

Mast Cells as an Indicator and Prognostic Marker in Molecular Subtypes of Breast Cancer

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Abstract. *Background/Aim: Mast cells (MCs) represent the most controversial non-malignant element of the tumor microenvironment. Our aim was to study how MCs density and distribution (intratumoral-MC_{it} versus peritumoral-MC_{pt}) relate to tumor grade and molecular subtypes. Materials and Methods: MCs tryptase immunohistochemistry was performed on 80 cases of breast carcinomas. Results: For Luminal A tumors, a partial correlation was detected between MC_{it} and progesterone receptor (PR) ($p=0.005$). Luminal B tumors showed a significant correlation between MC_{pt} and age ($p=0.009$), estrogen receptor (ER) ($p=0.017$) and PR ($p=0.035$). MC_{it} and MC_{pt} were strongly interrelated in this subtype ($p=0.002$) and in triple-negative breast cancers ($p=0.002$). In HER2 subtype, MC_{pt} tumors were significantly correlated with HER2 ($p=0.044$). In G2 tumors, MC_{pt} correlated with ER ($p=0.015$) and PR ($p=0.038$) while in G3 tumors ER correlated with both MC_{it} ($p=0.009$) and MC_{pt} ($p=0.000487$) tumors. Conclusion: MCs dynamics are strongly influenced by hormone receptors and HER2 status. MC_{it} increased in aggressive tumor types and is a worse prognostic factor.*

Breast cancer is the most common type of cancer in women and the second most common cancer overall. Over 2 million

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new cases of breast cancer appeared in 2018, and its incidence and mortality are rapidly growing worldwide (1). Breast cancer is a complex and heterogeneous disease in terms of microscopic features, therapeutic response, spreading to distant sites, and patients' outcomes (2). A possible explanation could be, in part, that we still lack a complete picture of the biological heterogeneity of breast cancer with respect to molecular changes and cellular composition. Importantly, this complexity is not entirely reflected by the main clinical parameters (age, lymph node status, tumor size, histological grade) and molecular markers. Molecular classification, introduced by Prat and Perou, is routinely used in the clinic to stratify patients for prognostic predictions and select treatments; however, it does not perfectly describe all the entities and there are still many debated issues (2, 3).

Cancer research is focused mainly on malignant cells, while non-malignant components of the tumor microenvironment are less investigated. Virtually, the contribution of some cells of the tumor stroma to the progression and metastasis is not yet understood. The tumor is a complex structure and it is now clear that stromal cells in the tumor microenvironment play an important role in cancer development (4). These could bring new insight concerning tumorigenesis and be used for the development of new therapeutic targets (4).

Mast cells (MCs) represent a controversial component of the breast cancer stromal compartment. Studies have shown that mast cells accumulate in a variety of malignant tumors, such as melanoma, Hodgkin's lymphoma and chronic lymphocytic leukemia, pancreatic, prostate and esophageal cancer (5). Some reports have shown that the high number of MCs is associated with a good prognosis (6), while others have noticed their association with poor prognosis and distant metastasis (7-9). Some reports suggest that mast cells play a promoting role in the occurrence and development of

malignant tumors (10, 11). Based on the controversies found in the literature, in the present work we tried to answer whether MCs are of good or bad prognosis in breast cancer.

Based on the expression of chymase and tryptase, the mast cell-specific serine proteases, human MCs are divided into MC_T, which express only tryptase, and MC_{TC}, which express both enzymes (6). Preliminary investigations have suggested that during breast cancer progression, MCs may contribute to stromal remodeling and differentiation of myofibroblasts through the release of tryptase in the stromal microenvironment (11). It has been shown that MC tryptase increases cell migration and invasion and promotes breast cancer invasion (6). MCs also take part in the inflammatory reaction occurring at the periphery of the tumor (12). Mast cell-secreted angiogenic cytokines facilitate tumor vascularization not only directly but also by stimulating other inflammatory cells of the tumor microenvironment to release other angiogenic mediators (12).

