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The Role of T Lymphocyte Subsets in Breast Cancer

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Abstract

Breast cancer, a leading type of malignant tumor, is highly heterogeneous, with each subtype having different prognoses and potential therapeutic targets. The tumor microenvironment plays a key role in tumor development. It consists of immune cells, stromal cells, the extracellular matrix, and blood and lymphatic networks, forming a complex immune regulatory network. In anti-tumor immunity, the cellular immune response led by T lymphocytes is crucial. An in-depth study of T lymphocytes in the tumor microenvironment may offer new insights for breast cancer treatment. This article reviews the role of T lymphocyte subsets in the current tumor microenvironment of breast cancer.

Keywords

Breast Cancer, Tumor Microenvironment, T Lymphocytes, Immune Cells

1. Background

Nowadays, an increasing number of people are affected by malignant tumors, which have become one of the leading causes of death worldwide. Among these, breast cancer (BC) is one of the most common cancers in women. It plays a dominant role among malignant tumors and is a primary contributor to the mortality of female cancer patients globally [1]. The complexity of breast cancer lies in its high heterogeneity. Currently, classification is based on three molecular markers: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2) [2]. Based on the expression of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 (HER2), breast cancer can be classified into the following molecular subtypes: 1) HER2 positive (ER negative, PR negative, HER2 positive); 2) Luminal A (ER positive and/or PR positive, HER2 negative, low Ki-67 expression); 3) Luminal B (ER

positive and/or PR positive, HER2 negative with high Ki-67 expression, or ER positive and/or PR positive, HER2 positive); 4) Triple negative (ER negative, PR negative, HER2 negative) [3]. Each subtype has distinct prognoses and potential therapeutic targets.

The tumor microenvironment (TME) plays a critical role in tumor development. It consists of immune cells, stromal cells, extracellular matrix, and blood and lymphatic networks, forming a complex immune regulatory network. Compared to the normal human internal environment, the TME exhibits distinct biochemical characteristics, including hypoxia, acidity, strong redox properties, abnormal enzyme metabolism, and immunosuppression [4]. In recent years, there has been a growing focus on understanding how immune cells, stromal cells, and cytokines regulate the proliferation, growth, metastasis, and invasion of tumor cells within the TME [5]. These components do not function in isolation; rather, they work together to form a cohesive whole. Importantly, the disruption of any part of this network can significantly impact overall tumor behavior [6]. It is noteworthy that immune cells, a vital component of the tumor microenvironment, play a crucial role in maintaining human health by identifying, targeting, and eliminating mutant cells *in vivo*. This function is achieved not only through indirect pathways—such as regulating the function and differentiation of other cells by secreting cytokines—but also through direct effects on the survival and progression of tumor cells [7]. In contrast to the complexity of indirect effects and the multifaceted interactions of mutual regulation, the direct cytotoxicity of immune cells offers a clearer path for tumor therapy. These direct interactions are typically unaffected by intermediate multistep regulations, resulting in potent cytotoxic effects or significant direct promotion [8]. However, this dual nature complicates immunotherapy and is closely linked to the current challenges in achieving effective treatment for certain malignancies [9].

The state of immune cells in the body is closely linked to the growth, invasion, and metastatic potential of tumor cells. In the process of anti-tumor immunity, the cellular immune response led by T lymphocytes plays a critical role [10]. T cells originate from pluripotent stem cells in the bone marrow, and during early human development, they may also derive from the yolk sac and liver. Some pluripotent stem cells or pre-T cells migrate to the thymus during fetal development and early postnatal periods. In the thymus, under the influence of thymic hormones, these cells undergo a differentiation process and ultimately transform into mature T cells with immune functions. The development of T cells in the thymus is a gradual process, primarily producing CD4+ and CD8+ T cell subsets. Upon antigen stimulation, naive T cells differentiate into CD4+ helper T cells, CD8+ cytotoxic effector cells, and memory cells, mediating direct killing, multiple immunoregulatory functions, and long-term protection [11]. T cells can be categorized based on their functions into helper T cells (Th cells), cytotoxic T cells (CTL cells), regulatory T cells (Treg cells), memory T cells, natural killer T cells (NKT cells), and others [12]. This article reviews the role of T lymphocyte subsets in the current tumor microenvironment of breast cancer.

