https://doi.org/10.15407/exp-oncology.2024.04.311

O. MUSHII [1, *], A. PAVLOVA [1], V. BAZAS [2], T. BORIKUN [1], N. LUKIANOVA [1]

[1] R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the NAS of Ukraine, Kyiv, Ukraine

[2] Kyiv City Clinical Oncology Center, Kyiv, Ukraine

* Correspondence: Email: sashamushy1@gmail.com

MAST CELLS AS A FACTOR IN REGULATION OF STROMAL COMPONENT ASSOCIATED WITH BREAST CANCER AGGRESSIVENESS

Background. It has been proven that changes in the morphology, representation, and organization of collagen fibers contribute to the formation of a unique microenvironment, which is associated with the metastatic potential of malignant neoplasms due to the initiation of cell migration and changes in polarization. Among the modulators of the collagen stroma, fibroblasts remain the most widely studied today. At the same time, much less attention is focused on the study of immune cells in the tumor microenvironment, in particular, mast cells (MCs). **Aim**. To investigate the relationship between the MCs status and the features of the collagen matrix of breast cancer (BCa). **Materials and Methods**. The study was conducted on the postoperative material of 78 patients with BCa stage I—II. MCs were assessed by a histochemical method using toluidine blue. For estimation of the functional activity of MCs, a degranulation index was calculated. COL1A1, COL3A1, and MMP-9 expression in tumor tissue was assessed immunohistochemically. A visualization of collagen fibers was performed using the staining by Malory. Microphotographs were pre-processed in Adobe Photoshop SS 2019 and analyzed using the software packages CurveAlign v. 4.0 and ImageJ. **Results**. Tumor tissue with a high density and functional activity of MCs was characterized by an increased expression of COL1A1 ($p < 0.05$), COL3A1 ($p < 0.05$), and MMP-9 ($p < 0.05$). In BCa tissue with the lower MCs degranulation index, collagen fibers become thicker ($p < 0.05$), shorter ($p < 0.05$), and denser ($p < 0.05$). At the same time, the existence of a relationship between the levels of miR-155-5p and the expression of COL1A1 (r = 0.703, $p = 0.009$), COL3A1 (r = 0.603, $p = 0.043$), and MMP-9 in tumor cells (r = 0.562, $p = 0.039$) and in the stroma (r = 0.546, p = 0.038), as well as the associations of the levels of this miRNA with the fiber length (r = –0.632, $p = 0.013$), width (r = –0.522, $p = 0.048$), and density (r = 0.699, $p = 0.014$) were found. Significantly higher rates of miR-155-5p expression ($p < 0.05$) were recorded in BCa tissue with a high index of MCs degranulation. **Conclusion**. During the BCa progression, the role of MCs in the manifestation of the tumor development increases. A growing number of infiltrated MCs contributes to the activation of MMP and fibrillar collagen expression. These changes lead to increased remodeling of the tumor stroma, which is directly reflected in the spatial organization of the collagen matrix. The increased activity of proteases causes a decrease in the length and width of fibrils, which is explained by a decrease in the number of mature fibers and their disorganization in three-dimensional space. The obtained data allow us to assert that MCs play a key role not only in the formation of a specific immune microenvironment of BCa but also in determining the direction of changes in the tumor stroma, which promotes cancer aggressiveness.

Keywords: breast cancer, mast cells, collagen matrix, MMP, extracellular matrix remodeling.

Citation: Mushii O, Pavlova A, Bazas V, Borikun T, Lukianova N. Mast cells as a factor in regulation of breast cancer stromal component associated with breast cancer aggressiveness. *Exp Oncol.* 2024; 46(4): 311-323. https://doi.org/10.15407/exp-oncology.2024.04.311

Over the past two decades, there has been an increasing trend in the number of studies devoted to identifying features of the tumor microenvironment (TME) associated with the aggressiveness of solid neoplasms of various histogenesis, including breast cancer (BCa). It has been proven that a dynamic interaction between malignantly transformed TME cells and its stromal components plays a key role in cancer progression, as well as the development of resistance to drug therapy [1]. The main fibrillar component of the stroma is collagen, which makes up 30% of the total protein mass in the human body. It has been established that the changes in the morphology, representation, and organization of collagen fibers contribute to the formation of a unique microenvironment, which is associated with the metastatic potential of malignant neoplasms due to the initiation of cell migration and changes in polarization. This is confirmed by the data of our research, according to which the straightening, shortening, and thinning of collagen fibers along with the increase in density, are associated with the tumor stage and the differentiation grade of malignant neoplasms of the mammary and prostate glands. At the same time, we showed that the growth of the stromal-tumor ratio is characteristic of an aggressive basal molecular subtype of BCa [2—5]. Also, it has been shown that both monomeric [6, 7] and polymeric [8] matricellular proteins play a key role in the formation of a three-dimensional matrix that serves as a scaffold for tumor cells modulating their migratory activity, proliferation, and apoptosis [9].

Among the modulators of collagen stroma, fibroblasts remain the most widely studied today. Under the influence of growth factors and signals from BCa cells, they are transformed into activated cancer-associated fibroblasts (CAFs), which simultaneously synthesize and reorganize matrix components such as collagen, fibronectin, and proteoglycans [10]. These changes lead to the formation of a rigid framework of the tumor, which promotes the migration and invasion of malignantly transformed cells and the maintenance of an inflammatory microenvironment, which promotes cancer progression [11].

At the same time, much less attention is focused by researchers on the study of immune cells in TME, in particular, mast cells (MCs), which, according to the literature data and the results of own research [12], are capable of systemically influencing the tumor development and participating in the formation of the BCa malignancy degree.

It was shown that MCs can control the state of the extracellular matrix and participate in its remodeling by secreting proteoglycans, specific proteases, and TGF-β. In particular, tryptase, which is synthesized by MCs, can destroy the connective tissue matrix [13, 14]. MCs also secrete professional extracellular matrix digesters, such as MMP-2, which convert pro-MMP-9 (inactive form) into MMP-9 (active form) [15]. The established relationship between the level of MCs infiltration and the intensity of fibrous changes in the tissue indicates their involvement in the regulation of the activity of collagen-producing cells as well as in the extracellular stages of fibrillogenesis under physiological conditions [16]. At the same time, the mechanisms of the effect of MCs on the main stromal components of the microenvironment of BCa remain unclear. The mechanisms of epigenetic regulation of collagen stroma rearrangements, mediated by MCs action, also remain unexplored. Given this, the aim of our work was to investigate the relationship between the MCs status and the features of the collagen matrix of BCa.

Materials and Methods

The study was conducted on the postoperative material of 78 patients with stage I—II BCa, whose detailed clinical characteristics are shown in Table 1. The patients were treated at the Municipal Non-Profit Enterprise "Kyiv City Oncology Center" during 2019—2022. All patients were examined using generally accepted clinical and laboratory methods in accordance with the Standards of diagnosis and treatment of oncological patients, approved by Order of the Ministry of Health of Ukraine No. 396 of 06.30.2015 (registration number GS 2015-396). No patient received neoadjuvant treatment. All donors of tumor material provided consent of agreement to conduct scientific research.

Histochemical method. Determination of MCs in the tissue of malignant neoplasms of the mammary gland was carried out by the histochemical method using toluidine blue (Sigma-Aldrich, USA). The preparations were examined using an AxioScope A1 light microscope (Carl Zeiss, Germany). The number of MCs was evaluated in

20 fields of view at ×200 magnification. The degree of MCs degranulation was determined at ×1000 magnification. MCs degranulation index (DI) was calculated using the Lindner formula [17]:

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

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

Table 1. **Clinical 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

Immunohistochemical study. The study of the COL1A1, COL3A1, and MMP-9 expression in BCa tissue was performed on paraffin sections with a thickness of 5 μm using appropriate monoclonal antibodies (Table 2).