Since the literature displays contradictory results regarding the role of MCs in breast cancer, the purpose of this study was to analyze how MCs' density and distribution relates with the main molecular types of breast cancer.

Materials and Methods

We investigated 80 cases of breast cancer from patients ranging between 37 and 84 years, mean of age being 66,04±8,65 years (Table I). Patients didn't go under radio- or chemotherapy before surgery. The study was approved by the Ethics Committee of Nicolae Testemițanu State University of Medicine and Pharmacy, Chișinău, Republic of Moldova (no. 33/ 37/ 12.02.2018).

Histology. Routinely processed and paraffin embedded specimens were used for creation of tissue microarrays (TMA) by means of TMA Grand Master (3DHISTECH Ltd., Budapest, Hungary). Tissue microarrays are paraffin blocks produced by extracting cylindrical tissue cores from different paraffin donor blocks and re-embedding them into a single recipient (microarray) block (13). Sections from these blocks were stained automatically by Leica Autostainer XL (Leica Biosystems, Newcastle UponTyne, UK) and were mounted automatically (Leica CV5030, Leica Biosystems, Newcastle UponTyne, UK). Tumor histology was reviewed by 3 pathologists and appropriate sections were selected for immunohistochemical stains.

Immunohistochemistry. Immunohistochemical staining was performed automatically using Leica Bond-Max (Leica Biosystems, Newcastle UponTyne, UK). Antigen retrieval was achieved using the Bond Epitope Retrieval Solution 2 (pH 9) (Leica Biosystems, Newcastle UponTyne, UK). We used the following antibodies to establish the molecular subtype and to identify MCs: ER – clone 6F11, PR – clone 16, CK5 – clone XM26, MCT (mast cell tryptase) – clone 10D11 (all antibodies were pre-diluted, source - Leica Bond, Leica Biosystems, Newcastle UponTyne, UK); HER2 – clone CB11, EGFR – clone EGFR.113 (both pre-diluted, source - Novocastra, Leica Biosystems, Newcastle UponTyne, UK). DAB (3, 3'-diaminobenzidine) (Leica Biosystems, Newcastle UponTyne, UK) was applied as a chromogen substrate and Mayer's hematoxylin was used for nuclear counterstaining.

Table I. Patients' data.

	Molecular subtype			
	Luminal A	Luminal B	HER2+	Triple-negative
N of cases	14	46	9	11
Age median	73.5	67.0	59.0	64.0
Tumor's grade				
G1	1	0	0	0
G2	9	29	3	4
G3	4	17	6	7

Table II. Mean±standard deviation values of intra- and peritumoral MCs.

Subtypes	MCit	MCpt
Luminal A	4.45±5.65	9.95±8.57
Luminal B	4.34±6.33	15.66±14.54
HER2+	1.34±1.85	5.22±3.62
Triple-negative	0.88±0.82	6.72±6.24

MCit: Intratumoral mast cells; MCpt: peritumoral mast cells.

Methods of quantification. Hormone receptors (ER-estrogen receptor and PR-progesterone receptor) were evaluated according to Allred score (14). HER2 (human epidermal growth factor receptor 2) protein was appreciated according to the recommendations of the American Society of Clinical Oncology (15).

Quantification of brown-stained MCs (they should show a moderate to strong cytoplasmic staining) was done using the Axio Imager A2 microscope (Carl Zeiss, Germany). Sections were initially analyzed at ×100 magnification in order to identify the area of highest MCs densities. We also analyzed the distribution of MCs in the tumor and peritumoral areas by counting the number of MCs from intratumoral and peritumoral stroma on 3 microscopic fields for each case at ×400 magnification. The arithmetic average of the three fields was the final result for MCs in each case. Morphology of MCs was analyzed in terms of shape and granulated/ degranulated appearance.

Data analysis. A MS Excel 2010 database was used to store the data that were statistically analyzed using the SPSS statistical software package (SPSS Statistics 23.0; IBM, Chicago, IL, USA). We used Pearson's correlation coefficient (r), Spearman's rank correlation coefficient (r_s) and considered a p-value of less than 0.05 as statistically significant.