2. CD4+ T Lymphocytes and Breast Cancer

2.1. CD4+ T Lymphocytes

CD4+ T helper cells (Th) are heterogeneous T cells that play a central role in nearly all aspects of the immune response. These cells can be activated by peptide-MHC class II complexes on antigen-presenting cells (APCs), costimulatory signals, and cytokine signaling [13]. CD4+ T cell subsets exhibit distinct surface molecules, cytokines, and key transcription factor expressions, including Th1, Th2, Treg, Tfh, Th17, Th9, Th22, and CD4+ cytotoxic T lymphocytes (CTL) [14].

2.2. The Double-Edged Sword Effect of CD4+ T Lymphocytes in Breast Cancer

In the progression of breast cancer, the predominant types of CD4+ T cells vary at different stages of the disease. For instance, in the early stages of breast cancer, Th1 cells play a crucial role, effectively activating the body's immune response against cancer. However, as the cancer advances, both Th17 and Treg cells begin to become more prominent [15]. These cells have a complex role in the immune system and may influence the efficacy of cancer treatments. Zhang *et al.* [16] utilized a T cell receptor α (TCRα)-deficient mouse model to demonstrate that CD4+ tissue-resident memory (TRM) cells can elicit a strong anti-tumor immune response, significantly inhibiting the progression of melanoma and breast cancer. The anti-tumor immunity initiated by CD4+ TRM cells relies on natural killer (NK) cells and interferon-gamma (IFN-γ). Furthermore, the CD4+ TRM/NK cell axis can coordinate the formation of the tumor microenvironment and inhibit the expansion of myeloid-derived suppressor cells (MDSCs). Research [17] indicates that CD4+ T cells and platelets (PLT) are associated with the pathological grade and lymph node metastasis of breast cancer. In breast cancer patients with high PLT levels, the CD4+ T cell levels are reduced, suggesting that both are involved in the progression of the disease. One possible explanation is that platelets promote mitosis and inhibit the expression of CD4+. Consequently, CD4+ T cells can influence the formation of the tumor microenvironment through interactions with NK cells, IFN-γ, and platelets, thereby exerting an anti-tumor effect.

However, CD4+ T cells also contribute to tumor growth and metastasis. A study [18] found that in experimental models of breast cancer and lung cancer in mice, Th cells produced IL-22, which maintained the expression of cancer cell CD155 and induced the internalization of the NK cell activation receptor CD226. This process impairs NK cell function and creates an immunosuppressive microenvironment, facilitating lung metastasis. Thus, CD4+ T cells can influence the immune microenvironment through NK cells, achieving both anti-tumor effects and promoting breast cancer metastasis. This indicates that CD4+ T cells act as a double-edged sword in breast cancer. Research [19] indicates that when the CD4/CD8 ratio exceeds 2, the risk of breast cancer is 0.475 times that of benign breast tumors. This finding further supports the notion that CD4+

lymphocytes can induce tumor immune tolerance and promote tumor growth in patients with breast tumors.

2.3. CD4+ T Cells and Breast Cancer Treatment

Similarly, changes in the parameters of CD4+ T cells may be linked to treatment responses in breast cancer. Research [20] found that in breast cancer patients receiving neoadjuvant chemotherapy (NAC), the expression of the multidrug resistance-1 (MDR1) transporter provided a selective advantage to Th1.17 and Th17 cells after paclitaxel treatment, both in vitro and *in vivo*. Single-cell RNA sequencing (scRNAseq) confirmed that MDR1 is associated with the characteristics of tumor Th1.17 and Th cells exhibiting cytotoxic properties. This suggests that MDR1 may play a significant role in regulating the function and drug resistance of CD4+ T cells during breast cancer treatment. A retrospective analysis [21] showed that levels of CD3+ T cells, CD4+ T cells, the CD4+/CD8+ ratio, NK cells, and lymphocyte-to-monocyte ratios (LMR) in breast cancer patients were significantly lower than those in women with benign breast tumors. Among breast cancer patients, those who achieved a pathological complete response (pCR) had higher levels of CD4+ T cells, NK cells, and LMR prior to neoadjuvant therapy (NAT). This suggests that a high infiltration of CD4+ T cells is associated with better therapeutic outcomes and prognosis.