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).

Parenchymal BCa cells MMP-9 expression was evaluated using the method of counting immunopositive cells on an AxioScope A1 light microscope (Carl Zeiss, Germany), with a magnification of ×400. The level of expression was calculated using the H-Score method by the formula:

$$S = N0\ (\%) + 1\times N1\ (\%) + 2\times N2\ (\%) + 3\times N3\ (\%),$$

where S is the H-Score indicator; N0 — the number of cells with no expression; N1, N2, and N3 — with low, medium, and high expression, respectively. The final result of the calculation was expressed in points: from 1 to 100 points — low, from 101 to 200 — medium, and from 201 to 300 — high level of expression [18, 19].

To evaluate the expression of COL1A1, COL3A1, and MMP-9 in the stromal component of breast neoplasms, 10 photomicrographs were made at ×400 magnification. Microphoto analysis was performed using the ImageJ software package (LOCI, University of Wisconsin, USA) and the IHC pro-

Table 2. **Monoclonal antibodies used for immunohistochemical study**

Antigen	Clone	Dilution	Manufacturer
COL1A1	1B2	1/300	MyBioSource, USA
COL3A1	5G2	1/400	Abcam, UK
MMP-9	MA5-15886	1/150	Thermo Scientific, USA

filer plug-in [20]. Quantitative stromal expression of the studied proteins was calculated using the optical density score formula:

$$ODS = P0 \times 1 + P1 \times 2 + P2 \times 3 + P3 \times 4,$$

where ODS is the optical density score; P0 is the number of pixels with missing expression; P1, P2, and P3 — with low, medium, and high expression, respectively. The value of the optical density score varied from 1 to 4 units.

Real-time reverse transcription polymerase chain reaction method. The study of hsa-miR-155-5p expression in BCa tissue was performed according to the protocol described in a previous study [21]. RT-PCR was performed on the hardware detection system Quant Studio DX5 Real-Time PCR System using a commercial kit for RT-PCR TaqMan MicroRNA Assay (ThermoScientific, USA) according to the manufacturer's protocol. Sequences of primers for the reverse transcription PCR were determined using the resource http://genomics.dote.hu:8080/mirnadesigntool/ and synthesized by Metabion (Germany). The primer sequence to hsa-miR-155-5p:

5'-GTGGGTTAATGCTAATCGTGAT-3'.

MicroRNA RNU48 was used as an endogenous control for objectifying expression indicators. Fold change (fold difference) between the expression of the investigated microRNA was calculated according to the formula $2^{-\Delta Ct}$ (hereinafter — a.u.). Errors for fold difference calculations show the range of ΔCt values based on including the standard deviation in these values [22].

Bioinformatics analysis. Possible targets of hsa-miR-155-5p were determined using the miRNet v. 2.0 resource (https://www.mirnet.ca/) and such databases as Tarbase v. 9.0, and miRTarBase v. 9.0. KEGG analysis of the miR-155-5p effects in pathways was investigated in DIANA miRPath v. 3.0 [23]. The functional enrichment analysis of the target genes was enforced using the DAVID tool [24]. Correlation analysis of the relationship between mRNA expression of collagen and MMP9 genes and miRNA-155-5p levels was performed using the open information resource ENCORI project(https://rnasysu.com/encori/index.php).

Statistical analysis. Processing of the obtained results was performed using the software package GraphPad Prism v. 10.00 (GraphPad Software Inc., USA) Quantitative comparison of two independent groups was performed using the Mann — Whitney U-test. The data are presented as M ± m, where M is the arithmetic mean, and m is the standard error of the mean. Quartile diagrams ("boxplot") graphically presented the results of the study, in which the central line marked the median and the lower and upper limits of the "box" — the first and third quartiles, respectively. The whiskers emerging from the rectangles indicate the minimum and maximum values of the indicators in the studied groups. The violin plots were used to demonstrate the distribution of the morphometric parameters of the BCa collagen matrix, in which the central line marked the median, and the upper and lower dashed lines — the first and third quartiles, respectively. Correlation analysis was performed using the Jamovi v. 2.4.11.0 program (Computer Software, https://www. jamovi.org) with the calculation of the Spearman correlation coefficient. The critical level of statistical significance was taken as equal to 0.05.

Results

The relationship of the levels of MCs infiltration and the functional activity with the expression of indicators of the TME stromal component markers and the collagen matrix spatial organization of the BCa tissue. Depending on the indicators of infiltration (Me = 7.899) and the degree of degranulation (DI (Me = 1.484)), MCs in the BCa tissue of all patients were divided into 2 groups (Table 3). Either of the groups contained 39 patients [12]. When analyzing the results of an immunohistochemical study of the TME stromal

Table 3. **Number of mast cells and DI values in experimental groups of patients with BCa**

MCs infiltration level, cells/mm²	
n = 39	n = 39
MCs low level	MCs high level
3.336 ± 0.4255	18.62 ± 1.927
DI, a.u.	
n = 39	n = 39
DI low	DI high
0.7681 ± 0.09366	1.929 ± 0.07627