Results

Most tumors (45 cases out of 80, 56.3%) were moderately differentiated (G2), 34 cases (42.5%) were poorly differentiated (G3) and only 1 case (1,3%) had a high grade of differentiation (G1). We identified the following histological types of breast

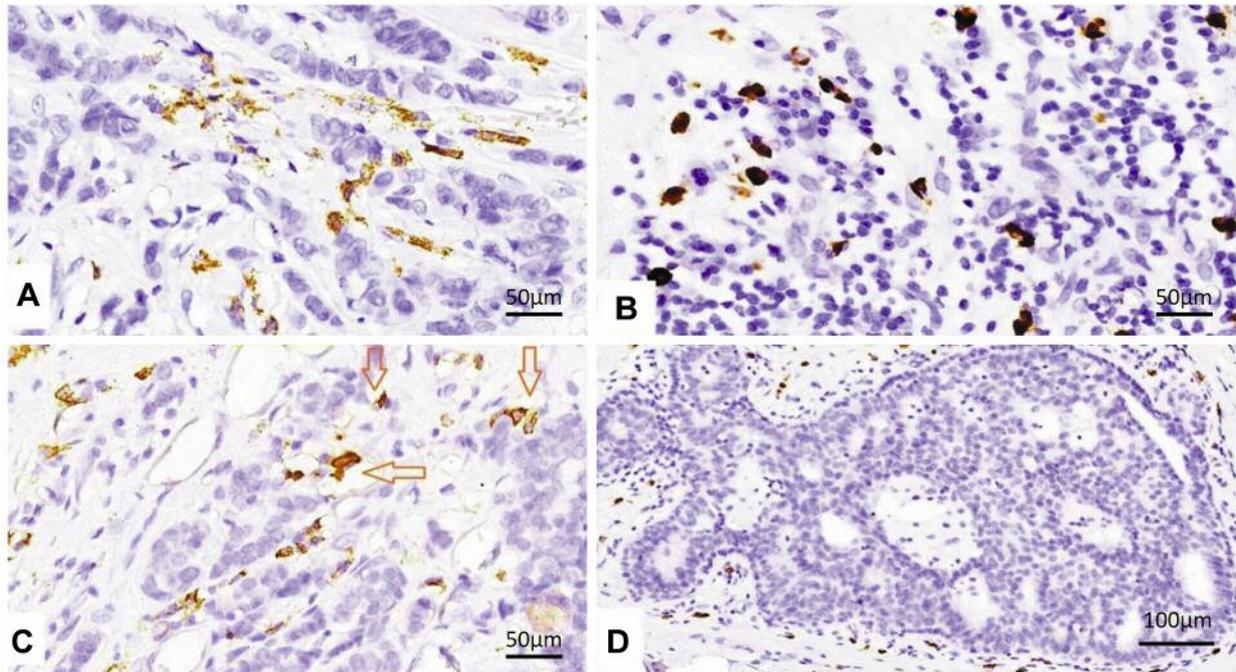


Figure 1. Mast cells (MCs) in breast carcinomas stained for anti-tryptase (brown) and, counterstained with Mayer's hematoxylin (purple). Different patterns of MCs: degranulated (A) and granulated (B). MCs in proximity to lymphatic vessels (arrows) (C). MCs surrounding the tumor nest (D). Scale bars: A-C=50 μm , D=100 μm .

carcinoma: i) ductal invasive in 74 cases (92.5%), ii) lobular invasive in 3 cases (3.75%), iii) lobular *in situ* in 2 cases (2.5%), and iv) ductal *in situ* in 1 case (1.25%).

Immunohistochemical staining revealed: i) 46 cases of luminal B subtype (57.5%), ii) 14 cases of luminal A (17.5%), iii) 9 cases of HER2+ (11.3%) and iv) 11 cases (13.8%) of triple-negative breast carcinomas.

Brown stained MCs were identified in all the slides. Most of them were oval- or spindle-shaped and appeared either as granulated or degranulated (Figure 1A and B). The degranulated MCs possessed an irregular shape with an uneven color and a non-complete cell membrane surface, while the granulated MCs were round or oval with a uniform color and an intact cell membrane surface.

