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S100 protein in molecular subtypes of breast cancer

Ecaterina Carpenco

Department of Histology, Cytology and Embryology, Laboratory of Morphology
Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Author's ORCID iD, academic degrees are available at the end of the article

Corresponding author: ecaterina.carpenco@usmf.md

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Abstract

Background: Cancer research is mainly focused on the tumor cells themselves, the tumor microenvironment being largely neglected. Antigen presenting cells are a heterogeneous population that infiltrates the tumor and can be identified due to the expression of the S100 protein. The aim of this study was to analyze the S100 protein expression (intratumoral vs peritumoral region) in different molecular subtypes, as well as its interrelations with various parameters (such as hormonal receptors expression and HER2 status, patients' age, tumor's grade).
Material and methods: 66 cases of breast carcinomas were examined in terms of their molecular profile (the expression of ER, PR, HER2) and the expression of S100 in the intra- (S100it) and peritumoral areas (S100pt). The data were analyzed using the SPSS program, the values being considered statistically significant in the case of $p < 0.05$.
Results: Maximum numerical values of S100it and S100pt were achieved in case of HER2+ and triple-negative carcinomas, respectively. In the case of luminal A subtype, an inverse correlation was established between S100it and age ($p=0.019$). In the HER2+ subtype, S100it correlated with HER2+ protein expression ($p=0.005$). In the triple negative subtype, the tumor grade influenced S100it ($p=0.022$), and S100it correlated positively with S100pt ($p=0.041$).
Conclusions: The dynamics of S100 positive intratumoral cells is strongly influenced by the HER2 status and age.
Key words: breast carcinoma, S100, HER2, peritumoral stroma, molecular subtypes, dendritic cells.

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Introduction

Cancer research is mainly focused on the tumor cells themselves, the tumor microenvironment being largely neglected. Latest studies suggest that tumors consist not only of neoplastic cells but also of a significantly altered surrounding stroma. Moreover, tumor microenvironment is considered to be a key player for tumor development and progression, as well as a measurable parameter of response to treatment. It is probably a combination of changes in both the epithelial and stromal elements which lead to tumor formation and progression [1].

The breast cancer microenvironment includes multiple cell types, such as fibroblasts, leukocytes, adipocytes, myoepithelial and endothelial cells. It also includes extracellular matrix (ECM), soluble factors (cytokines, hormones, growth factors and enzymes) and physical properties (pH and oxygen content). The interplay between epithelial and stromal cells is essential for the normal development and differentiation of the mammary gland. Physiological stroma maintains epithelial polarity and inhibits uncontrolled cell growth and neoplastic transformation [1, 2]. For example, myoepithelial cells form a natural border which is a semi-continuous protective sheet separating the human breast epithelium and the surrounding stroma. They suppress stromal invasion of tumor cells not only physically, but also by the secretion of various antiangiogenic and anti-invasive factors. Mast cells produce several proangiogenic (VEGFs – vascular endothelial growth factors) and lymphangiogenic factors. In addition, it was shown that VEGFs are chemotactic for mast cells, indicating that mast cells are a target, in addition to be a source for VEGF. Human mast cells produce different matrix metalloproteinases (e.g., MMP-9) and proteases (tryptase and chymase), which regulate the digestion of ECM favoring the migration of cancer cells [3]. Our previous study suggests that intratumoral mast cells increase especially in aggressive tumor types and serve as a worse prognostic factor [4].

Dendritic cells (DCs) are a heterogeneous population of leukocytes and play a crucial role in the initiation of an antitumor response because they are the most potent antigen-presenting cells to T lymphocytes, thus directing them to attack neoplastic cells [1, 5]. DCs are derived from hematopoietic bone marrow progenitor cells. These progenitor cells initially transform into immature dendritic cells, which are characterized by high endocytic activity and low T-cell activation potential [6]. Upon encounter with tumor antigens, immature DCs are induced to mature by inflammatory cytokines and prostaglandins released into the microenvironment. These mature DCs migrate in lymphoid organs where they interact with CD8+ and CD4+ T lymphocytes. They

also are able to stimulate and to generate memory T lymphocytes [5]. However, tumor-associated stroma shows an abundance of immature DCs with altered capacity to stimulate antitumor immunity. Moreover, immature DCs produce proangiogenic factors and increase endothelial cell migration, thus actively promoting tumor growth [1]. Studies revealed that in cancer patients, DCs present abnormalities that make T-cell activation against tumors difficult. On the other hand, the tumor microenvironment releases immunesuppressive factors that make antigen presentation difficult, with a negative impact on the immune response [5]. Despite the significant obstacles that T lymphocytes face in solid tumors, accumulating evidence indicates that natural/ induced/ and/ or engineered immune responses to cancer can dramatically change clinical outcomes [2]. As dendritic cells are considered the strongest stimulators of T-cell responses and play a crucial role in the initiation of primary immune response, different studies have exploited the potential effectiveness of DC-based vaccines in breast cancer [5].

