± 2.074	3.604 ± 0.849	8.914 ± 1.109	1.209 ± 0.340
	Lobular	OP low	9.662 ± 0.996	2.462 ± 1.308	7.339 ± 0.732	1.311 ± 0.208
		OP high	9.615 ± 1.737	4.647 ± 1.765	6.803 ± 0.608	1.384 ± 0.149
Molecular subtype	Lum A	OP low	9.314 ± 0.641	2.917 ± 0.458	6.337 ± 0.981	1.336 ± 0.108
		OP high	8.921 ± 0.922	3.106 ± 0.390	5.802 ± 0.506	1.205 ± 0.343
	Lum B	OP low	10.704 ± 0.919	2.892 ± 0.346	9.593 ± 0.466	1.493 ± 0.236
		OP high	10.981 ± 1.184	3.720 ± 0.688	9.708 ± 0.529	1.599 ± 0.104
	HER2 pos	OP low	11.001 ± 0.839	2.990 ± 0.706	7.464 ± 0.378	0.833 ± 0.473
		OP high	9.650 ± 1.317	1.841 ± 0.642	7.801 ± 0.407	1.602 ± 0.533
	Basal	OP low	11.108 ± 1.672	4.089 ± 0.619	9.079 ± 1.203	1.488 ± 0.609
		OP high	13.677 ± 1.948	3.227 ± 0.308	11.314 ± 0.944	1.782 ± 0.371

Note: The significant difference ($p < 0.05$) between OP low and OP high groups is highlighted in bold.

localization of MCs within the tumor tissue, regardless of the metastatic lesions in the regional lymph nodes. In addition, a greater number of MCs in the intratumoral zone of T1 tumors and the stromal component of T2 neoplasms was associated with an increase in OP expression. Along with this, a direct relationship was established between the expression levels of OP and DI and the BCa grade (Table 3).

It was shown that the high expression of OP is associated with a greater number of MCs in the stromal component of T2 category BCa patients, as well as patients with lymph node involvement. Along with this, a decrease in the overall level of

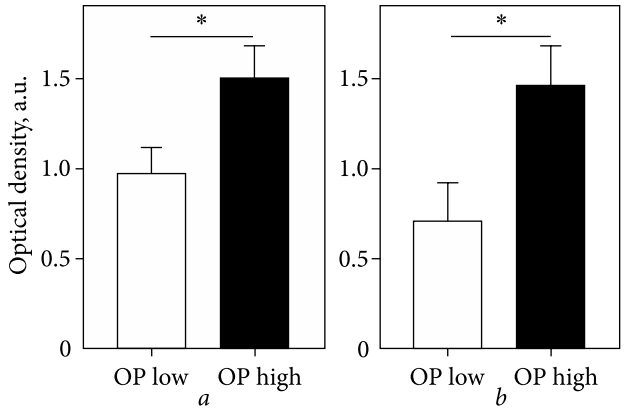

Fig. 7. The relationship between the topology of OP expression (parenchymal (*a*) and stromal (*b*)) and MCs degranulation in BCa tissue. * The difference is significant, $p < 0.05$

Table 4. **Relationship of the expression level of OP in the stromal component of BCa with the levels of MCs infiltration and degranulation in patients with different clinical status**

