

Expression Patterns of Immune Checkpoints in Breast Cancer Patients

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Abstract: Background: immunotherapy with immune checkpoint inhibitors (ICIs) for solid tumors had significantly improved overall survival (OS). Positive response to PD-1/PD-L1 blockades was observed in the treatment of solid tumors. Breast cancer (BC) patients are no exception. However, the efficacy of immunotherapy in BC patients remains poor. A particularly important factor is the lack of studies on the expression patterns of immune checkpoints in BC patients. Methods: This article summarizes the expression of immune checkpoints such as PD-1, PD-L1, STAT1, CTLA-4 in BC, and analyzes the relationship between the expression of these immune checkpoints and OS. Results: It was found that increased expression of PD-1, PD-L1, STAT1, CTLA-4 was associated with poor OS in BC patients. In addition, co-expression of PD-L1 with PD-1, STAT1 or CTLA-4 and co-expression of PD-1 with CTLA-4 was related to poor OS. We analyzed associations between the proportionate expression of PD-L1 and PD-1, PD-L1 and STAT1, PD-1 and CTLA-4, PD-1 and LAG3, PD-L1 and CTLA-4 in BC patients, there was significance in correlation in both of the BC patients. Conclusions: our results suggest that transcriptome-based co-expression of STAT1 and PD-L1 is a predictor for poor OS in BC patients, which might provide novel insight into designing combinational targeted therapy for BC.

Keywords: PD-L1, PD-1, STAT1, Immune checkpoint, Breast cancer.

1. Background

Over the past 5 years, immunotherapy with immune checkpoint inhibitors has revolutionized the management of various cancers [1]. The occurrence and development of cancer is closely related to immune escape and abnormal immune monitoring. A familiar cell surface receptor, called programmed cell death protein 1 (PD-1), may allow T cells to escape anti-tumor immunity when the cells associated with cancer cells are upregulated. Ligand for the PD-1 receptor, programmed death ligand 1 (PD-L1), is expressed in a variety of epithelial cancers [2]. In addition, other immune checkpoint molecules such as CTLA-4, LAG3, and FGL1 have also been studied [3, 4]. The PD-1/PD-L1 signaling pathway mainly causes the body to be in the state of immunosuppression [5]. Recently, Cancer immunotherapy employing PD-1/PD-L1 inhibitors has resulted in drastic improvements in clinical outcomes in multiple malignant tumors [6, 7]. Moreover, an anti-PD-1 mAb have shown good safety to multiline systemic therapy in patients with advanced refractory TNBC [8]. Previous reports have shown that PD-1 and PD-L1 correlated gene expression profiles and their association with clinical outcomes of BC [9]. In addition, PD-L1 expression is closely related to the positive response of PD-1/PD-L1 blockade in solid tumor therapy [10]. These findings suggest that PD-1/PD-L1 blockade may be a novel immunotherapeutic strategy for BC. However, different types of BC patients have different clinical responses to PD-1/PD-L1 blockade, clinical use of triple negative breast cancer (TNBC) is more [11, 12]. We believe that in addition to PD-1 and PD-L1, there are other factors that may aggravate its immunosuppression, affect its immunotherapeutic effect, and lead to poor prognosis in patients with other types of BC.

STAT1 exists in cells in the form of inactive monomer in

primarily. After phosphorylation by tyrosine kinases, STAT1 is activated to form homologous or heterodimer. Even after the formation of polymers and oligomers, STAT1 can also play a physiological role, especially in the interferon (IFN) system [13]. At the same time, STAT1 is defined as a growth inhibitor factor and a apoptosis factor due to its characteristics of promoting apoptosis and inhibiting cell growth and differentiation, and it plays an important role in inhibiting the occurrence and development of tumors. First of all, the overexpression of STAT1 is often observed in the pathogenesis of solid tumors, which indicates that STAT1 is indeed closely related to tumors [13]. Recently, STAT1 has been identified as a potential therapeutic target for malignancies, including breast cancer, mainly through the JAK/STAT1 signaling pathway that affects the body's normal immune system [13, 14]. The expression of phospho-STAT1 (p-STAT1) in the breast cancer microenvironment is a potential biomarker. Anti-PD-1/anti-PD-L1 monoclonal antibodies have been used in immunotherapy of cancer patients based on the expression of p-STAT1 and PD-L1 tumor cells [14]. In addition, PD-L1 is downstream of STAT1, and STAT1 inhibition facilitates the anti-tumor immune response in BC patients by suppressing PD-L1 expression [15]. Therefore, it is important to understand the correlation between STAT1 and PD-1/PD-L1 signal channel in BC patients.

In this study, the expression of STAT1 in breast cancer was compared between the expression of different types of BC, to illustrate the correlation between STAT1 and clinical outcomes of BC patients, and to reveal how STAT1 regulates the PD-1/PD-L1 signaling pathway. Furthermore, to investigate the effects of PD-1, PD-L1, STAT1, IDO, CTLA-4, LAG3 and FGL1 on clinical outcomes of BC, and to determine its potential as a biomarker for prognosis in BC patients.