We noticed a prevalence of MCs in the region of lymphatic vessels (Figure 1C). Evaluation of slides pointed out that MCs prevail in the peritumoral stroma, with a tendency to surround the tumor nest, as shown in Figure 1D. The mean of MCpt was 12.2 ± 12.5 cells, with a maximum of 65 cells, while the mean of MCit was 3.5 ± 5.5 cells, with a maximum of 27.6 cells.

Highest values of MCs were determined in the luminal B subtype (mean and standard deviation being 4.34 ± 6.33 for intratumoral and 15.66 ± 14.54 for peritumoral MCs). These were followed by the luminal A subtype (mean and standard deviation being 4.45 ± 5.65 and 9.95 ± 8.57 for the intra- and

peritumoral MCs respectively) (Table II). For Luminal A, a partial correlation was detected between MCit and PR ($r_s=0.703$, $p=0.005$). Luminal B subtype also revealed a positive correlation between MCit and MCpt ($r=0.446$, $p=0.002$) and a positive correlation between MCpt and hormone receptors (ER: $r=0.351$, $p=0.017$ and PR: $r=0.311$, $p=0.035$). MCpt positively correlated with the patients' age ($r=0.382$, $p=0.009$), as well as with HER2+ carcinoma cells ($r=0.680$, $p=0.044$) in HER2+ subtype, while both MCit and MCpt positively correlated with one another in triple-negative breast carcinomas ($r=0.825$, $p=0.002$).

Analyzing overall molecular subtypes, we found a positive correlation between MCit and MCpt ($r=0.442$, $p=0.000041$). In G2 tumors, MCpt correlated with ER+ carcinoma cells ($r=0.360$, $p=0.015$) (Table III) while in G3 tumors ER correlated with both MCit and MCpt ($r=0.439$, $p=0.009$ and $r=0.566$, $p=0.000487$, respectively) (Table IV).

Discussion

Mast cells (MCs) were first identified in human tumors and were named as such by Paul Ehrlich (16). These cells store and release a variety of biologically active substances that can affect various target cells. Although usually associated with allergic disorders, MCs are a major source of pro-tumorigenic (*e.g.*, angiogenic and lymphangiogenic factors) and anti-

Table III. Correlations and p-Values for G2 tumors.

		HistoType	Grade	ER	PR	HER2	MCit	MCpt
HistoType	r	1		0.086	0.201	-0.142	-0.092	0.022
	p			0.574	0.186	0.351	0.548	0.885
	N	45	45	45	45	45	45	45
Grade	r							
	p							
	N	45	45	45	45	45	45	45
ER	r	0.086		1	0.584	0.064	0.290	0.360
	p	0.574			0.000025	0.675	0.053	0.015
	N	45	45	45	45	45	45	45
PR	r	0.201		0.584	1	0.127	0.042	0.310
	p	0.186		0.000025		0.405	0.782	0.038
	N	45	45	45	45	45	45	45
HER2	r	-0.142		0.064	0.127	1	-0.041	0.267
	p	0.351		0.675	0.405		0.791	0.076
	N	45	45	45	45	45	45	45
MCit	r	-0.092		0.290	0.042	-0.041	1	0.423
	p	0.548		0.053	0.782	0.791		0.004
	N	45	45	45	45	45	45	45
MCpt	r	0.022		0.360	0.310	0.267	0.423	1
	p	0.885		0.015	0.038	0.076	0.004	
	N	45	45	45	45	45	45	45

R: Pearson's correlation coefficient; p: p-value; N: number of cases; HistoType: histological type of tumor; Grade: tumor's grade; MCit: intratumoral MCs; MCpt: peritumoral MCs.

