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The Role of Immune Escape and Immune Cell Infiltration in Breast Cancer

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Keywords

Breast cancer · Immune escape · Immune surveillance · Tumor microenvironment · Immune response

Summary

While detailed analysis of aberrant cancer cell signaling pathways and changes in cancer cell DNA has dominated the field of breast cancer biology for years, there now exists increasing evidence that the tumor microenvironment (TME) including tumor-infiltrating immune cells support the growth and development of breast cancer and further facilitate invasion and metastasis formation as well as sensitivity to drug therapy. Furthermore, breast cancer cells have developed different strategies to escape surveillance from the adaptive and innate immune system. These include loss of expression of immunostimulatory molecules, gain of expression of immunoinhibitory molecules such as PD-L1 and HLA-G, and altered expression of components involved in apoptosis. Furthermore, the composition of the TME plays a key role in breast cancer development and treatment response. In this review we will focus on i) the different immune evasion mechanisms used by breast cancer cells, ii) the role of immune cell infiltration in this disease, and (iii) implication for breast cancer-based immunotherapies.

Introduction

There is evidence that the low immunogenicity and the intense immunosuppressive tumor microenvironment (TME) of breast cancers (BC) limit the benefit of immunotherapies targeting the adaptive immune system, such as checkpoint inhibitors (CPI) [1, 2]. Immunosuppressive mechanisms are essential for the development of normal mammary glands; these strategies are also used by BC cells to promote tumor tolerance and escape from immune surveillance in the early stages of disease, suggesting an important role of adaptive and innate immunity in BC development and progression. Increased insights into the molecular mechanisms employed by cancer cells to subvert or escape from immune recognition have recently opened an array of new therapeutic interventions. These include the implementation of monoclonal antibodies directed against neoantigens, the development of cancer vaccines, adoptive T-cell transfer, and the use of immunomodulatory agents such as cytokines, costimulatory receptor agonists, and CPI. Therefore, we here review the different immune escape strategies of BC as well as the role of tumor-infiltrating immune cells in the TME and their clinical relevance, finally suggesting the development of novel strategies to overcome immunosuppression and to enhance the immunogenicity of tumors by reverting their immune escape with the aim to develop and/or improve immunotherapies for this malignancy.

Immune Escape Mechanisms of Tumors

Recent evidence has demonstrated an essential role of cells from the innate and adoptive immune system involved in the initiation but also progression of cancer. This is mediated by the suppression of immune rejection leading to enhanced tumor growth and spread including the formation of the primary metastatic lesions [3, 4]. In the early steps of tumor development, host immune factors, in par-

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ticular cells of the innate immune system such as natural killer (NK) cells, tumor-associated macrophages, tumor-associated neutrophils, and myeloid-derived suppressor cells, play a key role in the rejection of cancer cells [5], while in the equilibrium phase tumor cells survive in a quiescent state [6]. This is followed by an immune escape in which tumor variants grow out and evade immune recognition and establish an immunosuppressive TME [7, 8]. This so-called immunoediting process suggests that tumors develop distinct mechanisms to evade immune surveillance, induce tolerance, and survive in the host. Only recently, immune evasion has also been recognized as a hallmark of BC [9]. The underlying mechanisms include reduced expression of major histocompatibility complex (MHC) class I, adhesion and costimulatory molecules, loss of antigens, and increased expression of immunosuppressive components such as HLA-G, HLA-E, and PD-L1 and of other immunosuppressive factors such as cytokine and metabolites that contribute to the escape from immune recognition [10]. Due to these alterations and the influence of host immunity, tumors develop low immunogenicity and a strong immunosuppressive TME and do not elicit an adaptive immune response. Furthermore, the frequency of CD4+ T cells and CD8+ cytotoxic T lymphocytes (CTL) in the TME is low, and their phenotype is often associated with immune exhaustion [11]. Based on this information, a number of studies are currently ongoing analyzing the nature of immune cells in the TME and the peripheral blood of tumor patients in combination with the cytokine profile produced by these cells and the immunogenic phenotype of tumor cells.

Breast Cancer and Its Immunogenicity

BC has been suggested to be a very heterogeneous disease, and based on histology and the molecular and transcriptional profile, different BC subtypes have been identified and correlated with clinical outcome. Primarily, BC have been classified based on the presence or absence of certain hormone receptors, growth factor receptors, and mutational load resulting to the definition of different BC subtypes. Recently, a link between their molecular make up and immunologic features was reported based on RNA sequencing data of the The Cancer Genome Atlas (TCGA) [12].

