Mini-review

Yin-yang effect of tumor infiltrating B cells in breast cancer: From mechanism to immunotherapy

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ABSTRACT

Breast cancer cells secrete chemokines, such as CXCL13, and antigens or express high endothelial venules, attracting B cells to infiltrate into the tumor microenvironment and play a "yin-yang" effect. They not only enhance the anti-tumor immune effect via secreting antibodies and influencing the Fas/FasL, CXCR4/CXCL12 and perforin pathways but they also promote the tumor to form a suppressive milieu by producing immunomodulatory factors and cytokines or using cell-to-cell education to induce the generation of Tregs or myeloid-derived suppressor cells (MDSCs). Currently, most studies on breast cancer tissue have indicated that B cell infiltration could predict better survival and response to therapy, but two studies have reported opposite results. In a 4T1 tumor-bearing BALB/c mice model, B cell-based immunotherapies were administered, but the efficiency was unstable. Herein, we review the "yin-yang" effect of B cells in breast cancer and discuss B cell-based immunotherapy. B cells are complex aggregates, and breast cancer is a heterogeneous disease. Further studies are urgently required to define the B cell subsets and to discover ways to use B cell-based immunotherapy in breast cancer.

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Introduction

Breast cancer is a strongly heterogeneous malignant disease that accounts for many cancer-related deaths throughout the world [1]. Its development involves complex and contradictory biologic processes, which are not only controlled by genetic abnormalities but also by the interplay between cancer cells and the local microenvironment, including the extracellular matrix, stromal cells and immune cells [2]. Tumor infiltrating lymphocytes (TILs), an important component of immune cells in the breast cancer niche, have been widely studied [3]. Studies have shown that robust levels of TILs infiltrating breast cancer tissue were associated with favorable survival and good therapeutic responses, especially in the triple negative or HER-2 positive subtype [4,5]. Lymphocytes can be associated with breast cancer progression and prognosis, and the phenotype of lymphocytes is important. CD8$^+$ T cells have immunologic cytotoxic effects on breast cancer and predict improved survival outcomes [6]. CD4$^+$ T cells infiltrate tumors and secrete cytokines, which could facilitate the activation and proliferation of cytotoxic T cells to lyse tumor cells [7,8]. On the other hand, some subtypes of T cells have an immunosuppressive effect. IL-17-producing γδT cells drive neutrophil expansion and polarization and, subsequently, promote metastasis in distant organs [9]. Additionally, FOXP3$^+$ T lymphocytes (Tregs) can diminish the immunologic response to tumor specific antigens and create an immune privileged niche to facilitate breast tumor cell growth [10]. Except for T cells in humoral immunity, B cells produce antibodies as well as present antigens and secrete cytokines. Likewise, B cells constitute a central component of tumor infiltrating immune cells in ovarian, colorectal, hepatic and pancreatic cancer [11–13]. The interplay between B cells and the tumor microenvironment is complex. Cytokines, such as IL-6, could regulate CD5$^+$ B cells and promote tumor growth of B cells via STAT3 activation [14]. However, the hypoxic environment impacts B cell subsets and infiltration, modulating pancreatic carcinogenesis [15]. On the other hand,

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B cells can support pancreatic and hepatocellular tumor cell proliferation by producing IL-35 and IL-10 [13,16–18].

Nevertheless, B cells in the breast cancer microenvironment are less well understood. Recent research has indicated that approximately 20% of breast cancers have high levels of B cells [19], and the relationship between B cells and breast cancer progression and prognosis appears to be prominent [20]. However, the precise role of B cells in breast cancer development remains controversial. Hence, we discuss the unique functional properties of B cells in the breast cancer microenvironment and analyze B cell-based immunotherapy.

Origins and location of tumor infiltrating B cells (TiBCs) in breast cancer

B cell infiltration in malignant tumors (invasive breast cancer or ductal carcinoma in situ) was significantly higher than in benign breast lesions (50% vs. 13.33%, $P = 0.002$) [21,22]. We wondered about the mechanism for these traits. Antigens released from breast cancer cells were the most important factor driving B cell proliferation and infiltration into carcinoma tissue [23]. Cancer cells that secrete chemokines, such as CXCL13, also play a vital role in attracting B cells into the microenvironment [24]. Accumulating evidence suggests that the high endothelial venule (HEV) density in the tumor stroma is a strong predictor of infiltration by $CD4^+$ T, $CD8^+$ T and B cells. Therefore, HEVs may control lymphocyte infiltration into breast cancer [25]. In addition to these "chemotactic factors" that regulate B cell polarization, many other factors induce B cell subtype switching. Wejksza et al., using 4T1 mice breast cancer cells, found that breast non-metastatic cancer cells express and use metabolites of 5-lipoxygenase (5-LO) via the 5-LO/FLAP/leukotriene/PPARα pathway to induce tumor-associated regulatory B cell (Bregs) generation [26]. Of note, a study by Bodogai et al. showed that 4T1 tumor-bearing mice treated with an anti-CD20 monoclonal antibody enriched $CD20^{low}$ B cells and suppressed T cell effects. Cell co-culture experiments also verified these findings [27].

