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The Role of Innate Immunity in the Pathogenesis of Breast Cancer

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Keywords
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Abstract

Background: Breast carcinoma is the most common malignant disease in the female population and one of the leading causes of death among women worldwide. One crucial hallmark of cancer is chronic inflammation where the immunosuppressive environment is dominant. The immunosuppressive environment is largely achieved by the interaction of tumor cells and infiltrating leukocytes. ***Summary:*** Usually, human macrophages and natural killer cells are involved in antitumor immunity. The therapeutic potential of this population against cancers has stimulated their study and led to the discovery of several different tumor-associated macrophages and natural killer cell subsets, each of which is endowed with different immunoregulatory functions. Both heterogeneity and plasticity of the tumor-associated macrophages and natural killer cell compartment, which are both tightly linked to the tumor microenvironment of different breast cancer types. ***Key Messages:*** The identification of specific tumor-associated macrophages and natural killer cell subsets endowed with particular functional capabilities might help monitor tumor-mediated responses in breast cancer patients. Currently, one of the most used strategies for breast cancer of newly diagnosed patients is neoadjuvant chemotherapy.

Introduction

Breast carcinoma is the most common malignant disease in the female population and one of the leading causes of death among women worldwide. At present, various breast tumor classification systems are utilized; more commonly adopted are the TNM classification and immunohistochemistry classification. Traditional prognostic factors for breast carcinoma include: tumor size, axillary lymph node status, degree of tumor differentiation, and presence of lymph vascular invasion. In addition to the above prognostic factors for breast carcinoma, the treatment method is influenced by the molecular division of breast carcinoma in four subtypes. These include luminal A, luminal B (which can be human epidermal growth factor receptor 2 [HER2]-positive and HER2-negative), HER2-positive, and triple-

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negative cancer. Luminal A has the best prognosis of the listed carcinomas (hormone receptor positive and HER2 negative), while the most malignant type with quick relapse or even visceral disease dissemination is triple-negative cancer (hormone receptor negative and HER2 negative with a high proliferation marker- Ki67) [1]. Besides the prognostic significance of the above division, there is significance for the treatment of this disease. HER2-positive tumors are compulsorily treated with immunotherapy; i.e., using a monoclonal antibody for HER2, but what is new in today's treatment approach is the option for neoadjuvant chemotherapy, which also depends on the given carcinoma subtype. With this in mind, for luminal A subtype, neoadjuvant chemotherapy is not indicated because of its poor effect, while for malignant forms such as HER2-positive and triple-negative tumors, if advanced, neoadjuvant chemotherapy is beneficial and must be included for HER2-positive carcinomas along with immunotherapy (herceptin) [2, 3]. Determining the benefit of neoadjuvant chemotherapy is problematic for patients with carcinoma subtype luminal B HER2-negative tumors [4, 5]. In these patients, in order to determine the benefit of neoadjuvant chemotherapy, a whole palette of genes must be analyzed using genetic tests. Well-known tests include MammaPrint and OncotypeXD. Today, HER2 positivity is determined not only by immunohistochemistry, but also using molecular biology methods. Cases with weak membrane staining for HER2 protein should be subjected to molecular biology methods to test *HER2* gene activity. One of the above-mentioned molecular tests is in situ hybridization. A tumor is considered HER2 positive only if it is concluded that the gene which codes for HER2 protein synthesis is amplified [4, 6]. The common denominator of the development of cancer and autoimmune/neurodegenerative diseases is chronic inflammation, where the immunosuppressive environment is crucial [7–9]. Growing evidence supports the hypothesis that the local inflammatory processes modulate the microenvironment, which is the key trigger of the molecular etiopatogenetic mechanisms of tumor development [9–11]. Inflammation mediators transform normal cells by activating oncogenes or getting rid of the anti-oncogenic activity, inducing tumor progression of preneoplastic lesions, apoptosis resistance, and differentiation [9, 12]. The immunosuppressive microenvironment is largely achieved by the action of infiltrating leukocytes, which constitute a large percentage of cellular populations in tumor tissue [7, 12, 13].

In this review, we focus essentially on tumor-associated macrophages (TAM) and natural killer (NK) cells, providing a summary of the current knowledge of these cells and their possible therapeutic purpose.