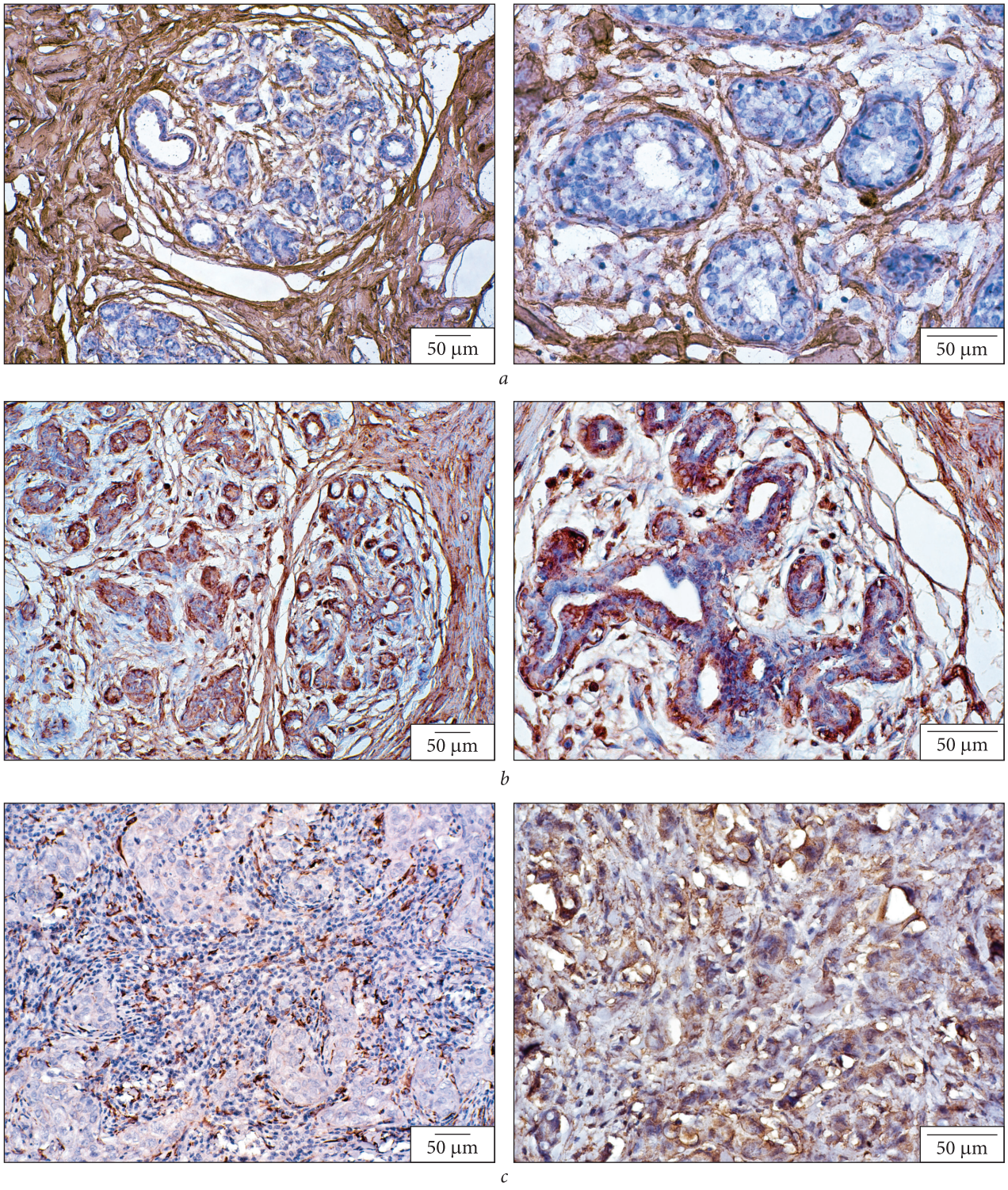


Fig. 1. Representative photomicrographs of COL1A1 (*a*), COL3A1 (*b*), and MMP-9 (*c*) expression in BCa tissue. Immunohistochemistry with chromogen 3-diaminobenzidine tetrachloride. Counterstaining with Meyer's hematoxylin

component expression (Fig .1) depending on the status of MCs, it was found that BCa with high infiltration of MCs was characterized by a 15.1% (p = 0.048) lower level of COL3A1 expression (Fig. 2). The high functional activity of MCs was associated with an increase in expression indicators of COL1A1 (by 15.5%, p = 0.011), COL3A1 (by 10.1%, p = 0.028), as well as MMP-9 in the parenchymal (by 63.4%, p = 0.045) and stromal (by 14.0%, p = 0.036) components of BCa.

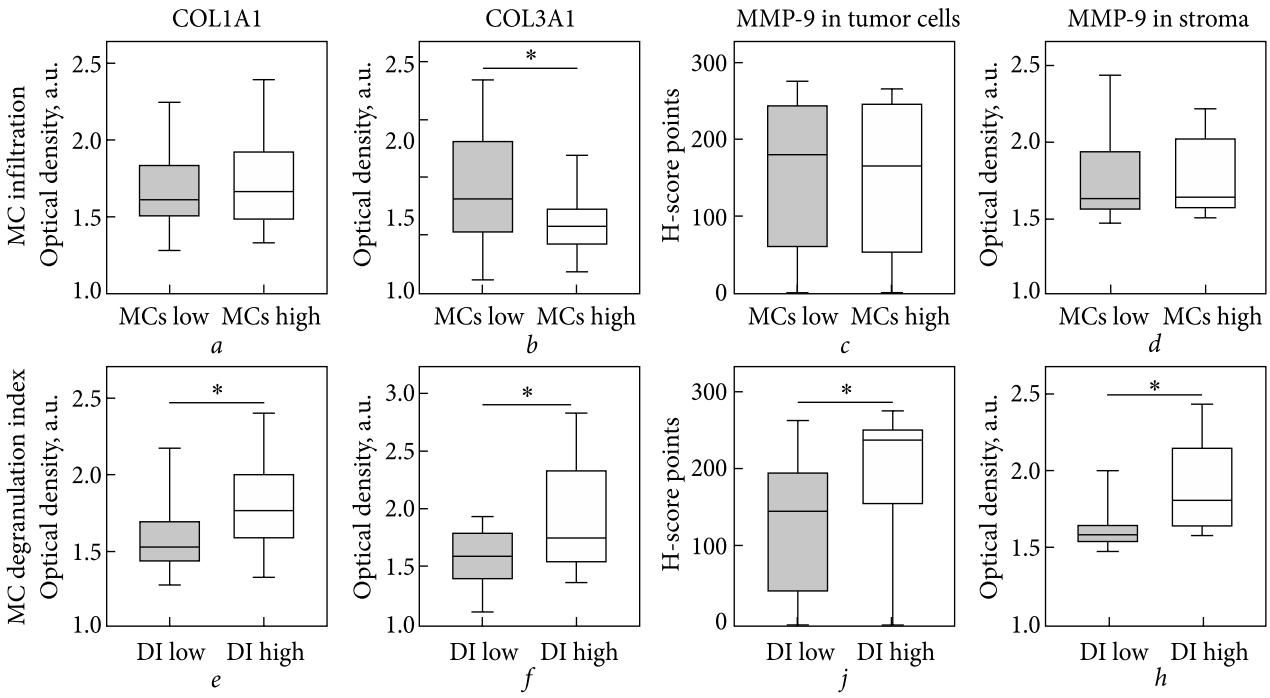


Fig. 2. The relationship of the levels of infiltration (*a*—*d*) and the DI (*e*—*h*) of MCs with the expression of indicators of fibrillar collagens (*a*—*b* and e—*f*), as well as MMP-9 in tumor cells (*c* and *j*) and stroma (*d* and *h*) of BCa. * $p < 0.05$, the difference is significant