2.4. The Two Sides of Treg to Breast Cancer

Among T cell subsets, regulatory T (Treg) cells are particularly noteworthy due to their ability to inhibit anti-tumor immunity, thereby creating a protective immune microenvironment for tumors. While targeting this T cell subset may enhance anti-tumor activity, it is important to recognize that Treg cells are vital for overall immune tolerance and play a crucial role in molecular signaling pathways [22]. Public single-cell sequencing datasets have shown an increased infiltration of Treg cells in triple-negative breast cancer (TNBC) tissues compared to normal breast tissues [23]. *In vivo* studies by Si *et al.* [24] demonstrated that blocking PD-L1 and/or STAT3 signaling can prevent *γδ*Treg-induced senescence and reverse tolerance functions in dendritic cells, thereby enhancing HER2 tumor-specific immune responses and immunotherapy in human breast cancer models. However, research [25] has found that the prognostic significance of FOXP3+ Treg cells in breast cancer is influenced by various factors, including molecular subtypes and Treg localization. In HER2+ breast cancer and triple-negative breast cancer, FOXP3+ Treg cells are associated with better pathological complete response (pCR) and overall survival (OS) rates. Additionally, FOXP3+ Treg cells located in the stroma are indicative of a favorable prognosis. Consequently, the primary role of Treg cells in triple-negative breast cancer (TNBC) and HER2-positive breast cancer is immune regulation, affecting cancer progression and treatment response by inhibiting immune system activity. However, an increase in Treg cells also correlates with a favorable prognosis, suggesting that the impact of Treg cells on breast cancer treatment has dual effects.

3. CD8+ T Lymphocytes and Breast Cancer

3.1. CD8+ T Lymphocytes

CD8+ T cells are a crucial component of tumor-infiltrating lymphocytes (TILs) and the tumor microenvironment (TME), primarily functioning to identify and eliminate tumor cells [26]. Upon antigen stimulation, naive CD8+ T cells undergo significant expansion, producing both effector T cells and memory T cells. CD8+ T cells (CD8+ CTLs) can induce the death of target cells through interactions involving Fas/Fas ligands and the secretion of cytolytic mediators, such as perforin. Perforin forms pores in the target cell membrane, facilitating the entry of granzyme, which subsequently triggers apoptosis. Memory CD8+ T cells provide rapid and robust protection upon re-encountering the same antigens, which is essential for effective and long-lasting immunity [27].

3.2. The Relationship between the Functional Status of CD8+T Cells and the Progression of Breast Cancer

With the rapid advancement of single-cell technology, extensive analyses of tumor-infiltrating lymphocytes have revealed a diverse spectrum of depleted CD8+ T cells in various cancers, including non-small cell lung cancer, melanoma, breast cancer, liver cancer, and colorectal cancer [28] [29]. In peripheral blood, Granzyme B and IFN-γ—markers associated with CD8+ T cell function in breast cancer patients—can reflect the functional status of these cells in the body. Analysis indicated that, compared to the Stage I-II group, the expression levels of CD8+ T cells, Granzyme B, and IFN-γ were significantly reduced in the Stage III-IV group. This suggests that as tumor progression becomes more severe, there is a corresponding increase in the inhibition of CD8+ T cell function [30]. The down-regulation of these indicators suggests that as breast cancer progresses, the immune function of CD8+ T cells is inhibited, which may impact the patient's treatment response and prognosis. Another study [31] analyzed the expression of CD8+ T cells. The results indicated that, compared to the negative group, tumors in the positive group were more likely to exhibit high or moderate differentiation. Most cases were classified as TNM stage I-II, with a relatively low proportion of tumors measuring ≥2 cm. Additionally, logistic analysis suggested that the expression of CD8+ T cells may be associated with both the degree of tumor differentiation and the TNM stage. This may be related to the cytotoxic effects of CD8+ T lymphocytes and the level of host inhibition of the tumor response. This may suggest that CD8+ T cells maintain a strong functional status in the early stages of breast cancer, which helps to control tumor development and spread.

