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Tumor infiltrating lymphocytes in early breast cancer

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ABSTRACT

Immunoediting represents a complex and dynamic process involving cancer and immune system cells, composed by three intertwined phases: elimination, equilibrium and escape. A large number of immune cell subtypes are involved, each playing a peculiar role in interacting with cancer cells: cytotoxic CD8$^+$ T cells play a main role in cancer killing by inducing tumor cell death, while FOXP3+ T-regulatory cells represent an immune-inhibitory cell subtype. The evaluation of tumor infiltrating lymphocytes (TILs) in H&E routine samples has been shown to represent a reliable surrogate of the immune anti-tumor activity and a robust independent prognostic biomarker in breast cancer (BC) patients, especially in the Tripe Negative and HER2+ subtypes. The present review addresses the mechanisms of breast cancer immunoediting, its cell complexity and prognostic/predictive relevance, providing evidence that TILs represent one the most promising biomarkers for BC patients.

1. Introduction

Inflammation plays a pivotal role in cancer recognition and eradication. More than 60 years ago, Dr. Thomas and Macfarlane Burnet introduced the concept of immunosurveliance, a process by which immune system recognizes transformed cells and inhibits their growth [1]. A rising body of experimental and clinical evidences suggest that cancer does elicit an immune response through the expression of new antigens or by modifying its microenvironment, eventually leading to a complex and dynamic interplay with the immune system (cancer immunoediting). This process is composed by three intertwined phases: elimination (that coincides with immunosurveliance), in which immune cells recruitment leads to the eradication of tumor cells; equilibrium, in which a cross-talk between cancer and immune cells is established, leading to mutual shaping; and escape, in which tumor cells begin to expand unrestrainedly as a result of acquired resistance to immune detection [2,3]. A large number of immune cell subtypes are involved in cancer immunoediting. Among them, cytotoxic CD8$^+$ T cells (CTL) play a main role in cancer killing by inducing tumor cell death *via* the release of IFN-γ and granzyme-perforin complex. CD8$^+$ T cells activation and maturation is in turn modulated through the IFN-γ produced by CD4$^+$ T-helper 1 (Th1) cells and specific tumor-associated antigens processed by dendritic cells. Chemotherapy (CHT), targeted therapies and radiotherapy are the standard of care for a large number of malignancies. Although they have been traditionally thought to exert their antitumoral effect by directly damaging and killing cancer cells, recent evidences point towards a close interaction with the immune system: in particular, CHT and radiotherapy lead to an "immunogenic" cancer cell death through immune system activation [4]. Likewise, HER2 antitumor activity is largely mediated by antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity, whose integrity may be in turn modulated by the innate or adaptive cancer immune status [5–7]. In this scenario, tumor-infiltrating lymphocytes (TILs) have been claimed to represent a morphological manifestation of anti-cancer immune response, and are associated with patient survival in a wide variety of tumor types (see Fig. 1). In this review, we will address the mechanisms of breast cancer (BC) immunity, its cell composition and prognostic/predictive relevance, emphasizing the potential role of TILs as a new biomarker for BC patients.

2. Breeding immunity: cancer mutational burden

Immunity recognizes cancer cells through their repertoire of antigenic non-self-peptides (neoantigens) deriving from damaged intracellular proteins [8] or mutated genes [9–15]. Accordingly,

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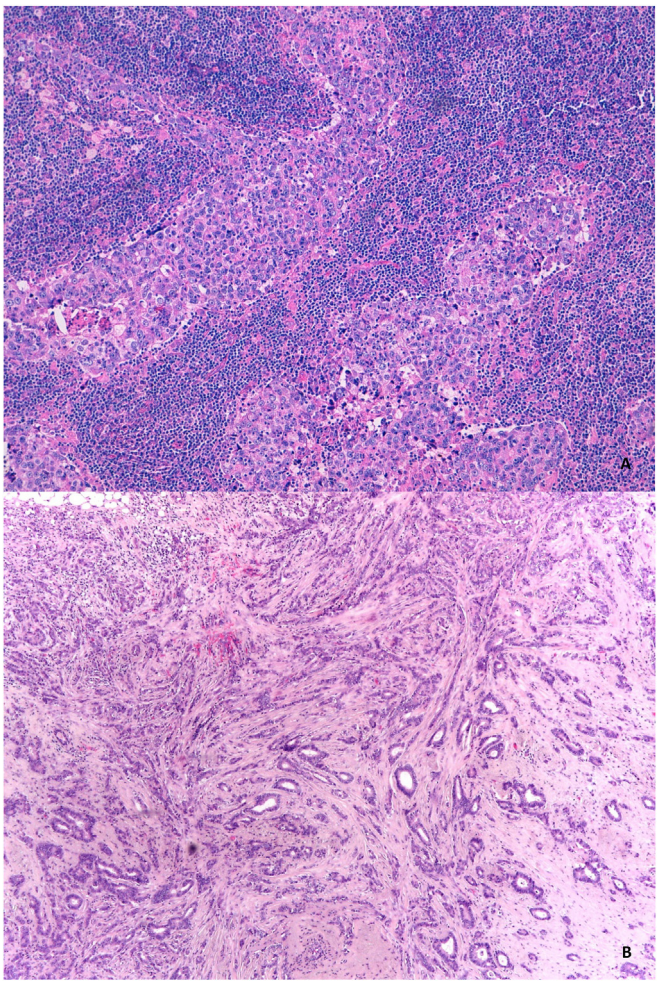