tumorigenic molecules (e.g., TNF- α and IL-90), thus, their role on breast cancer is controversial (5). Some scholars believe that mast cell infiltration suggests a good prognosis of breast cancer: similar to our results, three other groups have observed high MCs density in luminal A and B types of breast cancer, which can be hormonally treated and have a better prognosis (6, 10, 17). This suggests that MCs are associated with less aggressive tumors. On the other hand, we know that estrogen acts as a proliferative factor, and can stimulate breast cancer development (18). This can explain why in our study both MCit and MCpt interrelated with ER in G3 tumors. Positive correlations between ER and MCs suggest that estrogen is a chemotactic molecule for MCs. We were especially interested in the correlation between MCit and ER⁺ carcinoma cells, as some studies have proved that estrogen activates mast cells in ovarian endometriosis and that human uterine mast cells express ER β (19, 20). Others have shown that administration of estrogens leads to eosinophilic and MCs infiltrations (21). MCs play a pro-tumorigenic role in human bladder cancer through stimulating ER β and it has been demonstrated in a murine model of bladder cancer that a selective ER β antagonist can inhibit mast cell-promoted tumor growth (9).

Other groups, however, have shown a negative correlation between populations of MCs and tumor grade (6), data that was not confirmed by our study.

Some groups have concluded that the prognosis is worse with a higher density of mast cells in the breast cancer

tissue (10). Xiang *et al.* have observed more numerous peritumoral MCs in G3 breast cancers, increased tryptase being associated with higher tumor grade and more lymph node metastasis compared to lower grades. They have also noted that tryptase promotes the invasion and migration of breast cancer cells along with the activation of matrix metalloproteinase-2, and have concluded that tryptase promotes breast cancer migration and invasion (23). Raica *et al.* revealed strong positive correlations between populations of MCs and lymphatic vessels in some molecular subtypes of breast cancer, thus supporting the idea of MCs involvement in metastasis by lymphangiogenesis (11). Ribatti *et al.* have pointed out that angiogenesis increased in parallel with the number of tryptase-positive MCs particularly inside lymph nodes associated with micrometastases compared to non-metastatic lymph nodes (24). It has also been demonstrated that during breast cancer progression MCs may contribute to stromal remodeling and differentiation of myofibroblasts, through tryptase released in the stromal microenvironment (25). All these mean that targeting MCs could be involved in the inhibition of angiogenesis, lymphangiogenesis and many other negative effects of MCs' activation.

MCs' dynamics is strongly influenced by hormone receptors and HER2 status in breast cancer. Our findings suggest that MCit increased especially in aggressive tumor types and serves as a worse prognostic factor.

Table IV. Correlations and p-Values for G3 tumors.

		HistoType	Grade	ER	PR	HER2	MCit	MCpt
HistoType	r	1		0.166	0.484	0.364	-0.154	0.130
	p			0.349	0.004	0.035	0.386	0.465
	N	34	34	34	34	34	34	34
Grade	r							
	p							
	N	34	34	34	34	34	34	34
ER	r	0.166		1	0.572	0.139	0.439	0.566
	p	0.349			0.000404	0.434	0.009	0.000487
	N	34	34	34	34	34	34	34
PR	r	0.484		0.572	1	0.397	0.319	0.572
	p	0.004		0.000404		0.020	0.066	0.000406
	N	34	34	34	34	34	34	34
HER2	r	0.364		0.139	0.397	1	0.028	0.105
	p	0.035		0.434	0.020		0.875	0.553
	N	34	34	34	34	34	34	34
MCit	r	-0.154		0.439	0.319	0.028	1	0.570
	p	0.386		0.009	0.066	0.875		0.000430
	N	34	34	34	34	34	34	34
MCpt	r	0.130		0.566	0.572	0.105	0.570	1
	p	0.465		0.000487	0.00040	0.553	0.00043	
	N	34	34	34	34	34	34	34

R: Pearson's correlation coefficient; p: p-value; N: number of cases; HistoType: histological type of tumor; Grade: tumor's grade; MCit: intratumoral MCs; MCpt: peritumoral MCs.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Authors' Contributions

EC collected the data, interpreted the data, wrote the paper. RAC collected data, wrote the paper. AMC interpreted the data, wrote the paper. PNG collected the data. LS, VF and VD performed the statistical analysis. MR conceived and coordinated this study and wrote the paper.

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