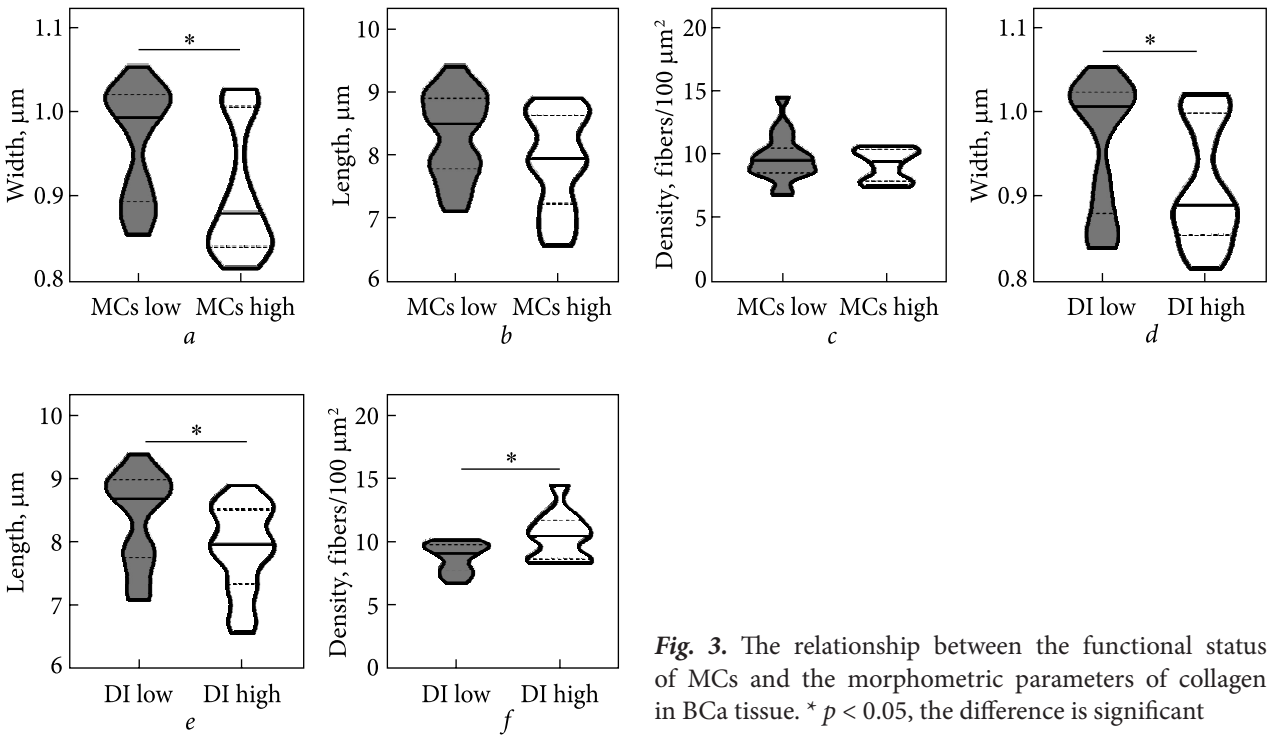
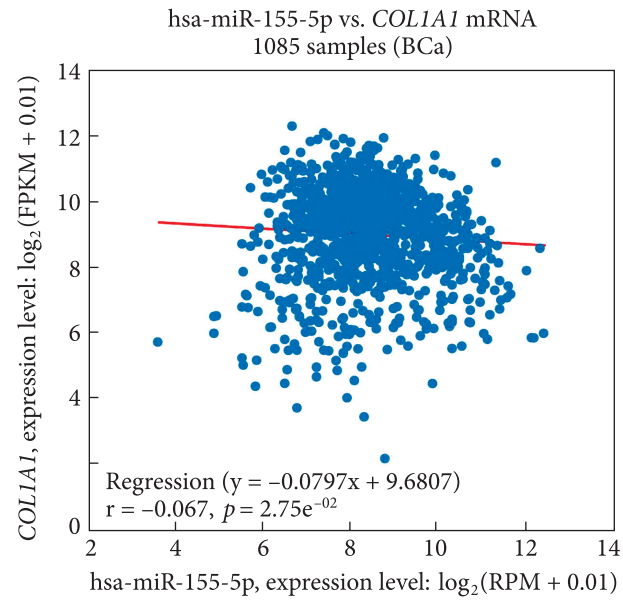
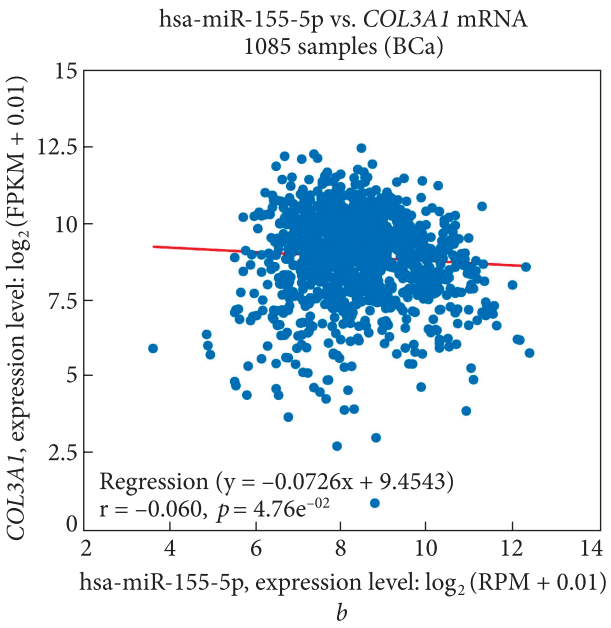


Fig. 3. The relationship between the functional status of MCs and the morphometric parameters of collagen in BCa tissue. * $p < 0.05$, the difference is significant

To find out the significance of the functional status of MCs in the processes of TME collagen matrix reconstruction, at the next stage, we studied the peculiarities of the organization of collagen fibers in the BCa tissue. As seen from the data shown in Fig. 3, the high MCs density is associated with a decrease in the width of collagen fibers by 13.7% ($p = 0.017$), while the low functional activity of MCs is associated with increasing their width (by 13.2%, $p = 0.015$) and length (by 9.2%,





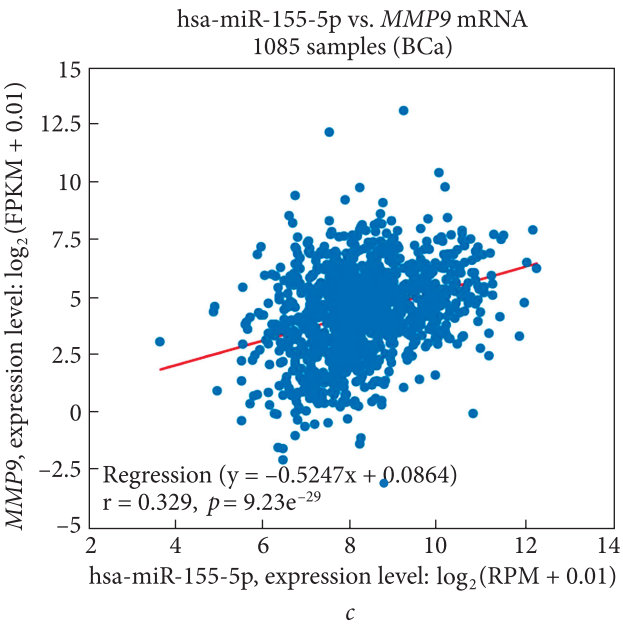


Fig. 4. Correlation between miR-155-5p and *COL1A1* (*a*), *COL3A1* (*b*), as well as *MMP9* (*c*) mRNA expression levels in BCa tissue samples: RPM — reads per million; FPKM — fragments per kilobase million (according to the open information resource ENCORI project (https://rnasysu.com/encori/index.php)

p = 0.016), as well as decreasing fiber density per unit area (by 14.5%, p = 0.017).

We identified the relationship between the MCs status and the morphometric parameters of the collagen matrix, probably indicating their participation in the regulation of the processes of breast neoplasm stroma remodeling. Tumor tissue with a high density and functional activity of MCs is characterized by the increased expression of both fibrillar collagens and MMP-9, involved in remodeling the extracellular matrix. This is confirmed by the dynamics of changes in the morphometric parameters of the collagen matrix depending on the MCs degranulation index.

The relationship of miR-155-5p levels with indicators of the TME stromal component marker expression and the collagen matrix spatial organization of the BCa tissue. Using open-source data Tarbase v. 9.0, miRNet v. 2.0, DIANA miRPath v. 3.0, and DAVID allowed us to establish that hsa-miR-155-5p is involved in the regulation of the formation of intercellular contacts, cell migration, proliferation, cytokine secretion by several immune cells, steroid biosynthesis, and in the modulation of signaling cascades of TGF-β and insulin.

Under physiological conditions, miR-155-5p is involved in the T-cell functional activity regulation and maintenance of cytokine balance in MCs [25].