Fig. 1. Examples of immunologically "hot" (A) and "cold" (B) breast cancer. A: Poorly differentiated invasive ductal carcinoma, showing a dense inflammatory infiltrate (lymphocyte predominant breast cancer). B: Well differentiated invasive ductal carcinoma, showing low TILs levels.

tumor mutational burden is associated with the formation of neoepitopes and with the magnitude of the immune infiltrate [16,17]. Rooney et al. exploited The Cancer Genome Atlas (TCGA) data to demonstrate a correlation between tumor mutational burden and T cell effector function [18]. Melanoma and colorectal cancer, which are characterized by the highest mutation rates, genomic instability and heterogeneity [19], are frequently associated with an extensive immune infiltration. Neoantigens are ultimately estimated by sophisticated in silico bioinformatic analyses which assess the likelihood of a particular mutant peptide to bind antigen presenting major histocompatibilty complex (MHC) class I molecules at high affinity. Only a small proportion of predicted neoantigens do elicit a T cell response in functional studies [20]. It has been estimated that approximately 1% of somatic mutations may result in antigenic peptides: in a study of 10 gastrointestinal tumors harboring a combined total of 1452 mutations, 18 mutations (1.2% of the total) were recognized by $CD4^+$ or $CD8^+$ lymphocytes [21]. In melanoma, that is characterized by a very high mutational load (130 mutations per case on average), only 0–3 mutations per sample are expected to result in immunogenic peptides [22–24]. Compared with other tumor types, BC shows a relatively low mutation rate [19]. In particular, HER2 enriched and basal-like molecular subtypes bear the highest mutation rate, followed by the luminal subtype, mirroring the typical distribution of TILs in BC [25]. Haricharan et al. interrogated 762 invasive BCs from the TCGA dataset, showing that ER-positive, but not ER-negative tumors with a high mutational load were associated with poorer overall survival (HR = 2.02) [26]. These data, coupled with the observation that TILs are not related to clinical outcome in luminal BC patients [27], lead to speculate that a high mutational load in ER-positive BC would result in clonal heterogeneity and proliferative advantage, rather than an effective anti-tumor immunologic response.

3. The BC immune cell paraphernalia

Tumor immunity is the result of a complex interplay between immune cells and their mediators, cancer cells and microenvironment, in a dynamic equipoise between tumor suppression and tolerance. A plethora of immune cell subtypes are on stage, each playing a peculiar role in interacting with cancer cells. Immune-mediated cancer cell elimination is mostly exerted by $CD8^+$ cytotoxic T lymphocytes. After recognition of their specific epitopes on MHC class I molecules expressed by antigen presenting cells and cancer cells, $CD8^+$ T cells release proteolytic and cytolytic enzymes, including perforin and granzyme, eventually leading to cancer cell death. Actually, the occurrence of a large number of infiltrating $CD8^+$ T cells, as assessed by immunohistochemistry, has been reported in a fraction of BC patients [28,29]. Th1 lymphocytes are crucial for the development and activation of $CD8^+$ T cells expressing IFN-γ, that in turn are capable of inducing cancer cell death by releasing chemotactic and angiostatic chemokines (CXCL9, CXCL10 and CXCL11) [30]. Likewise, tumor-associated macrophages (TAM) with M1 phenotype are involved in the anti-tumor immune response, through the secretion of type I cytokines, including tumor necrosis factor-α (TNFα) and nitric oxide synthase (NOS) [31], Natural killer (NK) T cells and T-follicular helper (Tfh) cells also exert an antitumor activity [32–34]. Tfh cells are antigen-experienced $CD4^+$ T cells located in germinal centers within tertiary lymphoid structures, which are frequently detectable in tumors with a dense inflammatory infiltration. They play a critical role in mediating the selection and survival of B-cells and their differentiation into plasma cells producing antibodies against foreign antigens. Ultimately, the aforementioned anti-tumor immune machinery produces a type 1 cytokine-predominant response, dominated by IFN-γ, TNF and interleukin 2 (IL-2). On the other hand, FOXP3+ T-regulatory (Treg) cells represent an immune-inhibitory cell subtype, affecting the selection of high-avidity $CD8^+$ T cells and their functionality. Treg lymphocytes also inhibit antigen presenting cells, $CD8^+$ T cells, NK, and Th1 cells. In addition, both Treg and tumor cells produce adenosine, which exerts inhibitory effects on T cells. Tumor cells can secrete cytokines and chemokines (e.g., TGF-β, CCL2), which enhance the activity of immune-inhibitory cells, including Tregs, myeloid-derived suppressor cells (MDSCs), and M2 macrophages. M2 macrophages and MDSCs inhibit T cell responses through nutrient sequestration, arginase, reactive oxygen species (ROS), and reactive nitrogen species (RNS) generation, as well as interference with trafficking into the tumor site. Interestingly, Crome et al. identified an innate NK CD56+/CD3-lymphocyte population that inhibited TILs in high-grade serous tumors and was associated with reduced T cell numbers and cytokine production in cell cultures [35].

4. TILs: the Occam razor of antitumor immune response?

Anti-tumor inflammatory response is a dynamic multifaceted process, including recognition of potentially harmful stimuli, as well as maturation, proliferation and activation of naïve leukocytes. In 1994, Baxevanis and colleagues isolated lymphocytes from

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