The results from Liu *et al.* [32] indicated that the activation of CD8+ T lymphocytes in the spleens and tumors of mice was significantly enhanced by the combination of compound Kushen injection (CKI) and chemotherapy. Furthermore, single-cell RNA sequencing (scRNA-seq) revealed that this combination could increase the proportion of tumor-infiltrating CD8+ T cells, inhibit tumor-promoting signaling pathways, promote T cell activation, and positively regulate immune

responses. Consequently, the functional changes and enhanced activation of CD8+ T cells in breast cancer can significantly improve the immune microenvironment, playing a crucial role in enhancing treatment responses and patient prognosis.

3.3. CD8+ T Cells and ICT

Anti-tumor immune checkpoint therapy (ICT) can alleviate immunosuppression and is an effective clinical treatment method [33]. Studies have shown that high doses of Vitamin C can regulate the infiltration of immune cells into the tumor microenvironment and slow cancer growth in a T cell-dependent manner [34]. Vitamin C not only enhances the cytotoxic activity of adoptively transferred CD8+ T cells, but it is also used alongside immune checkpoint therapies for various cancer types. Another study [35] performed single-cell RNA sequencing (scRNA-seq) analysis of breast cancer and identified five clusters of tissue-resident macrophages (RTMs) with a mixed M1-M2 phenotype. Comprehensive analysis of multi-omics data revealed that these RTM clusters exhibited characteristics of inflammatory responses and an increased reactive oxygen species pathway. Additionally, they were positively correlated with T cell toxicity and the infiltration of CD8+ T cells, indicating their sensitivity to ICT. In clinical experiments, Lu *et al.* [36] utilized molecular imaging to evaluate changes in CD4+ T cells and CD8+ T cells during immune checkpoint blockade (ICB) in breast cancer models. The results showed that after ICB treatment, CD8-specific PET signals increased within 6 days, while CD4-specific PET signals increased within 2 days in tumors that ultimately responded to immunotherapy.

In summary, studies have demonstrated that immune checkpoint blockade therapy can significantly enhance the activity of CD8+ T cells, which is highly beneficial for improving the therapeutic outcomes in breast cancer.

4. DPT Lymphocytes and Breast Cancer

4.1. Double Positive T-Cell

T lymphocytes are essential for combating a variety of pathogenic microorganisms and establishing tolerance to self-antigens, thus playing a crucial role in maintaining immune homeostasis. They undergo several developmental stages and checkpoints in the thymus, progressing from progenitor cells to mature T cells, transitioning from CD4-CD8 double-negative (DN) cells to CD4+CD8+ double-positive (DP) cells, and ultimately maturing into CD4+ or CD8+ single-positive (SP) cells. Once matured, SP cells migrate out of the thymus and further differentiate into various subsets, responding to distinct environmental signals to carry out specific functions [37]. While the expression of CD4 or CD8 co-receptors determines the functional specialization of mature T cells and excludes the other, a subset of peripheral T cells expressing both CD4 and CD8, known as DPT cells, has also been identified [38]. This unconventional T cell population is found in varying proportions in the peripheral blood of different species, including

approximately 3% in humans [39]. Notably, the frequency of intratumoral CD4+CD8+ T cells has been found to be elevated in patients with breast cancer, pancreatic cancer, and non-small cell lung cancer (NSCLC), compared to circulating CD4+CD8+ T cells [40].

4.2. The Role and Immune Response of DPT Cells in Breast Cancer Metastasis

Mature CD4+CD8+ double-positive T cells are present in healthy individuals and their numbers increase in disease contexts. However, their molecular characteristics and pathophysiological roles remain a topic of debate. In this study [40], various clone-amplified DPT cells were identified in human melanoma and lung cancer through single-cell RNA sequencing, demonstrating tumor reactivity in cytotoxicity assays. A comparable response was noted in breast cancer [41]. While intratumoral treatment with VC2 did not significantly decrease the average primary tumor size, there was a meaningful reduction in lung metastasis in mice treated with VC2, as opposed to those receiving UV-inactivated VC2. This reduction in metastasis was associated with an increased infiltration of T cells, including both CD4+ and CD4CD8 double-positive T cells. Another study [42] explored the mechanism by which RYF influences thymic immune function in breast cancer patients experiencing depression, anxiety, and other emotional disorders. In the CUMS stimulation group, RYF treatment significantly increased brain serotonin (5-HT) levels, enhanced thymic output, and elevated the counts of thymic epithelial cells (TECs, CK5+), as well as the proportions of CD3+CD4−CD8− (double-negative) and CD3+CD4+CD8+ (double-positive) T cells in the medulla. Thus, the increased infiltration of DPT cells appears to correlate with a significant reduction in breast cancer metastasis, highlighting their potential importance in tumor immune responses.