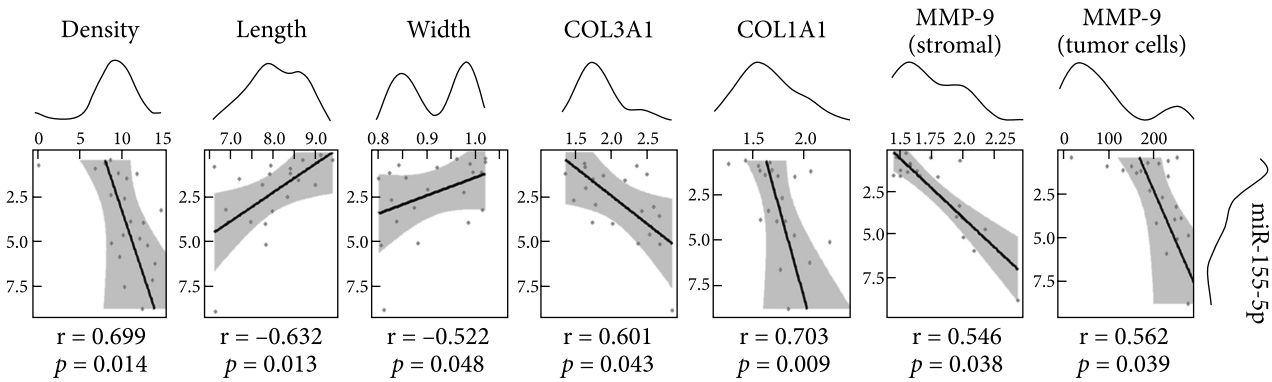


Fig. 5. Correlation between miR-155-5p expression level and features of the stromal component of BCa. The curves on the left and above the graphs characterize the group's values distribution

Dysregulation of this microRNA is associated with a change in the expression of MMP-9, which contributes to the emergence of inflammatory processes and tissue remodeling, especially in the presence of fibrosis [26]. In addition, this miRNA is involved in the epigenetic regulation of fibrous components of the stroma, in particular, collagen structures in fibrosis and angiogenesis [27, 28].

According to data from the ENCORI project resource, the existence of a positive correlation between the expression levels of MMP9 mRNA and miR-155-5p in breast tumor tissue has been proven ($r = 0.329$, $p = 9.23e^{-29}$). At the same time, the correlation between this miRNA and mRNA levels of the COL1A1 ($r = -0.067$, $p = 0.275e^{-02}$) and COL3A1 ($r = -0.060$, $p = 4.76e^{-02}$) genes in the BCa tissue samples is weakly negative but statistically significant (Fig. 4).

Taking into account the above, at the next stage, we analyzed the correlations between miR-155-5p expression indicators and the features of the stromal component of BCa (Fig. 5). A direct correlation of the miR-155-5p levels with the expression of COL1A1 ($r = 0.703$, $p = 0.009$), COL3A1 ($r = 0.603$, $p = 0.043$), and MMP-9 in tumor cells ($r = 0.562$, $p = 0.039$) and in stroma ($r = 0.546$, $p = 0.038$) of neoplasms, as well as with the density of the fibrous component of the BCa tissue ($r = 0.699$, $p = 0.014$) was identified. Along with this, an inverse relationship between the expression of this miR and the width ($r = -0.522$, $p = 0.048$) and length ($r = -0.632$, $p = 0.013$) of collagen fibers in BCa tissue was demonstrated.

To understand the peculiarities of MCs regulation and their role in the formation of TME, at the next stage, we investigated the relationship between

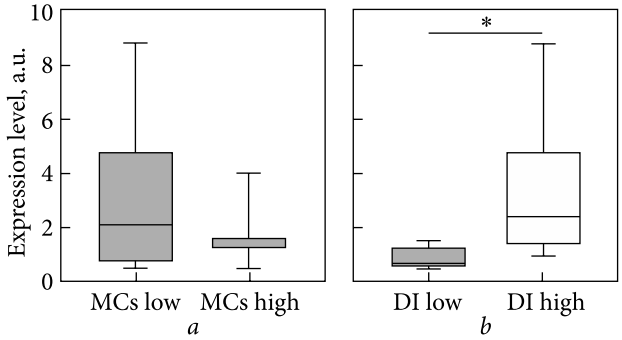


Fig. 6. Relationship between miR-155-5p expression level and infiltration (*a*) and degranulation (*b*) indices of MCs in BCa tissue. * $p < 0.05$, the difference is significant

miR-155-5p expression indicators (Fig. 6) and the status of MCs. Significantly higher rates of this miRNA expression were recorded in BCa tissue with a high index of MCs degranulation. No correlation of miR-155-5p expression indicators was found depending on the MCs infiltration into BCa tissue.

In summary, we can assert the existence of a relationship between the levels of miR-155-5p and the expression of COL1A1, COL3A1, and MMP-9 in BCa tissue. In addition, associations of the levels of this miRNA with the morphometric parameters of the spatial organization of the collagen matrix in samples of malignant breast tumors were found, which indicates its involvement in the regulation of tumor stroma remodeling processes.

Discussion

It is known that the development and progression of breast neoplasms are accompanied by inflammatory processes, the modulation of which is achieved

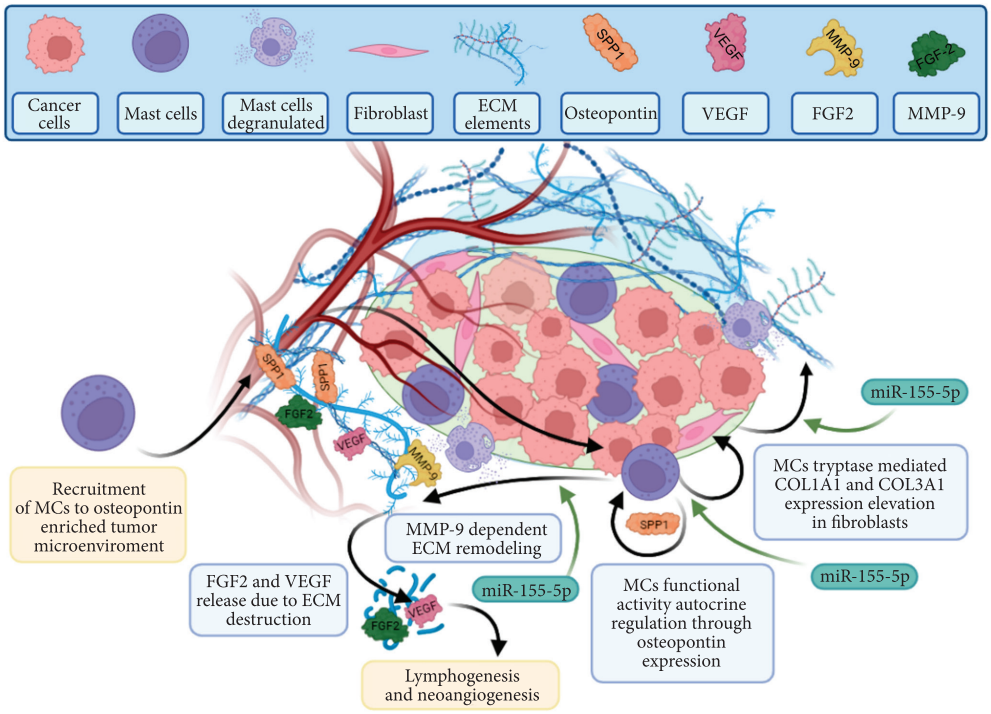


Fig. 7. The role of mast cells in the formation of TME of BCa

with the participation of immune cells infiltrated into the tumor tissue [29]. Inflammation is a key driver of remodeling of the microenvironment, which promotes the activation of tumor growth due to the migration and invasion of malignantly transformed cells [30, 31]. In recent years, there have been reports that prove that proteolytic cleavage of the extracellular matrix goes far beyond its rearrangements and significantly affects the immune component of the microenvironment [32].

MCs, which can modulate the composition of cellular and acellular components of neoplasms, are an integral part of the immune component of the BCa microenvironment. The peculiarities of the interaction of MCs with the components of the BCa microenvironment have not been finally clarified. The contact interaction of MCs with the elements of the extracellular matrix causes the release of not only a number of pro-inflammatory agents but also matrixins, which are involved in the rearrangements of the tumor stroma. Among them, MMP-9 is the most studied in the context of MCs action, because when activated by contact with T-cells, MCs can secrete MMP-9 [15, 33]. As known, MMP-9, in addition to its classic functions of remodeling the extracellular matrix, exhibits proinflammatory properties [34]. This suggests that MCs infiltration in the TME leads to the manifestation of inflammatory processes both intratumorally and systemically. At the same time, the increase in the number of MCs in the BCa tissue causes the activation of tumor stroma remodeling processes, which is confirmed by our data. Due to the destruction of the extracellular matrix fibers by MCs, the bound forms of VEGF and FGF-2 are released, which leads to increased neoangiogenesis and activation of tumors' metastatic activity [35].

