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Recent advances of immune checkpoint in breast cancer.

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Abstract

Many advances in local and systemic therapies have noticeably improved outcomes in breast cancer in women. However, early and late relapses continue to occur. Thus, novel therapeutic approaches are necessary. An understanding of the mechanisms of immune escape employed by tumour cells and the ability to regulate immune checkpoints with immunomodulatory monoclonal antibodies will provide very promising clinical results in different tumour types. In this review, we specify the rationale for targeting immune checkpoints in breast cancer and discuss their prospective use for the design of novel clinical trials.

Keywords: Breast cancer, Immunotherapy, Immune checkpoints, CTLA-4, PD-1.

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Introduction

According to published reports, the number of new breast cancer patients reached 1.67 million in 2012, accounting for 25% of total cancer patients [1-3]. Moreover, breast cancer has become the main cause of death in women cancer patients worldwide, including both low and middle-income countries [2]. Despite the fact that the less aggressive variants of this disease may be treated through surgery and other intensive therapies, the prognosis of patients remains dismal, and approximately one-third of women will develop metastases and die [4]. For example, inflammatory breast cancer and triple-negative breast cancer currently do not benefit from well-regarded treatments because of their aggressiveness and lack of defined molecular targets. Recently, the impacts of immunologic system targets on cancer have been extensively debated and have acquired increasing relevance in the context of cancer [5]. It is widely known that the primary function of immune cells is to eradicate transforming cells, and immune cells may exhibit antitumor or tumour-promoting activities during carcinogenesis [6]. Immunomodulation and immunotherapy have been shown to be beneficial in breast cancer treatment, such as the effects of pembrolizumab (MK-3475) in triple-negative breast cancer, which were reported by Nanda et al. [7]. The fine balance between co-stimulatory and co-inhibitory factors has an impact on the immune responses and the efficacy of immune responses, which are also referred to as immune checkpoints [8]. Immune checkpoint-targeted agents are new therapeutic drugs in the field of medicine. This review focuses on immune checkpoints and the monoclonal antibodies that block the Cytotoxic T-Lymphocyte-associated Antigen-4 (CTLA-4) and Programmed Death 1 (PD-1) pathway. We provide an overview of the rationale for targeting immune checkpoints in breast cancer, as well as the most recent developments in immune checkpoint inhibitors.

Checkpoint blockade

To date, the genomic revolution has enabled a comprehensive understanding of the molecular mechanism of tumour-associated immunologic responses. First, an antigen of the Major Histocompatibility Complex (MHC) is recognized by the T-Cell Receptor (TCR) immune response. Second, its quality and magnitude are determined by signals that are transmitted by immune checkpoints of the cell surface [9]. The checkpoints maintain the homeostasis of the host to avoid autoimmune reactions through a process that is regulated by the immune system. Ultimately, these checkpoints function in limiting the extension and severity of the immune response, particularly regarding the adaptive immune system [5].

Based on numerous studies regarding immunotherapies, it is generally accepted that CTLA-4 and PD-1 inhibitors lead to the most promising results and have been the most extensively tested in patients with breast cancer [10].

The CTLA-4 receptor

CTLA-4 (also referred to as CD152) is a protein receptor that is widely regarded as an archetypal T-cell intrinsic inhibitory checkpoint. It is expressed on most immune cells, particularly CD4+ and CD8+ T-lymphocytes. As a member of the immune regulatory system, CTLA-4 is homologous to the T-cell costimulatory protein CD28. Both of them can combine with CD80/CD86 on antigen presenting cells (APCs) [11,12]. CD28 on T cells combines with CD80/CD86 on the surface of APCs and stimulates T-lymphocyte proliferation and activation, whereas the binding of CD80/CD86 to CTLA-4 on T cells

leads to an anergic phenotype. CTLA-4 combines CD80/CD86 with a stronger affinity than CD28, giving rise to competition [13]. The tumour-specific target antigen stimulates the body, and specific antitumour T cells are activated by TCR and CD28. CTLA-4 is subsequently up-regulated and blocks the CD28 signalling pathway. In addition, the interaction between CTLA-4 and CD80/CD86 will induce the phosphorylation of the intracellular segment of CTLA-4, which results in a negative signal that blocks the activation and function of T-lymphocytes. As previously discussed, CTLA-4 is important for maintaining the normal immune homeostasis of the organism. This function has been demonstrated by the finding that a lack of CTLA-4 can cause mice to die from fatal lymphoproliferation [14,15]. Moreover, in preclinical models, the CTLA-4 antibody blockade resulted in antitumour immunity in various cancers, such as prostate, kidney, and urothelial cancers [16-18]. In breast cancer cells, higher CTLA-4 expression is associated with a poor prognosis, whereas a higher density of interstitial CTLA-4+ lymphocytes is associated with a good prognosis. However, when the tumour had a low expression of CTLA-4, a high density of CTLA-4+ lymphocytes was significantly correlated with a good prognosis [19].