The Role of TAM in Breast Cancer

In the last decade, TAM have been attributed to have a key role in supporting tumor growth and dissemination [13, 14]. They are heavily involved in cancer-related inflammation [10, 12]. As a critical component of tumor microenvironment (TME), TAM affect tumor growth, tumor angiogenesis, immune regulation, metastasis, and chemoresistance [13, 14]. Macrophages commonly represent the highest proportion of myeloid cells in the TME, and their function is mostly associated with pro-tumoral activity [11–3]. High plasticity and variety are the main characteristics of the monocyte lineage [15]. It is clear that macrophages are capable of displaying very different and even opposite phenotypes, depending on the microenvironment they are embedded in [15, 16]. Activated macrophages are often classified into the M1 (classical-activated macrophages) and M2 (alternative-activated macrophages) phenotypes [16, 17]. In general, M1 macrophages foster inflammation response against invading pathogens and tumor cells [16], whereas M2 macrophages tend to exert an immune-suppressive phenotype, favoring tissue repair and tumor progression [17, 18]. These two extremes of macrophages are distinct in their different markers, metabolic characteristics, and gene expression profiles. M1 macrophages secrete pro-inflammatory cytokines such as IL-12, tumor necrosis factor-α, CXCL-10, and interferon-γ and produce high levels of nitric oxide synthase (an enzyme metabolizing arginine to the "killer" molecule nitric oxide) [15, 16], while M2 macrophages secrete anti-inflammatory cytokines such as IL-10, IL-13, and IL-4 and express abundant arginase-1, mannose receptor (D206), and scavenger receptors [17, 18]. Through the production of different cytokines, chemokines, and other factors such as CCL2 and IL-13, tumor cells are able to modulate and polarize macrophage function and characteristics converting them into TAM [19, 20]. Many studies on genomic and proteomic levels showed that TAM usually display an M2-like phenotype [15]. Mantovani et al. [14] determinate that the TAM phenotype ($CD68^{+}/CD206^{high}/CD163^{high}$) contribute to tumor progression at different levels: promoting genetic instability, nourishing tumor stem cells, supporting molecular mechanisms of tumor dissemination, and inhibiting adaptive immunity. In HER^{+} breast cancer, CCL2 expression increases which results in the production of Wnt-1 by intra-epithelial macrophages, inducing the disruption of tumor cell-cell junctions leading to an early dissemination and lung intravasation even before the primary tumor becomes evident [19, 20]. Moreover, different studies showed that TAM infiltration correlated with poor prognostic breast cancer characteristics such as larger tumor size, higher tumor grade, lymph node metastasis, vascular invasion, hormone receptor

negativity,HER2 expressions, and basal phenotype [21, 22]. In addition, ER^- breast tumors demonstrate a higher infiltration of TAM, which correlates with a lack of pathologically complete response to neoadjuvant chemotherapy and a poorer outcome [22]. Medrek et al. [21] observed that $CD163^+$ macrophages in tumor stroma positively correlated with higher grade, larger tumor size, Ki67 positivity, estrogen receptor negativity, progesterone receptor negativity, triple-negative/basal-like breast cancer, and inversely correlated with luminal A breast cancer. Considering these data in general, high infiltration of TAM is associated with an unfavorable outcome and survival in patients with primary invasive breast cancer. Their polarization, localization, and the relative amount related to other types of breast cancer may have important clinical relevance and prognostic impact.

New Insights into Breast Cancer-Targeting Strategies of TAM

Collectively, many preclinical studies illustrated the pro-tumor function of TAM in breast cancer what makes these cells a good therapeutic target [23, 24]. Actually, during recent years, a huge effort has been done to develop efficiently drugs able to target TAM, eliminating them from the tumor tissue or even reprogramming their activation state [24]. Several pre-clinical studies indicate that targeting of TAM might be an efficient goal to limit tumor growth and the dissemination process [23, 24]. One promising agent could be zoledronic acid, which is used to repolarize TAM to the M1 type in breast cancer and other solid tumors [25]. In addition, one strategy could be blocking of the CCL2-CCR2 [26] or CXCL12/CXCR4 [27] signalling axis to re-educate macrophage polarization toward the pro-tumor phenotype and to block recruitment of TAM by genetic ablation or antibodies.

Many questions still remain open, and we need to extensively study the molecular mechanisms regulating the link between TAM and cancer to identify new prognostic targeting strategies and predictive biomarkers.