DCs can be identified by immunohistochemistry due to their expression of S100 proteins, a class of protein with emerging roles in human cancers. The first member of the S100 family was documented in the nervous system by Moore et al. in 1965 and the name refers its nature of a soluble protein in saturated ammonium sulfate**.** It is a multigenic family of Ca^{2+} binding proteins comprising at least 20 members. These proteins exhibit a high degree of structural similarity, but are not functionally interchangeable. It is well documented that S100 proteins have a broad range of intracellular and extracellular functions, and are implicated in multiple biological functions, including cell division, motility, secretion, protein synthesis, and membrane permeability [7-10]. The aim of this study was to analyze the S100 protein expression (intratumoral vs peritumoral region) in different molecular subtypes of breast cancer, as well as its interrelations with various parameters, such as hormonal receptors expression and HER2 status, patients' age, tumor's grade.

Material and methods

66 cases of breast carcinomas were collected at Arad Clinical Hospital, Romania between 2013-2016. Mean age of patients was 64.9 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).

Histological method. 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 by using a Leica RM2245 microtome (Leica Biosystems, Newcastle UponTyne, UK) and mounted on glass slides (Surgipath X-tra Adhesive, Leica Biosystems, Newcastle UponTyne, UK).

Staining was accomplished by Leica Autostainer XL (Leica Biosystems, Newcastle UponTyne, UK). Mayer's hematoxylin (Merck, Germany) and aqueous eosin (Merck, Germany) were used. 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.

Immunohistochemistry. Immunohistochemical staining was performed automatically by Leica Bond-Max (Leica Biosystems, Newcastle UponTyne, UK). For staining, antigen retrieval was carried out using the Bond Epitope Retrieval Solution 1 (pH 6) and 2 (pH 9) (Leica Biosystems, Newcastle UponTyne, UK). Primary antibody (ER, PR, HER2, S100) 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). Then sections were placed in absolute alcohol for 5 minutes, dried and clarified in benzene for 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).

Methods of quantification. Hormone receptors (ER – estrogen receptor and PR – progesterone receptor) were evaluated according to Allred score. This score accounts for the percentage of cells that test positive for hormone receptors, along with the intensity of staining [11]. HER2 protein was appreciated according to the recommendations of American Society of Clinical Oncology [12].

S100 requires cytoplasmic and nuclear staining for positive diagnosis. Positive staining is normal in case of neurons, Schwann cells, melanocytes, glial cells, myoepithelial cells, adipocytes, Langerhans cells, tissue dendritic cells and interdigitating dendritic cells, chondrocytes and notochordal cells [8].

Quantification of brown stained DCs was done by means of Axio Imager A2 microscope (Carl Zeiss, Germany). Sections were initially analyzed at a ×100 magnification in order to determine the most intensely stained regions. Then we analyzed intratumoral and peritumoral stroma, 2 microscopic fields for each one, at a ×400 magnification and counted DCs. The final value was the arithmetic mean of the values for the two fields. Expression was graded by two independent observers who were blinded to the patient's information.

Data analysis. We used a MS Excel 2010 database 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) and in all analyses, p values <0.05 were considered significant.

Results

Most of tumors (46 cases out of 66/ 69.7%) were moderately differentiated (G2). 19 cases (28.8%) were poorly differentiated (G3) and only 1 case (1.5%) was well differentiated. We established the following molecular subtypes: luminal A (15 cases/ 22.7%), luminal B/ HER2+ (30 cases/ 45.5%), luminal B/HER2 – (2 cases/ 3%), HER2+ (8 cases/ 12.1%) and triple-negative (11 cases/ 16.7%). Histologically, we identified 60 cases of ductal invasive, 1 case of ductal *in situ,* 3 cases of lobular infiltrative and 2 cases of lobular *in situ* carcinomas.