The PD-1 receptor

Similar to CTLA-4, PD-1 (also referred to as CD279) is an inhibitory receptor that negatively regulates the immune system. However, CTLA-4 mainly affects T cells, whereas PD-1 is more broadly expressed on B cells, Tregs and Natural Killer (NK) cells and regulates mature T-cell activity in the tumour microenvironment and peripheral tissues [8,20]. The PD-1 receptor combines two types of ligands: programmed cell death-1 ligand 1 (PD-L1) and Programmed cell Death-1 Ligand 2 (PD-L2) [21-24]. The ligands interact with CD28 and CTLA-4, respectively. PD-L1 binds CD80 with a substantially greater affinity than CD28, giving rise to competition [25]. The docking of CD80 causes the down-regulation of downstream pathways, which leads to cytokine release and T-cell activation [20]. Accordingly, by inhibiting this signalling pathway, the PD-1/PD-L1 interaction induces T-cell tolerance [25-27]. The intracellular enzymatic pathways of PD-1 and CTLA-4 are slightly different. However, they exert a similar immunosuppressive effect because both pathways induce an ineffective anergic T-cell response [28]. PD-L1 is expressed on many cancers, including ovarian cancer, hepatocellular carcinoma, renal cell carcinoma and gastric cancer [29,30]. PD-L1 expression in breast cancer has been detected by Ghebeh et al. who identified PD-L1 expression in 22 (50%) of the 44 tumours evaluated in their study. In 18 of these tumours (41%), PD-L1 was identified in tumour-infiltrating lymphocytes, and in 15 tumours (34%), it was restricted to the tumour epithelium [30]. Furthermore, PD-L1 mRNA expression was detected in substantially larger subsets of breast cancer, and the expression of PD-L1 was correlated with the clinicopathological parameters of highly proliferative breast cancer [31-36].

Other Checkpoint Molecules

LAG-3

LAG-3 (lymphocyte activation gene-3) is a cell surface molecule, also referred to as CD223, whose structure is similar to CD4 and is present on many immune cells [37]. It can be expressed by Tumour-Infiltrating Lymphocytes (TILs) and suppresses the activation of APCs [38]. Murine disease models and ovarian cancer patients showed synergism between the LAG-3 and PD-1 blockade. Both studies indicate that the antibody could block PD-1, and in combination with LAG-3 blockade, it showed higher efficacy than either factor alone. This method may become a useful strategy in some malignancies [38-40].

TIM-3

T-cell Immunoglobulin Mucin 3 (TIM-3) is a marker of Interferon-γ producing CD4+ and CD8+ T cells, which were first identified in mice and humans in 2002 [41,42]. The TIM-3 receptor may be combined with Gal-9 and subsequently plays a negative regulatory role in the survival and function of T cells [12]. TIM-3 is associated with nuclear grade, TNM stage, Progression-Free Survival (PFS) and recurrence-free survival by univariate analysis. The percentages of CD4+ T cells and TIM-3+PD-1+CD8 T cells were substantially higher in the tumour infiltrate than the peripheral blood obtained from patients with renal cell carcinoma [43]. However, to date, there are no sustained TIM-3 blockade clinical trials in patients with cancer [44].

KIR

The Killer-cell Immunoglobulin-like Receptor (KIR) is a receptor for MHC class I molecules located on NK cells, and it participates in the down-regulation of the immunological activity of NK cells [20]. In contrast to adaptive T and B cells, NK cells modulate and conduct their killing capacity independent of several activating and inhibitory receptors [45]. In addition, their cytotoxicity against multiple tumour cell lines is increased as a result of a mismatched KIR-ligand or NK cells that do not express the KIR-ligand [46,47].

Immune Checkpoint Blockade

Anti CTLA-4

Two monoclonal antibodies that target the CTLA-4 receptor are currently being investigated in breast cancer patients, namely, ipilimumab and tremelimumab.

Ipilimumab

Ipilimumab is a type of fully humanized IgG1 monoclonal antibody. It was recently used to treat metastatic melanoma [48]. Treatment with ipilimumab (3 mg/kg every 3 weeks) improved the median survival by 3.7 months in metastatic melanoma phase III clinical trials [49]. Ipilimumab was

reported to prolong the lifespan of some patients, and approximately 24% of the patients were alive after 2 y [50].

In addition, ipilimumab is being evaluated in breast cancer patients in active clinical trials. One aim is to evaluate the safety of ipilimumab alone or a combined application with cryoablation in women with curable early-stage breast cancer [51]. The other on-going clinical trial uses ipilimumab in stage IV breast cancer patients whose conditions are still developing although being treated with primary treatment [52]. In murine breast cancer models, the activity of ipilimumab was associated with fractionated radiotherapy [5,53,54]. Moreover, another study showed that 40% of mice underwent EMT6 breast cancer tumour regression when treated with ipilimumab only, whereas 100% of the mice treated with both ipilimumab and ixabepilone experienced complete tumour regression [55].