2. Expression of Immune Checkpoints in Breast Cancer

Female breast cancer has exceeded lung cancer, becoming the most often diagnosed cancer, and it is also the primary cause of female cancer-related death. In 2020, there were 2.26 million new cases of breast cancer in the world, surpassing lung cancer (2.21 million) to become the world's largest cancer, accounting for 11.7% of the global new cancer cases[16]. The high incidence of breast cancer has brought a heavy disease burden to women, which seriously affects women's physical and mental health and quality of life. The immune examination point also plays an important role in breast cancer. Some studies have found that targeting PD-L1 may be a promising treatment method for patients with breast cancer. And the conduction of the upstream STAT1 signal is the potential molecular mechanism of the lower PD-L1[17]. Studies have found that STAT1 breast cancer cell proliferation, invasion, blood vessel generation, metastasis and immune escape have a key role[18]. P-STAT1 expression may be potential biomarkers for patients to choose anti-PD-1/anti-PD-L1 monoclonal antibody immunotherapy[19].

Similarly, the occurrence of IDO, CTLA-4, LAG3 and FGL1 also plays an important role in breast cancer. The expression of IDO is related to ER and PR, while TNBC shows high expression of PD-L1 and IDO in TNBC. IDO expression is very common in TNBC, which is high-level three-negative breast cancer, and is often related to PD-L1, which indicates that IDO may be a mechanism for anti-PD-1/PD-L1 immunotherapy and drug resistance [20-21]. In Addition, The Literator Reports Some Associations Between CTLA-4, LAG3 and FGL1 in the Tumor Micro Environment

3. Discussion

STAT1 plays a significant role in the progression and prognosis of various tumors such as gastric cancer, colorectal cancer, ovarian cancer and other tumors [22-2]. We all know that the combined of PD-1 and PD-L1 has a negative regulatory effect on the immune system in cancer patients. However, little is known about the prognostic value of STAT1 in BC patients and how STAT1 affects the expression of PD-L1 in tumor cells [25]. This study investigated the relationship between STAT1 expression and OS in patients with BC. We found that overexpression of STAT1 predicts poor OS in BC, STAT1 was positively correlated with PD-L1. Therefore, STAT1 inhibitors can be used in combination with PD-L1 monoclonal antibodies to achieve optimal therapeutic efficacy. More importantly, immune checkpoints can be used as biomarkers to predict the prognosis of patients with BC. The expression of immune checkpoint molecules on T cells is considered to be one of the important regulatory mechanisms for immune cells to regulate their own antigen response [26, 27]. Some studies have shown that immune checkpoints such as PD-1, CTLA-4 and LAG-3 are upregulated on the surface of T cells in breast tumor tissues. The epigenetic modifications behind their upregulation are depends on the methylation of DNA and the distribution of inhibitory histone [28]. Therefore, the combination of targeted drugs and STAT1 blocking function is a new strategy for the treatment of tumors. Recent studies have shown that combination therapy of ICB with other targeted agents has the potential to enhance the anti-tumor response of BC [29, 30]. Besides that, PD-L1 has been proved to be a immediate target for STAT1-mediated

gene expression [31]. In this study, we found that the expression of STAT1 in all BC patients was increased. Then, it was also found that the expression of STAT1 was positively correlated with PD-1 and PD-L1. More importantly, STAT1 co-expression with PD-L1 and PD-1 also predicted OS in BC patients. In addition, STAT1 was overexpressed in BC patients regardless of type. These findings may provide accurate and valuable OS prediction for BC. On the basis of molecular typing, basal-like BC subtype showed highest expression of STAT1, IDO1, LAG3, which suggest that different subtypes possess various immune checkpoints status. In addition, the expression of PD-1 and PD-L1 was associated with recurrence and difficulty in BC patients [32]. Both PD-1 and IDO are highly expressed in the tumor microenvironment of BC patients, while LAG3 and PD-1 were highly expressed in T cells of BC patients, suggesting poor prognosis [33, 34]. Which not only confirm the critical role of immune checkpoint in BC progression and prognosis, but also associated with immune evasion in numerous. Especially in TNBC, PD-L1 and STAT1 are both highly expressed, while in conventional BC, PD-L1 and STAT1 are simultaneously low expressed, which may be related to the poor treatment of PD-L1/PD-1 inhibitors in some BC. Surprisingly, PD-L1 and STAT1 are also overexpressed in precancerous breast cancer cells. This suggests that the expression of immune checkpoint may be different in different subtypes of breast cancer or precancerous lesions. This may provide a new direction for our treatment strategy.

As is known to all, the research of immune checkpoint is becoming more and more important in the field of cancer. Tumor cells use various tricks to scape of immune system, such as activating immune checkpoint pathways that induce immunosuppressive function. PD-1 and PD-L1, CTLA-4 and LAG3 have been extensively evaluated as putative markers of response to immunotherapy with PD-1/PD-L1 and CTLA-4 blockade, respectively [35-38]. Abirami et al. demonstrations of clinical benefit of immunotherapy in female metastatic breast cancers (MBC) support the need for development and utilization of biomarkers to guide the use of immune checkpoint inhibitors (ICPIs) for these patients, such as PD-1, PD-L1, CTLA-4 and LAG3 [39]. In this study, both PD-1 and PD-L1 were positively correlated with STAT1 expression. Although not associated with short-term survival outcomes, it is significant for long-term survival, especially for PD-1 expression and co-expression PD-L1 or STAT1 expression. This may be due to inconsistencies in the subtypes of BC. In addition, we observed that PD-L1 was more expressed in TNBC. Therefore, in the near future, the application of molecular and biochemical markers in a personalized manner will improve optimal combination immunotherapy, providing customized treatments that may save the lives of patients with malignant breast cancer who have a poor prognosis.

4. Conclusion

In summary, we demonstrate that transcriptome-based co-expression of STAT1 and PD-1/PD-L1 could be a predictor of poor OS in BC patients. This finding will provide novel insights into designing combinational immuno-targeted therapy in BC patients.

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