Breast Cancer and Tumor-Associated Antigens

High throughput technologies allowed the identification of structural alterations, such as gene mutations, amplifications, and loss of heterozygosity (LOH), in BC subtypes. This can result in the development of neoantigens presented by human leukocyte antigen (HLA) class I molecules, which can then be recognized as foreign by CD8+ CTL. Next to these mutation-specific neoantigens, other tumor-associated antigens (TAA) are autoantigens which are expressed excessively, differentiation-specific antigens, cancer testis genes (CTA) that display normal expression in immune-privileged organs but aberrant expression in several types of cancer including BC-modified autoantigens, TAA that are highly expressed in tumors such as HER2/neu, and viral-associated antigens. For example, members of the CTA family of melanoma-associated antigens (MAGEs), MAGE-A9 and MAGE-A11, are less expressed in estrogen receptor(ER)-negative and HER2/neu-negative BC [13, 14]. In contrast, upregulation of MAGE-A9 in invasive ductal BC is correlated with an unfavorable outcome for patients [15]. Furthermore, mesothelin is overexpressed in triple-negative breast cancer (TNBC) via modifying the T-cell receptor (TCR) [16]. Other TAA expressed by BC cells include MUC1, Claudin, and HER2/neu [17, 18]. The latter is highly overexpressed in HER2/neu-positive BC, and its expression levels are positively correlated with histologic tumor grade and associated with poor prognosis [19–22]. Although low expression of HER2/neu might restrict the immune cell-mediated destruction of tumor cells, high levels of HER2/neu have been shown to diminish HLA class I surface expression resulting in reduced CTL activity [23]. Loss or modification of surface antigens may promote immune evasion via a lack of tumor cell recognition. These distinct TAA and mechanisms may become potential targets to broaden the immunotherapeutic strategies in BC.

Immunogenicity of Breast Cancer and Immune Escape

Recent data suggest that BC might also be an immunogenic tumor. Using RNA sequencing data from the TCGA obtained from >1,004 BC cases, 4 distinct immune phenotypes were identified based on the expression of immune-relevant genes. These Immunologic Constant of Rejection (ICR) phenotypes, ICR1–4, define an immune-favorable phenotype (ICR4) and immune-unfavorable phenotypes (ICR1) associated with tumor progression and survival of BC patients [12].

Abnormal Expression of MHC Class I and Components of the Antigen Processing and Presentation Machinery and Interferon Signal Transduction Pathway in BC

The MHC class I/TAA complex expressed on tumor cells is recognized by CD8+ effector CTL. Loss or downregulation of MHC class I surface antigens allows tumor cells to escape immune surveillance [24, 25]. Tumor cells of distinct origin are able to silence MHC class I surface expression, which is often caused by diminished expression of components of the MHC class I antigen processing and presentation machinery (APM) and of the interferon (IFN) signal transduction pathway and associated with a worse outcome [26]. Concerning BC, HLA class I expression levels have been shown to be significantly downregulated in BC, which is necessary for the transformation of normal cells into abnormal cells. Furthermore, downregulation of beta-2 microglobulin (β_2-m), calnexin, and transporter-associated antigen processing (TAP) subunit 1 leading to impaired HLA class I expression was found in metastatic brain lesions of BC, which was also negatively linked to the frequency of CTL infiltration [27]. The underlying molecular mechanisms responsible for deficient HLA class I expression are diverse and could be attributed to gene mutations, LOH, and tran-

scriptional, posttranscriptional, and translational control, and altered oncogenic and IFN signaling [28–33]. This results in decreased tumor immunogenicity, which is consequently associated with increased malignancy, metastasis formation, worse prognosis, and poorer response to (immuno-)therapies [34–37]. While downregulation of HLA class I antigens negatively interferes with T-cell responses only, the expression of the non-classical HLA-G, which consists of 4 membrane-bound and 3 soluble isoforms [38], results in escape of tumor cells from both NK- and T-cell-mediated recognition [39]. In BC, increased HLA-G expression levels were found [40] which were not only associated with poor prognosis [41, 42] but also with therapy response of BC treated with neoadjuvant chemotherapy [40]. In addition, high soluble HLA-G concentrations were detected in the serum of BC patients and correlated with occurrence of metastasis, suggesting their usefulness as prognostic biomarker [43]. However, whether HLA-G could be detected in exosomes and thus spread to other cells to reduce their immunogenic phenotype remains controversial [44]. The role of HLA-G in BC was further underlined by the fact that the 14 bp InDel polymorphism in the *HLA-G* gene was a risk factor for the development of BC [45]. Similar to HLA-G, the stress-induced MHC class I-related gene A (*MICA*) is also frequently upregulated in high-grade BC and is an indicator of poor prognosis [46]. Taken together, the link between low HLA class I/high HLA-G/MICA expression and worse BC prognosis postulates the induction of HLA class I expression and inhibition of HLA-G/MICA expression as a new therapeutic option in the treatment of BC [47].