It has been reported that tryptase from MCs granules can cause activation of myofibroblasts [36], enhance collagen expression [37], and contribute to the restructuring of the stroma of breast tumors. It was established that the decisive role in the regulation of the activity of the fibroblasts is determined precisely by their contact interaction with MCs, which leads to the contraction of the collagen matrix [38]. This is confirmed by the results of studies by Behzad et al. [39] regarding the effect of MCs on the synthetic 3D matrix of collagen. Feng et al. [40] showed that the amount of MCs in the peritumoral area of BCa is directly related to the amount of collagen there. Blocking the MCs degranulation also decreases the expression levels of COL1A1 and COL3A1 during wound healing [41]. Tissue enriched with MCs is also characterized by stabilization of COL1A1 mRNA and increased concentration of hydroxyproline

(a key component of collagen fibrils) in fibroblasts, [42]. The results of Ribeiro et al. are interesting as such associating the number of MCs infiltrated into human gingival tissue with a greater proportion of immature collagen fibers [43] characterized by shorter length and width [44]. These data confirm the positive relationship we identified between the number of infiltrated MCs and the expression of COL1A1 and COL3A1 and also explain the thinning and shortening of fibers destroyed by stromal MMP-9.

In addition to cytokines and proteins of the tumor stroma [6, 45, 46], miRNAs capable of regulating gene expression by RNA interference are also involved in MCs regulation. In particular, miRNA-155-5p is among the promising miRNAs that can modulate both the functions of cells of the immune system and elements of the stroma of several organs [47—49]. It has been reported that it is involved in the pathogenesis of oncological diseases [50, 51].

It should also be noted that miRNA-155-5p, among the several other short non-coding nucleotide sequences, is singled out as a key modulator of the formation of the inflammatory immune response [52]. Its participation in regulating the functional activity of MCs has also been proven [25, 53]. Along with this, a direct relationship between miRNA-155-5p and MMP-9 expression indicators was found [54, 55], as well as elements of the fibrous component of the stroma [27, 28, 56].

Summarizing the above discussed and taking into account our results, we can conclude that during the progression of BCa, the role of MCs in the manifestation of the tumor process increases. In addition, it can be confidently asserted that the growing number of infiltrated MCs contributes to the activation of the expression of MMP and fibrillar collagens in a monomeric form. These changes lead to increased remodeling of the tumor stroma, which is directly reflected in the spatial organization of the collagen matrix. The increased activity of proteases causes a decrease in the length and width of fibrils, which is explained by a decrease in the number of mature fibers and their disorganization in three-dimensional space. Some matricellular proteins, including osteopontin, as well as miRNA (Fig. 6), act as regulators of MCs' effects on the TME. The obtained data allow us to assert that MCs play a key role not only in the formation of a specific immune microenvironment of BCa but also in determining the direction of changes in the tumor stroma, which determines the high aggressiveness degree of the disease course.

Acknowledgments

This study was supported by the research programs «Development and Validation of Complex Treatment Technology for Breast Cancer Patients of Young Age» (0122U201203) financed by the Ministry of Education and Science of Ukraine, «Verification of Key Pathomorphological Characteristics of Tumor Growth as Innovative Elements of Artificial Intelligence in the Optimization of Breast Cancer Diagnostic Technology» (0121U110569), and «Stress-induced Tumor Microenvironment Factors as Risk Drivers of Breast Cancer Progression» (0124U000078) financed by the National Academy of Sciences of Ukraine.

REFERENCES

1. Tiwari A, Trivedi R, Lin SY. Tumor microenvironment: barrier or opportunity towards effective cancer therapy. *J Biomed Sci*. 2022;29(1):83. https://doi.org/10.1186/s12929-022-00866-3
2. Lukianova N, Mushii O, Zadvornyi T, Chekhun V. Development of an algorithm for biomedical image analysis of the spatial organization of collagen in breast cancer tissue of patients with different clinical status. *FEBS Open Bio*. 2024;14(4):675-686. https://doi.org/10.1002/2211-5463.13773
3. Lukianova N, Zadvornyi T, Mushii O, et al. Evaluation of diagnostic algorithm based on collagen organization parameters for breast tumors. *Exp Oncol*. 2022;44(4):281-286. https://doi.org/10.32471/exp-oncology.2312-8852.vol-44-no-4.19137
4. Zadvornyi T, Lukianova N, Mushii O, et al. Benign and malignant prostate neoplasms show different spatial organization of collagen. *Croat Med J*. 2023;64(6):413-420. https://doi.org/10.3325/cmj.2023.64.413
5. Naleskina LA, Lukianova NY, Zadvornyi TV, et al. Remodeling the architecture of collagen-containing connective tissue fibers of metastatic prostate cancer. *Cytol Genet*. 2023;57:406-412. https://doi.org/10.3103/S0095452723050031
6. Lukianova N, Zadvornyi T, Borikun T, et al. Significance of osteopontin for predicting aggressiveness of prostate cancer. *Exp Oncol*. 2023;45(3):312-321. https://doi.org/10.15407/exp-oncology.2023.03.312