			MCs infiltration level, cells/mm^2			DI, c.u.
			Total	Intratumoral	Stromal	
Stage	I	OP low	10.604 ± 0.702	2.885 ± 0.602	7.946 ± 1.003	**0.663 ± 0.076**
		OP high	10.297 ± 0.998	3.232 ± 0.477	7.311 ± 0.739	**1.552 ± 0.292**
	II	OP low	12.420 ± 1.324	2.574 ± 0.413	8.427 ± 0.659	1.617 ± 0.424
		OP high	11.009 ± 0.640	2.379 ± 0.761	8.771 ± 0.507	1.426 ± 0.137
Tumor size (T)	T1	OP low	10.590 ± 1.369	3.909 ± 0.904	**5.026 ± 1.303**	0.975 ± 0.269
		OP high	12.582 ± 1.663	3.257 ± 0.418	**10.45 ± 1.595**	1.469 ± 0.314
	T2	OP low	6.638 ± 2.401	2.059 ± 0.506	**3.746 ± 0.516**	1.258 ± 0.196
		OP high	8.583 ± 1.677	2.932 ± 0.499	**6.678 ± 0.702**	1.530 ± 0.235
Lymph node involvement (N)	N0	OP low	10.459 ± 1.118	1.996 ± 0.428	8.786 ± 0.921	1.045 ± 0.198
		OP high	9.782 ± 0.935	2.701 ± 0.476	7.093 ± 1.110	1.336 ± 0.185
	N1	OP low	**7.656 ± 0.847**	2.087 ± 0.483	**5.921 ± 1.073**	**1.260 ± 0.103**
		OP high	**11.812 ± 0.994**	2.689 ± 0.379	**10.317 ± 1.644**	**1.913 ± 0.200**
Tumor grade (G)	G1	OP low	**9.209 ± 1.208**	3.090 ± 0.372	**8.403 ± 1.533**	1.117 ± 0.462
		OP high	**13.948 ± 1.571**	3.233 ± 0.248	**15.784 ± 2.845**	1.395 ± 0.288
	G2	OP low	**7.754 ± 1.140**	2.260 ± 0.673	**6.611 ± 1.374**	**1.166 ± 0.111**
		OP high	**12.549 ± 1.715**	2.415 ± 0.357	**10.306 ± 1.387**	**1.933 ± 0.209**
	G3	OP low	**6.287 ± 0.926**	3.964 ± 0.974	**1.834 ± 0.781**	**1.386 ± 0.288**
		OP high	**9.921 ± 1.439**	4.729 ± 0.402	**6.202 ± 1.034**	**2.078 ± 0.294**
Histological type	Ductal	OP low	13.693 ± 2.780	2.980 ± 0.834	9.038 ± 1.394	1.033 ± 0.306
		OP high	11.457 ± 1.662	3.839 ± 0.576	8.117 ± 1.035	1.425 ± 0.377
	Lobular	OP low	8.994 ± 1.090	2.761 ± 0.579	7.295 ± 1.433	1.376 ± 0.280
		OP high	9.850 ± 1.386	2.109 ± 0.301	7.066 ± 1.290	1.313 ± 0.165
Molecular subtype	Lum A	OP low	7.713 ± 1.578	2.984 ± 0.784	5.096 ± 1.006	1.297 ± 0.062
		OP high	8.092 ± 1.936	2.883 ± 0.208	6.359 ± 0.959	1.356 ± 0.146
	Lum B	OP low	**10.600 ± 1.349**	**3.117 ± 0.934**	**6.898 ± 1.204**	1.711 ± 0.511
		OP high	**14.097 ± 1.046**	**5.274 ± 0.487**	**9.475 ± 1.238**	1.433 ± 0.384
	HER2 pos	OP low	9.424 ± 1.489	1.076 ± 0.399	6.501 ± 0.947	1.247 ± 0.472
		OP high	7.892 ± 0.894	1.742 ± 0.137	8.397 ± 1.831	1.289 ± 0.205
	Basal	OP low	**11.228 ± 2.463**	**3.408 ± 0.942**	**8.672 ± 1.575**	1.636 ± 0.450
		OP high	**17.836 ± 1.874**	**6.769 ± 1.253**	**12.312 ± 1.773**	1.524 ± 0.344

Note: The significant difference ($p < 0.05$) between OP low and OP high groups is highlighted in bold.

stromal infiltration and the degree of MCs degranulation was recorded in low-differentiated BCa with high expression of OP in the tumor stroma (Table 4). The relationship between OP expression indicators and the number of MCs in the tissue of Luminal B and basal molecular subtypes has been established.

Thus, the high expression of OP in the stromal component of BCa is associated with the growth of the tumor MCs population in patients with metastatic lesions of regional lymph nodes and neoplasms with a low differentiation grade. In addition, this matricellular protein is involved in the regulation of MCs in the tissue of luminal B and basal molecular subtypes. At the same time, the level of OP expression in the parenchymal component of BCa is associated with the number of infiltrated MCs in the presence of metastatic lesions of regional lymph nodes.

Discussion

Our data on high levels of MCs infiltration of BCa tissue compared to FAd coincide with the results of Kashiwase et al. [20], however, we did not find a relationship between the number of MCs and the histological type of BCa. We recorded a decrease in the number of MCs with an increase in the BCa size coinciding with the results obtained by Löfdahl et al. [21]. Fakhrjou et al. [22] showed that the number of MCs in tumor tissue elevated with increasing tumor grade, which is somewhat contrary to the decrease in their number found in the stromal component and BCa tissue in general. However, in the intratumoral zone, we also observed an increase in the number of MCs in the tissue of poorly differentiated neoplasms. Glajcar et al. [23] also showed a drop in the number of peritumoral MCs with a decrease in the tumor differentiation grade. A similar trend was noted by other researchers [21, 24]. There are also reports on the increased activity of tryptase, the main component of granules and a specific marker of MCs, in tumors of low differentiation grade, which indicates the need for further research into the features of MCs topology in a larger sample of patients [25].