Disturbance of Antiapoptotic Function in Breast Cancer

Next to alterations of classical and non-classical HLA class I genes, reduced apoptosis represents a major strategy for evading immune response in cancer development [48]. This includes altered expression of Fas (factor associated with suicide), also known as CD95, and its ligand FasL (CD95L) [49]. This system can activate apoptosis signaling and induce apoptosis in cells. Increased FasL levels in BC cells cause effector T lymphocytes to die, resulting in escape from immune surveillance [50, 51]. Furthermore, tumor cells can resist Fas-mediated apoptosis by silencing or downregulating the Fas/FasL signaling pathways, which has been reported to be associated with a worse prognosis in BC. It is noteworthy that CD95L exists not only at the transmembrane but also in a soluble form (sFasL) due to cleavage by metalloproteases. sFasL has been found in the serum of TBNC patients promoting metastatic dissemination [52]. In addition to Fas/FasL, other proteins such as Bcl-2, survivin, and caspase play an important role in apoptosis in BC. Bcl-2 is overexpressed in BC cells leading to prevention of apoptosis associated with neoplastic transformation and an enhanced cellular live span [53, 54]. Expression of survivin, another member of the antiapoptotic family, is increased in BC. This is accompanied by poor outcome, advanced tumor grade, increased metastasis formation, and a low survival rate of patients [55]. In contrast, decreased caspase activation due to downregulation of caspase expression represents a further strategy of tumors to resist apoptosis. In this context, it is noteworthy that caspase-3 and -7 expression is downregulated in BC [56], but this deregulation appear not to be significantly correlated with the clinicopathologic features of this disease [57].

Expression of Co-Inhibitory Molecules

Based on the successful implementation of antibodies directed against immune checkpoints, the programmed death receptor 1 (PD-1) and its ligand PD-L1 have come into the focus of research [58, 59]. The interaction between PD-1 on T lymphocytes with PD-L1 expressed on the surface of tumor cells inhibits the activation of effector T cells and induces FasL and the immunosuppressive cytokine interleukin (IL)-10. PD-L1 is expressed in many tumor cells of distinct origin and at a high frequency on BC cells [60] and circulating tumor cells [61]. Inhibition of PD-L1 significantly blocks T-cell apoptosis in tumor models [61, 62]. PD-L1 could directly synergize with FOXP3+ regulatory T cells [62], but could also be affected by ubiquitination and N-glycosylation [63]. In different BC subtypes, heterogeneous expression of PD-1/PD-L1 was shown [64–66]. In particular in TNBC, PD-L1 was found to be overexpressed [67, 68], which is related to tumor grade [69, 70], local cytotoxic immune response, and prognosis [71]. This was the rationale for the implementation of anti-PD1/anti-PD-L1 for the treatment of TNBC, stimulating tumor regression and improving patient outcomes. Indeed, results from clinical trials demonstrated an increase in lasting local antitumor responses accompanied by a promising clinical benefit [72, 73]. Other co-inhibitory molecules include the lymphocyte activation gene 3 (LAG-3) and the T cell immunoglobulin and mucin domain-3 (TIM-3) checkpoints, which have recently been investigated in a large cohort of BC patients and their expression pattern linked to clinicopathologic parameters. LAG-3-positive tumor-infiltrating lymphocytes (TILs) were particularly enriched in ER-negative BC and were negatively associated with young age, large tumor size, and high proliferation, while a high proportion of PD-1/PD-L1-positive tumors were co-infiltrated with LAG-3-positive TILs [74]; TIM-3 expression levels were high on tumor cells and significantly associated with clinicopathologic parameters such as age, axillary lymph node metastasis, and TNM stage [75]. In addition, other B7 family members, such as B7-H3 known to stimulate IL-10 secretion, also contribute to tumor immune evasion and tumor progression. Expression of B7-H3 was found at a high frequency of BC lesions, which was correlated with IL-10 [76]. Thus, other immune checkpoints also promote BC development and progression and might be used as independent prognostic factors for invasive ductal carcinoma (IDC) patients.

