THE INFLUENCE OF MAST CELLS ON TUMOR LYMPHATIC VESSELS IN BREAST CANCER

Carpenco Ecaterina

Department of histology, cytology and embryology, Laboratory of Morphology
Nicolae Testemitsanu State University of Medicine and Pharmacy,
Chisinau, the Republic of Moldova
ecaterina.carpenco@usmf.md

Abstract

Background: Mast cells (MCs) are frequently observed in the tumor stroma of cancers, their significance being a source of dispute because of both pro- and antitumoral roles. It is well known that MCs master angiogenic and lymphangiogenic functions. The goal of present study was to research on the distribution of MCs and lymphatic vessels in peri- and intratumoral areas as well as the relationships between LVD, MCs and the different molecular subtypes of breast cancer.

Material and methods: 62 cases of breast carcinomas were analyzed in terms of molecular classification by immunohistochemistry, followed by the identification of MCs and lymphatic vessels using the lymphatic endothelium marker D2-40 and the MC tryptase. These were counted in the intratumoral and peritumoral areas and results were compared with the molecular subtype.

Results: MCs numerically prevailed in the peritumoral stroma, highest values being noticed in case of luminal B/HER2+ subtype. Maximum numerical values of both D2-40it and D2-40pt were achieved in triple negative carcinomas. For luminal A, a positive correlation was detected between D2-40pt and MCpt (p=0.02). In HER2+ subtype intratumoral MCs correlated with both intratumoral and peritumoral lymphatic vessels (p=0.01 and p=0.03, respectively). In case of G2 tumors, MCit correlated with peritumoral lymphatic vessels (p=0,003).

Conclusions: MCs are a key player of the tumor microenvironment, involved in the development of lymphatic vessels for some molecular subtypes of breast cancer.

Key words: breast carcinoma, mast cells, LVD, lymphangiogenesis, D2-40, tryptase, molecular subtypes.

Introduction

Breast cancer is one of the most common causes of mortality and morbidity among women worldwide[1]. According to GLOBOCAN, there were registered over 2 million of new cases of breast cancer in 2018 [2].

Cancer development is a multistep process characterized by genomic instability, gene expression dysregulation and epigenetic abnormality that drive tumor progression. However, gene mutations do happen and mutant cells are constantly generated throughout life but the immune system detects and eliminates these cells. In case of cancer, immune-resistant cells develop sophisticated strategies to evade the immune system and go on to generate tumors. This process requires 2 mandatory "weapons": angiogenesis, the formation of new blood vessels, which is essential for tumor growth, and lymphangiogenesis, the development of new lymphatic vessels, which is essential in the formation of metastases. These 2 are a hallmark of cancer because their induction is indispensable to fuel tumor growth and spreading [3]. Lymph node metastasis is also one of the most important survival predictor in patients with cancers, this being crucial for tumor staging and therapy planning [4]. Lymphatic vessel density (LVD) is a quantitative measure of tumor lymphangiogenesis and is measured by directly counting lymphatic vessels using the D2-40, an IgG2a monoclonal antibody which has been reported to be a specific marker for lymphatic endothelium in normal and neoplastic tissue [5] [6]. The stromal microenvironment plays a major role in maintaining normal tissue homeostasis or promoting tumor growth [3]. But not that much time passed since researchers began to focus upon alterations in the surrounding stroma or tumor microenvironment. These alterations are now recognized as a critical element for breast cancer development and progression, as well as potential therapeutic targets [7]. As in other cancers, mast cells (MCs) are frequently observed in the tumor stroma of breast cancers, and their accumulation and prognostic significance have been a source of dispute because of both pro- and antitumoral roles. Up to now, there is not yet a