The location of B cells in breast cancer has been investigated. A tissue immunohistochemistry study implied that infiltrating immune cells consist of T and B lymphocytes. $CD8^+$ T cells were closely linked to cancer cells, and $CD4^+$ T cells were located inside the tumor. While B and plasma cells (effector B cells, secreting antibodies) tended to be located around the neoplasia, they mainly aggregated within the fibrotic areas. This finding not only suggested the location of TILs, it also indicted a time sequence wherein T cells respond first, followed by B cells [28]. The construction of TILs in breast cancer is influenced by histological characteristics. Medullary breast cancer (MBC) is a high-grade cancer with a relatively favorable prognosis. Compared to atypical medullary breast cancer (AMC), studies demonstrated that $CD8^+$ T cells are more abundant in MBC; nevertheless, B cells are more prevalent in AMC [29]. In TILs, the ratio of $CD8^+$ T cells to B cells was reciprocally related to *HER2/neu* gene expression [30]. This evidence indicated that TILs and B cells in breast cancer participate in the complex heterogeneity throughout space and time.

Immunopotentiation role of TiBCs in breast cancer

Immunoglobulin (Ig) secretion plays a major role in the B cell adaptive immune response. Similarly, Ig has been identified as one of the top genes in breast cancer, and it is related to a favorable prognosis and better chemotherapy response [31]. Kotlan et al. performed an Ig repertoire analysis in MBC and found that ganglioside D3 (GD3), a useful marker in breast cancer cells and breast cancer stem cells, is a tumor antigen that could drive tumor

infiltrating B lymphocytes to express antibodies [32]. This phenomenon indicted that antibodies secreted by tumor-infiltrating B cells in breast cancer have an important effect. Both human breast cancer specimens and a 4T1 BALB/c murine model indicated that tumor cell-activated B cells secreting immunoglobulin G (IgG) mediate tumor cell-specific lysis and apoptosis [33,34] In addition to their direct "killing" function, antibodies binding to tumor antigens can attract many immune cells, which can then present antigens to T cells in triple negative breast cancer. Carmi et al., using murine mammary tumor models, revealed that tumor-binding IgG enable dendritic cells (DCs) to internalize antigens and subsequently activate T cells, inducing powerful antitumor immunity [35]. In a 4T1 interleukin-10 KO ($IL-10^{-/-}$) BALB/c murine model, purified B cells from the tumor draining lymph node (TDLN) can directly kill tumor cells via the Fas/FasL pathway, which is regulated by interleukin-10 [36]. When using adoptive transfer of B cells for 4T1 BALB/c mice, activated B cells could also kill tumor cells, which involved the CXCR4/CXCL12 and perforin pathways. Additionally, effector B cells can express IL-2R, and exogenous IL-2 administration significantly augmented the suppression of breast cancer lung metastasis [37]. The study presented here revealed that B cells can act via antibodies and the Fas/FasL, CXCR4/CXCL12 and perforin pathways to kill breast cancer cells, but the factors that trigger B cells to express FasL or CXCR4 remain unknown (Fig. 1).

Immunosuppression role of TiBCs in breast cancer

B cells that exert immunosuppressivity in cancer development have been studied recently. 4T1 mouse breast cancer cells act via metabolites of 5-lipoxygenase (5-LO) to induce the generation of Bregs through peroxisome proliferator-activated receptor α, promoting cancer escape, progression and metastasis [26]. A murine 4T1 breast cancer model indicted that tumor cells actively induce Bregs to release TGF-β, converting resting $CD4^+$ T cells to Tregs. This effect can successfully downregulate protective immune responses and promote breast cancer metastasis [38]. In an EMT-6 mammary tumor murine model, tumor cells attract normal B cells into the tumor bed and convert them into Bregs. Those newly generated Bregs could suppress $CD4^+$ T cell proliferation by immune suppressive ligands (PD-L1 and TGF-β), promoting tumor growth [39]. Bregs also use TGF-β to induce TGF-β receptors on MDSC and upregulate ROS and NO production in MDSC. As a result, MDSC is fully suppressive for T cells, while promoting breast cancer metastasis [40]. Lindner et al. reported that granzyme B producing B cells are induced by IL-21, which suppresses T cell proliferation via interfering with the T cells receptor-ζ-chain [41]. Other than secreting regulating cytokines, B cells can interact through cell-to-cell contact to domesticate immunosuppressive cells. Myeloid and B cells from healthy peripheral blood that are then educated by breast cancer cells are capable of inhibiting T cell proliferation. However, when B cells were depleted, this inhibition effect vanished. These results indicated that myeloid-derived suppressor cells (MDSCs) acquire immune suppressive functions through "education" by Bregs [42,43]. Hence, under some circumstances or for some special subtypes, B cells, especially Bregs, promote breast cancer cells to form a suppressive milieu. This appears to occur through production of immunomodulatory factors and cytokines or through cell-to-cell education, which induces the generation of Tregs or MDSCs (Fig. 1).

TiBCs predict prognosis for breast cancer

Higher levels of B cells in breast cancer were associated with poor prognostic biomarkers, such as a high tumor grade, negative estrogen receptor (ER) status, IL-10 secretion and PD-L1 expression