Tumor Microenvironment in Breast Cancer

Different components of the TME have been show to play a key role in tumor development and progression. These are cellular, soluble, and physical factors which are shaped by specific structures, functions, and metabolic properties of neoplastic lesions [77, 78]. Autocrine and paracrine mechanisms of tumor cells alter the inter-

play between tumor and immune cells thereby maintaining conditions essential for the survival, development, proliferation, and progression of tumors. Furthermore, advances in molecular biologic as well as immunologic features of BC have helped elucidate the association between malignant BC cells and immune factors or their modulators around the tumor. Different types of immune responses have been linked to a distinct cytokine microenvironment: While tumor-specific B-cell responses were correlated with increased transforming growth factor (TGF)-β1, reduced IFN-α, absence of T-cell responses, and worse prognosis, high IFN-γ levels were associated with a strong presence of T-cell infiltration and activity [79]. Recently, a number of studies characterized the BC TME concerning suppressive immune cells, re-programmed fibroblast cells, altered extracellular matrix, and soluble factors, which negatively interfere with an effective antitumor response and promote BC progression and metastasis. It has been suggested that changes in the TME not only have an important impact on BC development and progression but appear to also serve as prognostic factors for the clinical outcome of patients and response to (immuno-)therapies. Substantive tissue and tumor subtype-specific differences of multiple cell types, in particular TILs, have been identified in BC subtypes, and in particular TNBC and HER2/neu-negative BC exhibit a unique TME distinct from that of other BC subtypes. Immunohistochemical analyses have demonstrated that CD8+ CTL, known to contribute to tumor clearance, were associated with good prognosis and long-term survival [80] and are thus of clinical relevance [81]. Furthermore, the number of tumor-infiltrating CD8+ T cells was associated with primary tumor size, lymph node metastasis, WHO (World Health Organization) grade, Ki-67, and molecular classification. Although the major focus of most studies was on CD8+ CTL, Th1 CD4+ T cells could also contribute to the elimination of BC by the production of IFN-γ, resulting in reduced angiogenesis and enhanced T-cell and M1 macrophage activity [82]. In contrast, the role of Th2 CD4+ T cells has not yet been identified in detail in BC, but might be more tumor-promoting than tumor-suppressive. There is also evidence that infiltration with CXCL13-expressing CD4+ follicular helper cells in BC predicts improved patient survival [83] and could serve as a prognostic marker in this disease [84]. The function of B-cell infiltration is also poorly understood, and discrepant results have been reported regarding its importance, or lack thereof, for poor/good clinical prognosis [85]. Therefore, more detailed analyses have to be performed in order to conclusively determine the prognostic and predictive potential of immune cells in BC. Expression profiling of BC lesions further revealed a differential expression pattern of genes associated with immune cells, such as IFN-regulated genes [86, 87], B-lymphocyte marker [86], as well as T-lymphocyte-associated genes [87], which further underpins the crucial role even of host immune responses in this disease. This was also underlined and extended by a recent report comparing the expression of immune-relevant genes in normal mammary tissues, ductal carcinoma in situ (DCIS), and IDC [88]. A decrease in CD8+ signatures in IDCs versus DCIS and of activated GZMB+CD8+ T cells in IDC was detected. These were accompanied by significantly higher TCR clonotype diversity in DCIS than in IDCs. Furthermore, a link between the frequency of TILs in the stroma and prognosis as well as response to chemotherapy has been shown [89–91].

Perspectives and Conclusion

The main hurdle for BC in generating a broad and robust antitumor immune response is to overcome the evasion of immune surveillance. However, the underlying molecular mechanisms leading to immune escape in BC have to still be elucidated in detail. Despite some treatment success with CPIs, the response seen in patients is limited. The cause of intrinsic and extrinsic resistance mechanisms of BC have not yet been analyzed. However, first evidence in melanoma patients suggests that resistance to anti-PD1 therapy is mediated by structural alterations or reduced expression of HLA class I APM and/or IFN signaling components [92]. Future perspectives to improve patient outcome and monitor therapy response are therefore based on the molecular make up in combination with the expression of immune-relevant molecules in both immune cells and tumor cells and the thorough analysis of the composition of the immune cell infiltrate as well as the peripheral blood of BC patients. This might lead to the development and design of therapies in which CPIs will be combined with each other or with targeted therapies, chemotherapy, and/or radiation. Examples are anti-PD-1/anti-PD-L1 or inhibitors of MAP2K and glucocorticoid-induced tumor necrosis factor receptor (GITR) [93, 94], which are currently under way and will be covered by a separate article in this issue.

Disclosure Statement

We hereby state that both authors have no conflicts of interest.

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