We identified brown stained S100 positive cells in all the slides. In normal breast tissue adjacent to the tumor S100 protein expression was detected in a variety of structures: myoepithelial cells, adipocytes, nerves. These were used for internal positive control. Peritumoral DCs were usually accompanied by lymphocytes and had an irregular shape with a lot of cytoplasmic processes. They had a strong staining. Intratumoral DCs were less stained and had a foamy cytoplasm.

Intratumoral DCs were most numerous in case of HER2+ molecular subtype (maximum numerical value – 80.6). Peritumoral DCs were most numerous in the triple-negative subtype (maximum numerical number – 66.0).

For luminal A subtype, statistical analysis revealed a negative correlation between S100it and age (p=0.019, r=-0.594). In case of HER2+ subtype, S100it negatively correlated with the expression of HER2 protein (p=0.005, r=-871). In triple-negative carcinomas, S100it inversely correlated with tumor's grade (p=0.022, r=-0.678) and positively correlated with S100pt (p=0.041, r=0.621).

In G2 tumors, S100it negatively correlated with age (p=0.041, r=-0.302), while in G3 tumors S100it positively correlated with the molecular subtype (p=0.048, r=0.459).

Discussion

Breast cancer is the most common type of cancer among women. Despite the huge improvement in its outcome approximately 20–30% of patients still relapse, even many years after diagnosis [5]. Moreover, breast cancer remains one of the most enigmatic and poorly predictable cancers in its evolution due to the elevated biological heterogeneity along with varied responses to therapies across patients [6]. Thus, new biomarkers useful in clinical setting and for breast cancer management are coming up to explore [7].

Despite the promising potential of the S100 family as a biomarker panel, there are few studies that analyzed the interplay between the expression of S100 protein and different clinical parameters.

Masuda et al. showed that that expression of S100A2 (a member of S100 family) mRNA in colorectal cancer is significantly higher in cancerous tissue than in neighboring non-neoplastic tissue. The overexpression of S100A2 in colorectal cancer cells was associated with significantly worse overall survival and could be a biomarker of poor prognosis in stage II and III colorectal cancer recurrence. Their results suggest also the potential of the S100A2 protein as a target for molecular-targeted drugs for colorectal cancer [13]. This is supported by the idea that immunotherapy is an emerging and increasingly promising approach to treat cancer [2].

In lung adenocarcinoma, the expression of S100 proteins was higher in neoplastic cells than in bronchiolar epithelial cells. According to Tetsukan et al., S100A11 levels were significantly higher in adenocarcinomas with KRAS (Kirsten rat sarcoma viral oncogene homolog) gene mutations and strong proliferating activity. Their results suggested that the upregulation of S100A11 was involved in tumor progression and correlated with shorter disease-free survival [14].

As of breast cancer, Cancemi et al. demonstrated that patients which developed distant metastases showed a general tendency of higher S100 protein expression, compared to the disease-free group. They also found significantly higher S100 expression levels in ER negative tumors, in higher grade tumors and in basal-like and HER2 tumors, while lower S100 expression levels were found in Luminal A and Luminal B tumors [7].

Pedersen et al. found that high levels of S100A4 significantly correlated with histological grade and loss of estrogen receptor, but not to the time interval between surgery and development of distant metastasis or to patient's survival. They also demonstrated a significant correlation between the S100A4 immunoreactivity and the high histological grade. S100A4 staining was not correlated to the patients' age at the time of presentation, PR, lymph node involvement or tumor diameter [15]. Our study showed an inverse correlation between S100it and patients' age. However, the cited studies analyzed different S100 family members, while we payed attention to localization of S100 positive cells, thereby intratumoral and peritumoral areas.

Conclusions

S100 positive cells are more numerous in hormone-negative tumors (HER2+ and triple-negative molecular subtypes). The dynamics of S100 positive intratumoral cells is strongly influenced by the HER2 status and age.

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Author's ORCID iD and academic degree
Ecaterina Carpenco, MD, PhD Applicant, Assistant Professor – https://orcid.org/0000-0003-1464-3149.

Authors' contribution
EC acquired, interpreted the data and drafted the first manuscript. The author revised manuscript critically and approved the final version.

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Ethics approval
This study was approved by the Ethics Committee of Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, Moldova (No 33/ 37/ 12.02.2018).

Conflict of interests
No competing interests were disclosed.