The literature reports a direct relationship between the expression status of ER and PR and the number of MCs in BCa tissue. At the same time, we have shown the absence of such a connection [26]. However, it is worth noting the inverse relationship between the HER2/neu expression status and the number of MCs found in our study. Interestingly, several studies indicated an increased level of infiltration by chymase- and tryptase-positive MCs in neoplasms of luminal subtypes [23], while we found a reliable increase in their number during detection using toluidine blue specifically in the tissue of triple-negative and luminal B BCa, which is likely associated with their high proliferative activity.

Particular attention should be paid to the relationship between MCs and the frequency of BCa metastasis to regional lymph nodes. High MCs infiltration levels in malignant neoplasms of the mammary gland are associated with enhanced neovascularization and lymphangiogenesis [27], which is consistent with our data on the direct relationship between the number of MCs in BCa tissue and the presence of metastatic lesions of regional lymph nodes

In addition to the importance of MCs in the development and progression of BCa, much less attention has been paid to their role in the formation of TME. The results of our previous studies indicated the involvement of OP in MCs migration to the TME in prostate cancer [28]. We also showed the relationship between OP expression and the degree of malignancy of BCa *in vitro* [29] and *ex vivo* on clinical material [19]. One of the receptors capable of binding to OP on the MCs surface is integrin α4β1 [30]. A study by Abonia et al. [31] showed the crucial role of integrin α4β1 and VCAM in the migration and adhesion of MCs to the foci of inflammation. The data we obtained evidence that OP in the stroma of neoplasms contributes to the recruitment and activation of MCs in BCa. In addition, it has been reported that MCs themselves can produce OP and thus activate themselves and surrounding tumor cells through autocrine and paracrine regulation [32]. Further study of the MCs infiltration density and functional activity in BCa tissue will allow us to find out their prognostic potential and will become the basis for optimizing the treatment of patients.

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ОСТЕОПОНТИН-РЕГУЛЬОВАНІ ЗМІНИ В ПОПУЛЯЦІЇ ОПАСИСТИХ КЛІТИН, АСОЦІЙОВАНІ З РАКОМ МОЛОЧНОЇ ЗАЛОЗИ

Стан питання. Доведено, що розвиток раку молочної залози (РМЗ) значною мірою визначається особливостями пухлинного мікрооточення, яке зазнає суттєвих змін під час прогресування захворювання. Одним з найменш вивчених компонентів пухлинного мікрооточення, роль якого при РМЗ залишається дискусійною, є опасисті клітини (ОК). **Метою** роботи було визначити прогностичне значення показників інфільтрації та функціональної активності ОК при РМЗ і встановити роль остеопонтину (ОПН) в їх регуляції. **Матеріали та методи**. Дослідження проведено на операційному матеріалі 15 хворих на фіброаденому та 78 хворих на РМЗ I—II стадії. Ідентифікацію ОК у тканині доброякісних і злоякісних новоутворень молочної залози проводили гістохімічним методом з використанням толуїдинового синього. Для оцінки функціональної активності ОК розраховували індекс дегрануляції. Визначення експресії ОПН у пухлинній тканині проводили імуногістохімічним методом. **Результати.** Отримані дані свідчать, що рівень інфільтрації ОК та їхня функціональна активність асоційовані з такими показниками злоякісності РМЗ, як розмір пухлини, ураження лімфатичних вузлів, ступінь диференціювання, молекулярний підтип, проліферативна активність, експресія рецепторів прогестерону та епідермального фактору росту (HER2/neu). Високі показники експресії ОПН у стромальному компоненті РМЗ асоціюються зі зростанням популяції пухлинних ОК з метастатичним ураженням регіонарних лімфатичних вузлів та низьким ступенем диференціювання. Крім того, ОПН бере участь у регуляції ОК у тканинах люмінального В і базального молекулярного підтипів. При цьому рівень експресії ОПН в паренхіматозному компоненті пухлини асоціюється з показником інфільтрації ОК РМЗ при наявності метастатичного ураження регіонарних лімфатичних вузлів. **Висновки.** Подальше вивчення особливостей інфільтрації та функціональної активності ОК в тканині РМЗ дозволить з'ясувати їхній прогностичний потенціал і може стати основою для оптимізації лікування хворих.

Ключові слова: рак молочної залози, опасисті клітини, остеопонтин, прогнозування перебігу.