Tremelimumab

Tremelimumab is a fully humanized IgG2 monoclonal antibody that blocks the binding of CTLA-4 to CD80 and CD86 and enhances human T-cell activation [8]. Tremelimumab has antitumour activity in patients with advanced melanoma [56]. In addition, tremelimumab was the first CTLA-4 abrogating agent, and its activity in combination with the aromatase inhibitor exemestane has shown mild adverse reactions (constipation, diarrhoea, fatigue and pruritus) in 42% of breast cancer cases [57]. As a result of the low response rate to tremelimumab, it is no longer used as a single immunotherapeutic agent in patients with advanced solid tumours, and the evaluation of the combination of tremelimumab with the anti-PD-L1 antibody MEDI-4736 is on-going.

Anti PD-1 signalling

Various monoclonal antibodies that block PD-1 signalling are under evaluation in clinical trials. Compared with anti-CTLA-4 agents, anti-PD-1 agents have more advantages, such as high tumour specificity and weak immune toxicity [58].

Nivolumab

Nivolumab was the first monoclonal anti-PD-1 antibody and showed minimal toxicity in large phase I research in small-cell lung cancer, renal cell carcinoma, advanced melanoma and other solid tumours [59-61]. Melanoma patients treated with ipilimumab and nivolumab showed a 32% overall response rate compared with an 11% response rate for chemotherapy (dacarbazine or carboplatin/paclitaxel) [62]. The objective response was approximately 18% in non-small cell lung cancer, 27% in renal cell carcinoma and 28% in melanoma. Moreover, this response is associated with the PD-L1 positivity of tumour cells [63]. However, no preclinical or clinical data are available for breast cancer treatment to date.

BMS-936558

BMS-936558 (formerly MDX-1106) is another monoclonal anti-PD-1 antibody that blocks the activity of PD-1 [64]. In previous research, 39 patients with advanced colorectal cancer, non-small cell lung cancer, metastatic melanoma, renal cell carcinoma or castrate-resistant prostate cancer were treated with BMS-936558. The results provided good safety and ideal evidence in support of its clinical application [65]. BMS-936558 exhibited a high response rate, which was approximately 18% in non-small cell lung cancer, 27% in renal cell carcinoma and 28% in melanoma [59]. It has been reported that BMS-936558 and MPDL3280A are both specific monoclonal antibodies of PD-L1, which showed substantially better safety than ipilimumab in clinical trials, including breast cancer trials (NCT00729664 and NCT01375842) [64].

Pidilizumab (CT-011)

Pidilizumab is a humanized IgG-1k recombinant monoclonal antibody that specifically blocks PD-1. It is currently approved for the treatment of metastatic non-small cell lung cancer by the US Food and Drug Administration (FDA) [66]. A phase II trial, which investigated its use for diffuse large B-cell lymphoma treatment, also evaluated the activity of pidilizumab in different tumours, including colorectal cancer, melanoma and many other solid tumours [67]. Pembrolizumab showed an acceptable toxicity profile in melanoma, non-small cell lung cancer and advanced renal cell carcinoma in an initial early phase trial [68]. Currently, many clinical trials are on-going for breast cancer treatment with pidilizumab, whose activity is associated with a p53 vaccine as a combinatorial therapy.

Other modulators

There are other checkpoint modulators, such as BMS-663513, which is specific for CD137 (4-1BB). Moreover, monoclonal anti-PD-1 antibodies, such as CT-011, AMP-224, MK-3475 and MPDL3280A, have been evaluated in phase II clinical trials in melanoma. Moreover, an agonist, an anti-OX-40 antibody, has been evaluated in prostate cancer [69]. There are no published data regarding the predictive value of PD-L1 expression in breast cancer for immune checkpoint inhibitor therapy. However, all phase I trials in breast cancer that reported clinical outcomes required PD-L1 expression for eligibility.

Conclusions

The human immune system functions as an immune surveillance, exhibiting a protective role against cancer by identifying and eliminating abnormal cells. However, tumour cells may escape attack through immune checkpoints as described in this review. Therefore, checkpoint blockade has emerged as a promising approach to renovate antitumour immunity. Reactivation of effector T cells through checkpoint blockade may overcome the development of resistant mutations and benefit cancer patients. Immune checkpoint inhibitors, which target regulatory immune cells to enhance antitumour immune responses, have achieved promising results and demonstrate significant clinical efficacy in various malignancies, including breast cancer. To date, the treatment of breast cancer remains challenging, as the research on breast

cancer is limited, and most research is in the basic research stage. Thus, there are many problems that remain to be solved. However, we have reason to believe that with continued research and clinical validation of immune checkpoint inhibitors, immunotherapy will bring more hope to breast cancer patients in the near future.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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