clear verdict on this ongoing debate [1] [3]. Protumoral functions are supported by the facts that MCs: master tumor angiogenesis and lymphangiogenesis; facilitate stromal remodeling and stromal invasion via proteolytic enzymes, such as tryptase; and suppress antitumor immune responses by stimulating immunosuppressor cell migration to the tumor microenvironment. MCs are the first cells to migrate to the TME during carcinogenesis, and they play a critical role particularly during the transition from the *in situ* carcinoma to invasive tumor stage [8] [9].MCs in human tumors were initially described by Paul Ehrlich and extended by Eugen Westphal [3] [10]. Researchers have demonstrated that MCs produce several proangiogenic (VEGF-A, VEGF-B, and FGF-2) and lymphangiogenic factors (VEGF-C and - D). In addition, it was shown that VEGFs are chemotactic for MCs, indicating that MCs are a target, in addition to be a source, for VEGF. Human MCs produce also different matrix metalloproteinases (e.g., MMP-9) and proteases (tryptase and chymase), which regulate the digestion of extracellular matrix favoring the migration of cancer cells [11]. MCs tryptase is a marker used for the identification of these cells [12].

The goal of present study was to research on the distribution of MCs and lymphatic vessels in peri- and intratumoral areas as well as the relationships between LVD, MCs and the different molecular subtypes of breast cancer.

Material and methods

We analyzed 62 cases of breast carcinomas, collected at Arad Clinical Hospital, Romania between 2013-2016. Mean age of patients was 65.4 years (range 37–83). All patients did not undergo chemo- or radiotherapy before surgery. Clinical data were obtained from the medical records of each patient. The current research is a part of a larger study of stromal changes in molecular subtypes of breast cancer that was approved by the Ethics Committee of Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, Moldova (no. 33/ 37/ 12.02.2018).

Specimens were obtained after surgery, fixed in 10% formalin and paraffin embedded (Paraplast High Melt, Leica Biosystems). Paraffin blocks were later used for creation of tissue microarrays by means of TMA Grand Master (3DHISTECH Ltd., Budapest, Hungary). Sections from these blocks were cut and mounted on glass slides. After automatic staining with Mayer's hematoxylin (Merck, Germany) and aqueous eosin (Merck, Germany), slides were mounted automatically (Leica CV5030, Leica Biosystems, Newcastle UponTyne, UK). Tumor histology was reviewed by 3 independent pathologists and suitable sections were selected for immunohistochemical stains. Immunohistochemical study included several antibodies necessary for molecular classification and for identification of MCs and lymphatic vessels. For staining, antigen retrieval was carried using the Bond Epitope Retrieval Solution 1 (pH 6) and 2 (pH 9) (Leica Biosystems, Newcastle UponTyne, UK). Primary antibody (ER, PR, HER2, mast cell tryptase, D2-40) was followed by 3% hydrogen peroxide in order to quench endogenous peroxidase activity. DAB (3, 3'- diaminobenzidine) was applied as a chromogen substrate for 10 minutes. Mayer's hematoxylin was the additional dye used for counterstaining (5 minutes). Lastly, slides were mounted automatically (Leica CV5030, Leica Biosystems, Newcastle UponTyne, UK) using an ENTELLAN–like mounting medium (Leica CV Mount, Leica Biosystems, Newcastle UponTyne, UK). Hormone receptors (ER – estrogen receptor and PR - progesterone receptor) were evaluated according to Allred score. This score accounts the percentage of cells that test positive for hormone receptors, along with the intensity of staining [13]. HER2 protein was appreciated according to the recommendations of American Society of Clinical Oncology [14]. Quantification of brown-stained MCs (they should show a moderate to strong cytoplasmic staining) and lymphatic vessels was done using the Axio Imager A2 microscope (Carl Zeiss, Germany). Sections were initially analyzed at ×100 magnification in order to identify the area with the greatest number of distinctly highlighted vessels (hot spots) and MCs. We also analyzed their distribution 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 data used for analysis. Morphology of MCs was also analyzed in terms of shape and granulated/ degranulated appearance.