5. DNT Lymphocytes and Breast Cancer

5.1. Double Negative T-Cells

Double-negative T cells (DNT) represent a rare subset of T lymphocytes that lack both CD4 and CD8 markers, yet they express either $\alpha\beta$ or $\gamma\delta$ T cell receptors (TCR). This population constitutes only about 3% - 5% of T lymphocytes in peripheral blood. Recent findings indicate that DNT cells can originate via both thymus-dependent pathways—by evading the negative selection process—and thymus-independent pathways, which may involve activated peripheral lymphocytes that, in some cases, lose their CD4 or CD8 expression [43]. There is also evidence suggesting that DNT cells could be derived from autoreactive CD8+ T cells, particularly in autoimmune conditions [44]. While DNT cells have not been classified into specific phenotypes, a recent study [45] utilized single-cell RNA sequencing to identify a subset of naïve and activated TCR$\alpha\beta$+ DNT cells in mice. In this research, naïve DNT cells were characterized as resting, helper, intermediate, cytotoxic, or innate T cells, distinguished by their unique transcriptomic profiles,

whereas active DNT cells were categorized into cytotoxic and pro-inflammatory subsets.

5.2. Immune Surveillance and Therapeutic Potential of DNT Cells in Breast Cancer

The study [46] found that the frequency of granzyme B (GZMB)+CD8+ T cells and GZMB+ DNT cells in breast cancer tissue was lower than that observed in patient blood samples (PB). Conversely, the frequency of programmed cell death protein 1 (PD1)+ CD8+ T cells and PD1+ DNT cells in tumor tissue (CA) was higher than in PB. DNT cells from healthy volunteers (HBs) exhibited cytotoxicity against MDA-MB-231 cells. These findings suggest that DNT cells have potential as a standalone treatment or in combination with LAG3Ab for novel adoptive cell therapies targeting triple-negative breast cancer (TNBC). Additionally, it has been reported that the proportion of DNT cells in peripheral blood is significantly elevated in various malignant tumors, including lung, stomach, liver, and intestinal cancers [47]. Domestic studies [48] have shown that breast cancer patients exhibit a notable increase in DNT cells in peripheral blood when compared to healthy individuals and those with benign breast tumors, indicating the likely significance of DNT cells in breast cancer. Another study [49] analyzed 132 samples from breast cancer patients and found that as tumor size increased, the absolute number of DNT cells decreased significantly ($P < 0.05$). Moreover, the higher the malignancy of the breast cancer, the lower the frequency of DNT cells observed.

In summary, the relationship between double-negative T cells and breast cancer highlights their potential role in regulating immune responses and participating in tumor immune surveillance. While the precise anti-tumor mechanisms are not yet fully understood, the observed changes in their expression among breast cancer patients suggest that these T cells may play a significant role in the progression of breast cancer.

6. Summary

T lymphocytes, as crucial components of the immune system, play a significant role in immune surveillance and response to breast cancer. In recent years, research has deepened our understanding of T lymphocyte subsets and their multiple functions in the occurrence, progression, and treatment of breast cancer. Studies have demonstrated that these subsets are integral to tumor progression, distant metastasis, assessment of therapeutic efficacy, and future immunotherapy approaches. Investigating the relationship between T lymphocyte subsets and breast cancer is vital for elucidating the immune regulatory mechanisms involved in this disease and lays the groundwork for developing novel immunotherapy strategies. Future research could focus on enhancing breast cancer treatment by modulating the proportions and functions of T cell subsets. Moreover, employing single-cell sequencing technology to analyze T cell heterogeneity within tumors will provide critical molecular insights to support these strategies. In summary, the role of T

lymphocyte subsets in breast cancer is gaining increasing attention. Their involvement in disease development and treatment responses suggests that a deeper understanding of the regulatory mechanisms governing these cells may yield important clues for the advancement of new therapeutic strategies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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