7. Chekhun V, Pavlova A, Zadvornyi T, et al. Expression of SPP1 and SPARC genes in tumor tissue of patients with breast cancer. *Exp Oncol*. 2024;46(1):13-21. https://doi.org/10.15407/exp-oncology.2024.01.013
8. Chekhun V, Mushii O, Zadvornyi T, et al. Features of COL1A1 expression in breast cancer tissue of young patients. *Exp Oncol*. 2023;45(3):351-363. https://doi.org/10.15407/exp-oncology.2023.03.351
9. Janjanam J, Pano G, Wang R, et al. Matricellular protein WISP2 is an endogenous inhibitor of collagen linearization and cancer metastasis. *Cancer Res*. 2021;81(22):5666-5677. https://doi.org/10.1158/0008-5472.CAN-20-3982
10. Simon T, Salhia B. Cancer-associated fibroblast subpopulations with diverse and dynamic roles in the tumor microenvironment. *Mol Cancer Res*. 2022;20(2):183-192. https://doi.org/10.1158/1541-7786.MCR-21-0282
11. Xu R, Yin P, Wei J, Ding Q. The role of matrix stiffness in breast cancer progression: a review. *Front Oncol*. 2023;13:1284926. https://doi.org/10.3389/fonc.2023.1284926
12. Mushii O, Pavlova A, Bazas V, et al. Osteopontin-regulated changes in the mast cell population are associated with breast cancer. *Exp Oncol*. 2024;46(3):209-220. https://doi.org/10.15407/exp-oncology
13. Yoshii M, Jikuhara A, Mori S, et al. Mast cell tryptase stimulates DLD-1 carcinoma through prostaglandin- and MAP kinase-dependent manners. *J Pharmacol Sci*. 2005;98(4):450-458. https://doi.org/10.1254/jphs.fpj05002x
14. Blair RJ, Meng H, Marchese MJ, et al. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. *J Clin Invest*. 1997;99(11):2691-2700. https://doi.org/10.1172/JCI119458
15. Baram D, Vaday GG, Salamon P, et al. Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF-alpha. *J Immunol*. 2001;167(7):4008-4016. https://doi.org/10.4049/jimmunol.167.7.4008
16. Atiakshin D, Buchwalow I, Tiemann M. Mast cells and collagen fibrillogenesis. *Histochem Cell Biol*. 2020;154(1):21-40. https://doi.org/10.1007/s00418-020-01875-9
17. Linder DP, Poberiĭ IA, Rozkin MIa, Efimov VS. Morfometricheskiĭ analiz populiatsii tuchnykh kletok [Morphometric analysis of a mast cell population]. *Arkh Patol*. 1980;42(6):60-64. PMID: 6998431
18. McClelland RA, Wilson D, Leake R, et al. A multicentre study into the reliability of steroid receptor immunocytochemical assay quantification. British Quality Control Group. *Eur J Cancer*. 1991;27(6):711-715. https://doi.org/10.1016/0277-5379(91)90171-9
19. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue — a review. *Diagn Pathol*. 2014;9:221. https://doi.org/10.1186/s13000-014-0221-9
20. Seyed Jafari SM, Hunger RE. IHC Optical Density Score: A new practical method for quantitative immunohistochemistry image analysis. *Appl Immunohistochem Mol Morphol*. 2017;25(1):e12-e13. https://doi.org/10.1097/PAI.0000000000000370
21. Chekhun V, Borikun T, Zadvornyi T, et al. Osteonectin (SPARC) prognostic value in prostate cancer. *Pathol Res Pract*. 2024;254:155053. https://doi.org/10.1016/j.prp.2023.155053
22. Zhang JD, Ruschhaupt M, Biczok R. ddCt method for qRT–PCR data analysis. Available from: https://www.bioconductor.org/packages/devel/bioc/vignettes/ddCt/inst/doc/rtPCR.pdf Accessed October 29, 2024
23. Vlachos IS, Paraskevopoulou MD, Karagkouni D, et al. DIANA-TarBase v7.0: indexing more than half a million experimentally supported miRNA:mRNA interactions. *Nucleic Acids Res*. 2015;43:D153-D159. https://doi.org/10.1093/nar/gku1215
24. Sherman BT, Hao M, Qiu J, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res*. 2022;50(W1):W216-W221. https://doi.org/10.1093/nar/gkac194
25. Mohammed Z, McHale C, Kubinak JL, et al. miR-155 is a positive regulator of FcεRI-induced cyclooxygenase-2 expression and cytokine production in mast cells. *Front Allergy*. 2022;3:835776. https://doi.org/10.3389/falgy.2022.835776
26. Csak T, Bala S, Lippai D, et al. MicroRNA-155 deficiency attenuates liver steatosis and fibrosis without reducing inflammation in a mouse model of steatohepatitis. *PLoS One*. 2015;10(6):e0129251. https://doi.org/10.1371/journal.pone.0129251
27. Chen Y, Xu D, Yao J, et al. Inhibition of miR-155-5p exerts anti-fibrotic effects in silicotic mice by regulating meprin α. *Mol Ther Nucleic Acids*. 2020;19:350-360. https://doi.org/10.1016/j.omtn.2019.11.018
28. Lou R, Chen J, Zhou F, et al. Exosomal miRNA-155-5p from M1-polarized macrophages suppresses angiogenesis by targeting GDF6 to interrupt diabetic wound healing. *Mol Ther Nucleic Acids*. 2023;34:102074. https://doi.org/10.1016/j.omtn.2023.102074
29. Jiang X, Shapiro DJ. The immune system and inflammation in breast cancer. *Mol Cell Endocrinol*. 2014;382(1):673-682. https://doi.org/10.1016/j.mce.2013.06.003
30. Ortiz-Montero P, Londoño-Vallejo A, Vernot JP. Senescence-associated IL-6 and IL-8 cytokines induce a self- and cross-reinforced senescence/inflammatory milieu strengthening tumorigenic capabilities in the MCF-7 breast cancer cell line. *Cell Commun Signal*. 2017;15(1):17. https://doi.org/10.1186/s12964-017-0172-3
31. Murray JI, West NR, Murphy LC, Watson PH. Intratumoural inflammation and endocrine resistance in breast cancer. *Endocr Relat Cancer*. 2015;22(1):R51-R67. https://doi.org/10.1530/ERC-14-0096
32. Adair-Kirk TL, Senior RM. Fragments of extracellular matrix as mediators of inflammation. *Int J Biochem Cell Biol*. 2008;40(6-7):1101-1110. https://doi.org/10.1016/j.biocel.2007.12.005