Data was stored in a MS Excel 2010 database and were statistically analyzed using the SPSS statistical software package (SPSS Statistics 23.0; IBM, Chicago, IL, USA). We used Pearson's correlation coefficient (r) and in all analyses, p values <0.05 were considered statistically significant.

Results and discussions

Most of tumors were moderately differentiated (42 cases, 67.7%). 19 cases were poorly differentiated (30.6%) and 1 case well differentiated (1.6%). We identified the following histological types of tumors: ductal invasive (56 cases, 90.3%), ductal in situ (1 case, 1.6%), lobular infiltrative (3 cases, 4.8%) and lobular in situ (2 cases, 3.2%). Immunohistochemical staining revealed 12 cases of luminal A (19.4%), 31 cases of luminal B/HER2+ (50%), 1 case of luminal B/HER2- (1.6%), 11 cases of triple-negative (17.7%) and 7 cases of HER2+ (11.3%) subtypes. Brown stained MCs were identified in all slides and showed morphological heterogeneity. Thus, two types of cells were highlighted: degranulated, with an uneven, lightly stained cytoplasm, multiple granules outside of cell and granulated, with a darker uniform cytoplasm. MCs were preferentially located along the tumor border, trying to surround the tumor island. Thus, MCs numerically prevailed in the peritumoral stroma, highest values being noticed in case of luminal B/HER2+ subtype (tab.1).

Table 1. Mean±standard deviation values of intra- and peritumoral MCs and lymphatic vessels in different molecular subtypes of breast cancer

Molecular subtype	n*	Mast cells		Lymphatic vessels	
		Intratumoral	Peritumoral	Intratumoral	Peritumoral
Luminal A	12	4.72±5.98	7.47±6.22	0.18±0.39	1.68±2.85
Luminal B/HER2+	31	5.24±7.34	**16.68±15.36**	0.57±1.16	1.16±1.41
Luminal B/HER2-	1	1.6	6.0	1.6	0
Triple-negative	11	0.88±0.82	6.72±6.24	**3.65±5.93**	**3.95±5.57**
HER2+	7	0.72±0.71	4.67±3.55	2.12±3.63	0.61±1.49

n* - number of cases

The D2-40-positive lymphatic vessels were unevenly distributed, had irregular morphology and thin-walled lumens. Intratumoral lymphatic vessels were very rare, with open lumens and occasionally contained invading tumor-cell clusters. The peritumoral lymphatic vessels were enlarged and dilated. Remarkably, maximal numerical values of both D2-40it and D2-40pt were achieved in case of triple negative breast carcinomas (tab.1). For luminal A, a positive correlation was detected between D2-40pt and MCpt (r=0.657, p=0.02). In HER2+ subtype intratumoral MCs correlated with both intratumoral and peritumoral lymphatic vessels (r=0.875, p=0.01 and r=0.788, p=0.03, respectively). In case of G2 tumors MCit positively correlated with peritumoral lymphatic vessels (r=0,453, p=0,003). Studies indicate that tumor-associated lymphangiogenesis does occur in various human cancers, promotes metastasis and that this has prognostic importance for patients. Peritumoral lymphatic vessels was also identified as a key prognostic indicator for the survival outcomes of patients with breast cancer [15]. Few data regarding the specific profile of lymphangiogenesis in different molecular subtypes of breast cancer are available and the contribution of microenvironment to this process was less investigated. Similar to our findings, Raica *et al*. found significant positive correlations between peritumoral MCs and LVD for the luminal A breast cancers (p=0.025) and also for basal-like carcinomas (p=0.029) [16]. Schoppmann *et al.* showed that HER2 overexpression is associated with high VEGF-C expression and high LVD [8]. In

our study, in HER2 molecular subtype, Mcit were linked to D2-40 positive intratumoral lymphatic vessels. Keser *et al.* have indicated that MCs may have at least some effect on lymphangiogenesis, which appears to be a predictor of tumor progression in breast cancer [8].

Conclusions

Mast cells are a key player of the tumor microenvironment, involved in the development of tumor lymphatic vessels for some molecular subtypes of breast cancer.