The role of NK Cells in Breast Cancer

NK cells are innate immune cells and play a role in the early immune response in tumors [28, 29]. NK cells respond to inflammatory stimuli and are best known for their role in tumor immunosuppression. Phenotypically, NK cells are defined as $CD56^+$ $CD16^+CD3^-$ in humans [28, 30]. NK cell activity depends on the interplay of their activating and inhibiting receptor repertoire [28, 30]. After activation, NK cells secrete proinflammatory cytokines such as interferon-γ, tumor necrosis factor-α, granulocyte macrophage colony-stimulating factor, and chemokines (CCL1, CCL2, CCL3, CCL4, CCL5 and CXCL8) that can modulate the function of other innate and adaptive immune cells [29, 31, 32]. CD94/NK group 2 member A (NKG2A) heterodimer receptor is one of the NK inhibitory receptors that binds to nonclassical minimally polymorphic HLA class I molecules such as HLA-E that is overexpressed in many solid tumors [29, 30]. In addition, HLA-E expression may represent an important regulatory mechanism of tumors to evade immune surveillance [30]. Mamessier et al. [32] determinate the importance of tolerogenic NK cells in the control of invasive breast tumors resulting in impaired malignant cellular immunogenicity. Crucial receptors for tolerogenic properties of NK cells are the cell Ig-like receptors and/or NKG2A as important factors for the recognition of breast tumor cells [32, 33]. Muraro et al. [34] showed an increased percentage of NK cells in women with $HER2^+$ tumors and observed that reduced NK cell infiltration into tumor tissue may be a predictive indicator of failure of chemotherapy treatment in breast cancer. Many questions on the interaction of tumor cells and NK cells are still open. Every day, scientists gain new insights into the function of NK cells in breast cancer. Their functional changes and infiltration localization related to different immunophenotypes of breast cancer may be important clinical prognostic factors.

New Insights into Breast Cancer-Targeting Strategies of NK Cells

In the last decade, increasing evidence from clinical studies supports the potential efficacy of NK cell therapies in multiple cancers [35, 36]. NK cells represent an attractive target for cancer immunotherapy owing to their innate capacity to eliminate malignant tumors in a non-major histocompatibility complex (MHC) and non-tumor antigen-restricted manner. NK cell-based immunotherapy has been associated with the treatment of hematologic cancers as well as in patients with breast cancer [37]. The most widely used strategy in cancer immunotherapy is to employ tumor-specific antibodies that promote ADCC through the ligation of CD16 receptors on NK cells. Rituximab (mAb to the B-cell marker CD20), trastuzumab (mAb to *ErbB2*/*HER2*) and cetuximab (mAb to EGFR) have demonstrated marked efficacy in the treatment of various solid and hematological tumors [34]. One of the studies showed that downregulation of NKG2A significantly enhances the anti-tumor capacity of NK cells, so it could be one of the promising therapeutic targets [29]. Another, PD-L1 blockade is known to enhance anti-tumor efficacy of NK cells and may open new lines in immunotherapy with the goal of overcoming im-

mune suppression in cancer, thereby improving patient outcomes [36]. NK cells have become an important factor in the treatment of cancer. Adaptive NK cell therapy offers great opportunities in the treatment of cancer. Furthermore, NK cells are effective in preventing disease relapse [37]. The decrease in the percentage of NK cells in immune infiltration in tumor tissue may be a predictive indicator of chemotherapy treatment outcome for breast cancer [38].

Conclusion

The innate immune cell infiltration, in particular TAM and NK cells, exerts essential roles in the development and regulation of tumor growth [39] The expected role of innate immune cells is anti-tumor response against tumor growth; however, data collected over the years demonstrate that cancer cells can modulate the anti-tumoral properties of innate immune cells. TAM acquire an M2-like phenotype in the tumor bed and become the major orchestrator for creating an appropriate TME, promote survival of cancer cells, angiogenesis, and cancer cell dissemination. NK cells are less cytotoxic, possibly with immunosuppressive properties facilitating cancer growth. Finally, accumulating evidence demonstrated that innate immune cells play a pivotal role in the immunosuppressive TME and correlate with tumor progression. All together, these observations strongly suggest that targeting tumor-infiltrating TAM and NK cells represents a promising therapeutic tool against cancer.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization, D.G., T.G.; investigation, D.G., T.G., A.S., M.A.; data curation, P.V.Z., D.V.V; writing – original draft preparation, D.G., T.G., A.S; writing – review and editing, G.B.Z., P.V.Z., D.V.V; supervision, D.G.

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