33. Kanbe N, Tanaka A, Kanbe M, et al. Human mast cells produce matrix metalloproteinase 9. *Eur J Immunol*. 1999;29(8):2645-2649. https://doi.org/10.1002/(SICI)1521-4141(199908)29:08<2645::AID-IMMU2645>3.0.CO;2-1
34. Xu L, Cai Z, Yang F, Chen M. Activation-induced upregulation of MMP9 in mast cells is a positive feedback mediator for mast cell activation. *Mol Med Rep*. 2017;15(4):1759-1764. https://doi.org/10.3892/mmr.2017.6215
35. Hu G, Wang S, Cheng P. Tumor-infiltrating tryptase+ mast cells predict unfavorable clinical outcome in solid tumors. *Int J Cancer*. 2018;142(4):813-821. https://doi.org/10.1002/ijc.31099
36. Mangia A, Malfettone A, Rossi R, et al. Tissue remodelling in breast cancer: human mast cell tryptase as an initiator of myofibroblast differentiation. *Histopathology*. 2011;58(7):1096-1106. https://doi.org/10.1111/j.1365-2559.2011.03842.x
37. Abe M, Kurosawa M, Ishikawa O, et al. Mast cell tryptase stimulates both human dermal fibroblast proliferation and type I collagen production. *Clin Exp Allergy*. 1998;28(12):1509-1517. https://doi.org/10.1046/j.1365-2222.1998.00360.x
38. Yamamoto T, Hartmann K, Eckes B, Krieg T. Mast cells enhance contraction of three-dimensional collagen lattices by fibroblasts by cell-cell interaction: role of stem cell factor/c-kit. Immunology. 2000;99(3):435-439. https://doi.org/10.1046/j.1365-2567.2000.00973.x
39. Behzad H, Sharma A, Mousavizadeh R, et al. Mast cells exert pro-inflammatory effects of relevance to the pathophysiology of tendinopathy. *Arthritis Res Ther*. 2013;15(6):R184. https://doi.org/10.1186/ar4374
40. Feng TY, Azar FN, Dreger SA, et al. Reciprocal interactions between the gut microbiome and mammary tissue mast cells promote metastatic dissemination of HR+ breast tumors. *Cancer Immunol Res*. 2022;10(11):1309-1325. https://doi.org/10.1158/2326-6066.CIR-21-1120
41. Dong X, Geng Z, Zhao Y, et al. Involvement of mast cell chymase in burn wound healing in hamsters. *Exp Ther Med*. 2013;5(2):643-647. https://doi.org/10.3892/etm.2012.836
42. Gordon JR, Galli SJ. Promotion of mouse fibroblast collagen gene expression by mast cells stimulated via the Fc epsilon RI. Role for mast cell-derived transforming growth factor beta and tumor necrosis factor alpha. *J Exp Med.* 1994;180(6):2027-2037. https://doi.org/10.1084/jem.180.6.2027
43. E Ribeiro LSF, Dos Santos JN, Rocha CAG, Cury PR. Association between mast cells and collagen maturation in chronic periodontitis in humans. *J Histochem Cytochem*. 2018;66(6):467-475. https://doi.org/10.1369/0022155418765131
44. Partusch L, Michler JK, De Spiegelaere W. Collagen remodeling in equine exuberant granulation tissue characterized by picrosirius red staining 34th EAVA Congress, Abstracts, 2023. Available from: https://biblio.ugent.be/publication/01HMGZGGXKHMXV3YJM1B4ZRDPP
45. Lukianova N, Zadvornyi T, Kashuba E, et al. Expression of markers of bone tissue remodeling in breast cancer and prostate cancer cells in vitro. *Exp Oncol*. 2022;44(1):39-46. https://doi.org/10.32471/exp-oncology.2312-8852.vol-44-no-1.17354
46. Nagasaka A, Matsue H, Matsushima H, et al. Osteopontin is produced by mast cells and affects IgE-mediated degranulation and migration of mast cells. *Eur J Immunol*. 2008;38(2):489-499. https://doi.org/10.1002/eji.200737057
47. Tong Y, Zhou MH, Li SP, et al. MiR-155-5p attenuates vascular smooth muscle cell oxidative stress and migration via inhibiting BACH1 expression. *Biomedicines*. 2023;11(6):1679. https://doi.org/10.3390/biomedicines11061679
48. Guo P, Qiao F, Huang D, et al. MiR-155-5p plays as a "janus" in the expression of inflammatory cytokines induced by T-2 toxin. *Food Chem Toxicol*. 2020;140:111258. https://doi.org/10.1016/j.fct.2020.111258
49. Cazzanelli P, Lamoca M, Hasler J, et al. The role of miR-155-5p in inflammation and mechanical loading during intervertebral disc degeneration. *Cell Commun Signal*. 2024;22(1):419. https://doi.org/10.1186/s12964-024-01803-7
50. Kalantzakos T, Hooper K, Das S, et al. MicroRNA-155-5p targets JADE-1, promoting proliferation, migration, and invasion in clear cell renal cell carcinoma cells. *Int J Mol Sci*. 2023;24(9):7825. https://doi.org/10.3390/ijms24097825
51. Li Y, Zhang L, Dong Z, et al. MicroRNA-155-5p promotes tumor progression and contributes to paclitaxel resistance via TP53INP1 in human breast cancer. *Pathol Res Pract*. 2021;220:153405. https://doi.org/10.1016/j.prp.2021.153405
52. Wang X, Chen Y, Yuan W, et al. MicroRNA-155-5p is a key regulator of allergic inflammation, modulating the epithelial barrier by targeting PKIα. *Cell Death Dis*. 2019;10(12):884. https://doi.org/10.1038/s41419-019-2124-x
53. Wang Z, Yi T, Long M, et al. Involvement of the negative feedback of IL-33 signaling in the anti-inflammatory effect of electro-acupuncture on allergic contact dermatitis via targeting microRNA-155 in mast cells. *Inflammation*. 2018;41(3):859-869. https://doi.org/10.1007/s10753-018-0740-8
54. Zhou X, Zhang J, Liu J, et al. MicroRNA miR-155-5p knockdown attenuates Angiostrongylus cantonensis-induced eosinophilic meningitis by downregulating MMP9 and TSLP proteins. *Int J Parasitol*. 2021;51(1):13-22. https://doi.org/10.1016/j.ijpara.2020.07.013
55. Bala S, Zhuang Y, Nagesh PT, et al. Therapeutic inhibition of miR-155 attenuates liver fibrosis via STAT3 signaling. *Mol Ther Nucleic Acids*. 2023; 33:413-427. https://doi.org/10.1016/j.omtn.2023.07.012
56. He W, Huang H, Xie Q, et al. MiR-155 knockout in fibroblasts improves cardiac remodeling by targeting tumor protein p53-inducible nuclear protein 1. *J Cardiovasc Pharmacol Ther*. 2016;21(4):423-435. https://doi.org/10.1177/1074248415616188

Submitted: October 18, 2024

О. Мушій [1], А. Павлова [1], В. Базась [2], Т. Борікун [1], Н. Лук'янова [1]

[1] Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького НАН України, Київ, Україна

[2] Київський міський клінічний онкологічний центр, Київ, Україна

ОПАСИСТІ КЛІТИНИ ЯК ФАКТОР РЕГУЛЯЦІЇ СТРОМАЛЬНОГО КОМПОНЕНТУ РАКУ МОЛОЧНОЇ ЗАЛОЗИ, ПОВ'ЯЗАНИЙ З АГРЕСИВНІСТЮ ПЕРЕБІГУ ПУХЛИННОГО ПРОЦЕСУ

Вступ. Доведено, що зміни морфології, репрезентації та організації колагенових волокон сприяють формуванню унікального пухлинного мікрооточення (ТМЕ), яке пов'язане з метастатичним потенціалом злоякісних новоутворень внаслідок ініціації клітинної міграції та зміни поляризації. Серед модуляторів колагенової строми на сьогодні найбільш вивченими залишаються фібробласти. Водночас значно менше уваги дослідники приділяють вивченню імунних клітин ТМЕ, зокрема опасистих клітин (ОК). **Мета**: дослідити зв'язок між функціональним станом ОК та особливостями колагенового матриксу раку молочної залози (РМЗ). **Матеріали і методи**. Дослідження проведено на післяопераційному матеріалі 78 хворих на РМЗ I—II стадії. Визначення ОК проводили гістохімічним методом з використанням толуїдинового синього. Для оцінки функціональної активності ОК використовували розрахунок індексу дегрануляції. Експресію COL1A1, COL3A1 та MMP-9 у пухлинній тканині визначали імуногістохімічним методом. Візуалізацію колагенових волокон проводили гістохімічним методом фарбування тканин за Малорі. Попередню обробку мікрофотографій проводили в програмі Adobe Photoshop SS 2019. Аналіз оброблених зображень виконували за допомогою програмних пакетів CurveAlign v. 4.0 та ImageJ. **Результати**. Пухлинна тканина з високою щільністю та функціональною активністю ОК характеризується підвищеною експресією COL1A1 ($p < 0,05$), COL3A1 ($p < 0,05$) та MMP-9 ($p < 0,05$). У тканині РМЗ з нижчим індексом дегрануляції ОК колагенові волокна стають товщими ($p < 0,05$), коротшими ($p < 0,05$), а колагенова матриця щільнішою ($p < 0,05$). Водночас виявлено зв'язок між рівнями miR-155-5p та експресією COL1A1 (r = 0,703, $p = 0,009$), COL3A1 (r = 0,603, $p = 0,043$), MMP-9 в пухлинних клітинах (r = 0,562, $p = 0,039$) та в стромі (r = 0,546, $p = 0,038$), а також показано асоціацію рівнів цієї мікроРНК з довжиною (r = –0,632, $p = 0,013$), шириною (r = –0,522, $p = 0,048$) колагенових волокон та щільністю колагенової матриці (r = 0,699, $p = 0,014$). Достовірно вищі показники експресії miR-155-5p ($p < 0,05$) зареєстровано в тканині РМЗ з високим індексом дегрануляції ОК. **Висновки**. При прогресуванні РМЗ зростає роль ОК у пухлинному процесі. Зростаюча кількість інфільтрованих ОК сприяє активації експресії матриксних металопротеїназ і фібрилярного колагену. Ці зміни призводять до посиленого ремоделювання строми пухлини, що безпосередньо відображається на просторовій організації колагенового матриксу. Підвищена активність протеаз викликає зменшення довжини і ширини фібрил, що пояснюється зменшенням кількості зрілих волокон і їх дезорганізацією в тривимірному просторі. Отримані дані дозволяють стверджувати, що ОК відіграють ключову роль не лише у формуванні специфічного імунного мікрооточення РМЗ, а й у визначенні напрямку змін у стромі пухлини, що зумовлює високий ступінь агресивності перебігу захворювання.

Ключові слова: рак молочної залози, опасисті клітини, колагеновий матрикс, матриксні металопротеїнази, ремоделювання позаклітинного матриксу.