Acknowledgements

This work was supported by CNFIS-FDI-2018-0459 grant offered by Romanian Ministry of Education and Research. Special thanks to the Department of Microscopic Morphology/ Histology, Angiogenesis Research Center, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania.

References

[1] A. Aponte-López, E. M. Fuentes-Pananá, D. Cortes-Muñoz, and S. Muñoz-Cruz, "Mast Cell, the Neglected Member of the Tumor Microenvironment: Role in Breast Cancer," *J. Immunol. Res.*, vol. 2018, 2018.

[2] F. Bray, J. Ferlay, and I. Soerjomataram, "Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries," *Ca Cancer J Clin*, vol. 68, pp. 394–424, 2018.

[3] G. Sammarco *et al.*, "Mast cells, angiogenesis and lymphangiogenesis in human gastric cancer," *Int. J. Mol. Sci.*, vol. 20, no. 9, p. 2106, 2019.

[4] C. N. Qian *et al.*, "Preparing the 'soil': The primary tumor induces vasculature reorganization in the sentinel lymph node before the arrival of metastatic cancer cells," *Cancer Res.*, vol. 66, no. 21, pp. 10365–10376, 2006.

[5] K. H. Pak, A. Jo, H. J. Choi, Y. Choi, H. Kim, and J. H. Cheong, "The different role of intratumoral and peritumoral lymphangiogenesis in gastric cancer progression and prognosis," *BMC Cancer*, vol. 15, no. 1, pp. 1–8, 2015.

[6] Y. M. El-Gohary, G. Metwally, R. S. Saad, M. J. Robinson, T. Mesko, and R. J. Poppiti, "Prognostic significance of intratumoral and peritumoral lymphatic density and blood vessel density in invasive breast carcinomas," *Am. J. Clin. Pathol.*, 2008.

[7] E. Carpenco, "The key players of tumor microenvironment and their role in breast cancer," *Mold. Med. J.*, vol. 62, no. 3, pp. 67–71, 2019.

[8] S. F. Schoppmann *et al.*, "HER2/neu expression correlates with vascular endothelial growth factor-C and lymphangiogenesis in lymph node-positive breast cancer," *Ann. Oncol.*, vol. 21, no. 5, pp. 955–960, 2009.

[9] J. Folkman and Y. Shing, "Angiogenesis," *Biol. Chem.*, vol. 267, no. 16, pp. 10931–10934, 1992.

[10] E. Paul, "Beiträge zur Kenntniss der granulirten Bindegewebszellen und der eosinophilen Leukocythen," *Arch Anat Physiol*, vol. 3, pp. 166–169, 1879.

[11] G. Varricchi *et al.*, "Are mast cells MASTers in cancer?," *Front. Immunol.*, vol. 8, no. 424, pp. 1–13, 2017.

[12] D. Ribatti *et al.*, "Angiogenesis and mast cells in human breast cancer sentinel lymph nodes with and without micrometastases.," *Histopathology*, vol. 51, no. 6, pp. 837–842, 2007.

[13] V. Fulga, *Eterogenitatea tipurilor histologice a cancerului de sân: origini, cauze și aplicare practică.* Chișinău: Impressum, 2016.

[14] A. C. Wolff *et al.*, "Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer : American Society of Clinical Oncology / College of American Pathologists Clinical Practice Guideline Focused Update," *J. Clin. Oncol.*, vol. 36, no. 20, pp. 2105–2122, 2019.

[15] A. Christiansen and M. Detmar, "Lymphangiogenesis and Cancer," *Genes and Cancer*, vol. 2, no. 12, pp. 1146–1158, 2011.

[16] M. Raica, A. M. Cimpean, R. Ceauşu, D. Ribatti, and P. Gaje, "Interplay between mast cells and lymphatic vessels in different molecular types of breast cancer," *Anticancer Res.*, vol. 33, no. 3, pp. 957–964, 2013.